



Seroprevalence of *Leptospira* spp. in horses from Rio Grande do Norte, Brazil¹

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ABSTRACT. Rizzo H., Rocha L.L.L., Diniz D.D.M., Lima G.S., Jesus T.K.S., Pinheiro Júnior J.W. & Castro V. 2022. **Seroprevalence of *Leptospira* spp. in horses from Rio Grande do Norte, Brazil.** *Pesquisa Veterinária Brasileira* 42:e06784, 2022. Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Manuel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: hubervet@gmail.com

This study aimed to determine the prevalence and risk factors associated with the presence of anti-*Leptospira* spp. antibodies in horses reared in the Eastern and Western Potiguar mesoregion, State of Rio Grande do Norte, northeastern Brazil. Seven hundred and eighty-five animals (785) were used from 90 properties from 2018 to 2019. The serological diagnosis was performed by the microscopic agglutination technique (MAT), using a collection of live antigens composed of twenty serovars belonging to sixteen serogroups. Variable data associated with risk factors were obtained from questionnaires carried out to herd owners and statistically analyzed. The absolute and relative frequencies were determined by descriptive analysis and risk factors by univariate analysis of the variables using the Pearson's chi-square test and Fisher's exact test, when necessary, and then assessed by Poisson regression. The positivity frequency among animals was 97.2% (763), and from all properties, there was at least one seropositive animal. The main reactive serogroup was Icterohaemorrhagiae (72.88%), followed by reactive animals to Australis (14.94%), Sejroe (4.43%), Shermani (2.77%), Autumnalis and Pomona (1.29%). It was observed occurrences lower than 1% to serogroups Canicola, Ballum, Grippotyphosa and Hebdomadis, with titles ≤ 400 in 78,97% of cases. The significant occurrence of seropositive horses and the serogroup Icterohaemorrhagiae predominance highlights the importance of vaccination and hygiene in Potiguares herds facilities to prevent leptospirosis cases and the consequent elimination of the agent in the environment.

INDEXING TERMS: Seroprevalence, *Leptospira* spp., horses, Brazil, Icterohaemorrhagiae, macroscopic seroagglutination, serogroup.

RESUMO.- [Soroprevalência de *Leptospira* spp. em equinos do Rio Grande do Norte, Brasil.] Este estudo objetivou determinar a prevalência e os fatores de risco associados à presença de anticorpos anti-*Leptospira* spp. em equinos criados na mesorregião Leste e Oeste Potiguar, estado do Rio Grande do Norte, Nordeste do Brasil. Foram utilizados 785 animais oriundos de noventa propriedades durante o período

de 2018 e 2019. O diagnóstico sorológico foi realizado pela técnica de soroprecipitação microscópica (MAT) utilizando coleção de antígenos vivos composta por vinte sorovares, pertencentes a dezesseis sorogrupos. Os dados das variáveis associadas aos fatores de risco que podem predispor a presença de equinos soropositivos, foram obtidos a partir de questionários aplicados aos proprietários dos rebanhos e analisados estatisticamente. As frequências absolutas e relativas foram determinadas por análise descritiva e os fatores de risco por análise univariada das variáveis de interesse pelo Teste de Qui-quadrado de Pearson e Exato de Fisher, quando necessário, e em seguida submetidos à análise de regressão de Poisson. A frequência de equinos soropositivos foi de 97,2% (763), sendo que em todas as propriedades houve pelo menos um animal com presença de anticorpos. O sorogrupo predominante foi o Icterohaemorrhagiae (72,88%), seguido

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do Australis (14,94%), Sejroe (4,43%), Shermani (2,77%), Autumnalis e Pomona (1,29%). Ocorrências menores que 1% foram observadas para os sorogrupos Canicola, Ballum, Grippytyphosa e Hebdomadis, com títulos ≤ 400 em 78,97% dos casos. Devido à alta ocorrência de equinos soropositivos e a predominância do sorogrupo Icterohaemorrhagiae, ressalta-se a importância da vacinação e da higiene das instalações nos rebanhos Potiguares na prevenção de casos de leptospirose e a consequente eliminação do agente no ambiente.

TERMOS DE INDEXAÇÃO: Soroprevalência, *Leptospira* spp., equinos, Brasil, Icterohaemorrhagiae, soroaglutinação macroscópica, sorogrupo.

INTRODUCTION

Leptospirosis is a zoonosis caused by different species of the genus *Leptospira* spp. It affects humans, horses, ruminants, canines, felines and other domestic, wild and synanthropic species (Pinna et al. 2008). It belongs to the family Leptospiraceae, order of Spirochetes and is an obligate aerobe with an ideal growth range between 28 and 30°C (82.4-86°F) (Hines 2014). There are 64 identified species (Vincent et al. 2019); *L. interrogans* is the most epidemiologically important in Brazil (Vasconcelos et al. 2012).

Leptospirosis is considered the most widespread zoonosis globally, causing more than one million severe cases a year in humans, with a lethality between 5 and 20%. Outbreaks usually occur in flooding, increased rainfall and contact with flooded areas. The predominantly humid environment allows leptospire to survive for several weeks after being eliminated in the urine of carrier animals (Abela-Ridder et al. 2010, Castro 2010). The most common form of infection is the penetration of mucous membranes and skin and can also occur via inhalation and ingestion (Hines 2014). After infection, colonization of renal tubules occurs, leading to leptospiruria and animal reservoirs (Vallejo et al. 2008).

In Brazil, the disease is endemic in all states. In rainy periods in rural areas, it has an epidemic character due to intense contact with livestock, devolving into an important occupational disease (Hartskeerl et al. 2011). A survey carried out in 2007 showed that US\$ 439,956.47 was spent on hospitalized patients who died from leptospirosis in the country (Souza et al. 2011).

In urban areas, synanthropic rodents (*Rattus norvegicus*, *Rattus rattus* and *Mus musculus*) are the main sources of infection for humans (Vasconcelos et al. 2012). In rural areas, livestock such as cattle, pigs and horses are responsible for 16.3%, 9.1% and 3.6% of infections, respectively, and wild or free-living animals account for 11% of the cases (Hartskeerl 2005).

In livestock, the disease impact depends on the infection's origin. In the case of infection by serovars not adapted to the species in question, they will develop the acute form of the disease leading to severe kidney complications that may lead to death. In the case of infection by a serovar adapted to the species, the host will develop the disease in a subclinical or asymptomatic form, with long periods of elimination of the agent in the urine, acting as reservoirs that constitute the most critical source of contamination of soil, water and infection to other animals (Divers 2015).

Horses may play a relevant role in the transmission of leptospirosis by eliminating the agent in the environment, even in cases of infection by non-adapted serovars. Unlike other production species, high titers of circulating antibodies in horses are not antagonistic to the survival of *Leptospira* spp. in the renal tubules, resulting in elimination in the urine between one- and three-months post-infection (Yan et al. 2010, Hamond et al. 2013). The economic impacts of leptospirosis are relevant for equine agribusiness. Although the infection is more common than the clinical disease, the latter, when it occurs, can cause abortions, renal and hepatic dysfunction, a drop in athletic performance and recurrent uveitis, which is considered the most significant cause worldwide of vision loss in horses (Artiushin et al. 2012, Polle et al. 2014).

The horse's relevance in the leptospirosis transmission chain, the magnitude of the economic impact resulting from the infection and the relationship between this species and the man encourage the importance of studies on the prevalence and research of factors associated with infected animals. Therefore, the objective was to evaluate the seroprevalence of anti-*Leptospira* antibodies in horses raised in the East and West Potiguar mesoregions and their predisposing factors.

MATERIALS AND METHODS

The study was carried out in the State of Rio Grande do Norte (latitude -5°45'0 S, longitude -36°30'0 W), located in the Northeast of the Northeast region of Brazil, with a territorial area of 8,510,820,623km² divided into 167 municipalities and four mesoregions. It has a humid climate in the Eastern mesoregion, semi-arid in the Agreste and Central region and a dry sub-humid in the West, with an average rainfall of more than 870mm on the coast to up to 600mm in the West of the State (Lucena et al. 2018). Only municipalities that had at least five hundred horses registered with the "Instituto de Defesa e Inspeção Agropecuária do Rio Grande do Norte" (IDIARN), belonging to the West Potiguar (n=400) and East Potiguar (n=385) mesoregions were included in the study. Added up to sixteen municipalities (Fig.1), where samples of 3 to 9% of the total number of horses

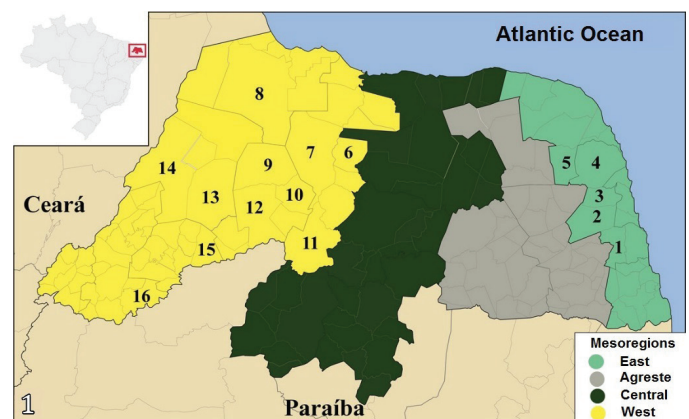


Fig.1. Map of Rio Grande do Norte divided into its four mesoregions. The municipalities where blood samples were collected from horses for serological diagnosis by microagglutination test (MAT) for *Leptospira* spp. are numerically indicated. East mesoregion: São José de Mipibú (1), Macaíba (2), São Gonçalo do Amarante (3), Ceará-Mirim (4), Taipú (5). West mesoregion: Ianguacú (6), Assu (7), Mossoró (8), Upanema (9), Paraú (10), Jucurutu (11), Campo Grande (12), Caraúbas (13), Apodi (14), Patu (15) and Alexandria (16).

were collected. The studied properties were selected according to the availability of allowing the harvests.

All horses from the properties visited, aged over six months and not vaccinated against *Leptospira* spp., participated in the study, totaling 785 blood samples in ninety herds of the two mesoregions. An investigative epidemiological questionnaire was applied to each property containing questions related to the breeder, the property, the animal and the adopted sanitary, reproductive and nutritional management. The collections took place from August 2018 to February 2019 and were performed using a dry tube vacuum bottle by aseptic venipuncture of the jugular vein. The samples were transported to the laboratory under refrigeration. Then, they were centrifuged at 3,000 rpm for fifteen minutes to obtain the serum, stored in 2ml Eppendorf[®] centrifuge microtubes, identified and stored at -20°C until the serological tests were performed.

The sera were sent to the “Laboratório de Doenças Bacterianas da Reprodução” (Laboratory of Bacterial Diseases of Reproduction) of the “Instituto Biológico de São Paulo”, São Paulo, where the serological diagnosis was carried out to detect the presence of serum antibodies against *Leptospira* spp. The microscopic agglutination technique (MAT) was applied using a collection of live antigens composed of twenty serovars. Of the twenty serovars, eighteen were pathogenic: *L. interrogans* serovars Bataviae, Bratislava, Butembo, Canicola, Copenhageni, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes, Sentot and Whitcombi; *L. borgpetersenii* serovars Autumnalis, Castellonis, Hardjo and Javanica; *L. santarosai* serovars Guaricura and Shermani. Two were non-pathogenic saprophytic serovars: *L. biflexa* serovars Andamana and Patoc, belonging to sixteen serogroups, cultivated in modified Ellinghausen, McCullough, Johnson, Harris (EMJH) medium (Alves et al. 1996). Samples with evident agglutination at a dilution equal to or greater than 1:100 were considered positive (Faine et al. 1999). In the samples that reacted to more than one serovar, the one with the highest titer was considered infecting. When two or more serovars with identical titers were identified, the samples were computed to calculate the prevalence – but not the most frequent serovar. Since serovars from the same serogroup can cross-react in MAT, the *Leptospira* serogroups were considered.

The number of horses needed to carry out the study was calculated considering an expected seroprevalence of 50%, with a confidence level of 95% and a statistical error of 5% (Thrusfield 2004). Thus, a minimum sample of 385 animals per mesoregion was determined.

For the study of factors associated with horses with anti-*Leptospira* spp. antibodies, a univariate analysis of the variables of interest was performed using Pearson’s chi-square test and Fisher’s exact test, when necessary. Subsequently, a regression analysis using the Poisson model with robust estimation was performed, considering the serological test (positive or negative) as the dependent variable. The independent or explanatory variables considered in the model were those with a statistical significance of $P < 0.05\%$ (Hosmer & Lemeshow 1987). The IBM SPSS Statistics program, version 21.0 (Armonk, NY: IBM Corp.), was used to perform the statistical calculations.

The Ethics Committee on the Use of Animals of the “Universidade Federal Rural de Pernambuco” approved the research project, with registration number 100/2018, on August 22, 2018.

RESULTS

The seroprevalence of horses seropositive to *Leptospira* spp. was 97.2% (763/785; CI: 0.96-0.98) in the East (97.9%, 377/385) and West (96.5%, 386/400) mesoregions of Potiguar, with horses infected in all ninety properties surveyed.

Were identified 221 (28.96%) samples that presented two or more serovars with comparable titers so that 542 (71.04%) animals were considered to calculate the most frequent serogroup, with seroagglutination reactions against ten serogroups. The most predominant was Icterohaemorrhagiae, with 72.88% of the reactions. The Australis, Sejroe, Shermani, Pomona, Canicola, Autumnalis, Ballum, Grippotyphosa and Hebdomadis serogroups represented the other 27.12%. The titers ranged from 100 to 3,200, and 78.97% (428/542) of the animals analyzed had titers ≤ 400 (Table 1).

After univariate analysis based on epidemiological questionnaires applied during the collections, Poisson regression analysis was performed to assess the relationship between the statistically significant variables. However, none of them proved to be a predisposing factor for horses with antibodies anti-*Leptospira* spp. ($P > 0.05\%$).

DISCUSSION

Studies with the same methodology (agglutination evident at dilution equal to or greater than 1:100) were carried out in the Northeast of Brazil, in the states of Bahia (Gomes et

Table 1. Descending distribution of the 542 reactions and titers of anti-*Leptospira* spp. agglutinins by the microagglutination test (MAT) in horses raised in the East and West Potiguar mesoregions, Rio Grande do Norte, Brazil, 2018-2019

Serogroup	Titration (%)						Total (%)
	100	200	400	800	1,600	3,200	
Icterohaemorrhagiae	89 (16.42)	149 (27.49)	100 (18.45)	45 (8.30)	9 (1.66)	3 (0.55)	395 (72.88)
Australis	1 (0.18)	12 (2.21)	36 (6.64)	15 (2.77)	11 (2.03)	6 (1.11)	81 (14.94)
Sejroe	-	4 (0.74)	9 (1.66)	6 (1.11)	3 (0.55)	2 (0.37)	24 (4.43)
Shermani	2 (0.37)	4 (0.74)	9 (1.66)	-	-	-	15 (2.77)
Autumnalis	-	4 (0.74)	2 (0.37)	-	-	1 (0.18)	7 (1.29)
Pomona	-	1 (0.18)	1 (0.18)	-	3 (0.55)	2 (0.37)	7 (1.29)
Canicola	-	-	2 (0.37)	1 (0.18)	-	2 (0.37)	5 (0.93)
Ballum	-	-	1 (0.18)	1 (0.18)	2 (0.37)	-	4 (0.74)
Grippotyphosa	-	-	2 (0.37)	-	1 (0.18)	-	3 (0.55)
Hebdomadis	-	-	-	-	-	1 (0.18)	1 (0.18)
TOTAL	92 (16.97)	174 (32.10)	162 (29.89)	68 (12.55)	29 (5.35)	17 (3.14)	542 (100)

al. 2007, Siqueira 2012), Pernambuco (Souza 2012, Alves et al. 2016) and Paraíba (Oliveira Filho et al. 2014, Morais et al. 2019b), described prevalences ranging from 8% to 62.5%. The highest occurrence of seropositive horses was found in the current Rio Grande do Norte survey, presenting a more significant number of horses and municipalities researched compared to other studies.

A serological survey using a bank of sera of 1,264 horses from four Northeastern states (Piauí, Ceará, Paraíba and Pernambuco) showed an occurrence between 27% and 33.7%. The Rio Grande do Norte (n=252) had the highest seropositive rate. In this study, most of the samples from the State (82% of 252) were from West Potiguar, which had a rate (26%; 53/206) well below this survey (96.5%), as well as from East Potiguar (56%, 13/26), despite the lower number of samples (Morais et al. 2019a). Since the climatic conditions are the same in both regions, this difference may be related to the type of samples studied (random/convenience X sent to a diagnostic laboratory) and the sanitary management to which these animals were submitted. It is assumed that animals submitted to laboratory tests receive better health support from their breeders.

The data heterogeneity found in studies of the Northeast region can be attributed to its edaphoclimatic trait. Due to the diversity of the collection sites, we can observe abrupt variations in biome and precipitation rate within the same State. The most humid and with higher rainfall levels were more prone to high prevalence values (Alves et al. 2016).

According to Hashimoto et al. (2010), the most relevant factors for the high frequency of infections have been the absence of vaccination and the presence of carrier animals in the environment. Such animals are highly susceptible to infection by the adapted serogroup and usually present the subclinical form of the disease with long periods of leptospiruria. Thus, high prevalence rates of a serogroup in a given region are positively correlated with its natural host's presence (Hines 2014).

The predominance of the *Icterohaemorrhagiae* serogroup corroborates serological surveys carried out in the states of Pernambuco (Souza 2012), Bahia (Gomes et al. 2007, Siqueira 2012), Goiás (Linhares et al. 2005), Mato Grosso (Jorge et al. 2011), São Paulo (Silva et al. 2010, Coiro et al. 2012) and Minas Gerais (Caselani et al. 2012). It also corroborates a serological survey on donkeys slaughtered in Pernambuco, destined for meat exportation for human consumption (Morais et al. 2019c). It was the main serogroup in the Central Potiguar mesoregion and the second most diagnosed in East and West Potiguar (Morais et al. 2019a). Such expressive numbers of prevalence for this variant warn to the management and hygiene practices in the studied mesoregions. This predominance positively correlates with rodents, precarious or deficient cleaning conditions, and inadequate food storage (Siqueira 2012, Alves et al. 2016, Morais et al. 2019a). Infection of the equine species by this serovar leads to high production of antibodies, and a greater possibility of clinical manifestations (Chiareli et al. 2008), a situation that was not observed in any animal in this study at the time of sampling.

The results suggest synanthropic rodents in the Potiguar properties of the East and West mesoregions. These properties are the main natural reservoirs of the *Icterohaemorrhagiae* serogroup (Vasconcelos et al. 2012). In addition, the questionnaires

carried out with breeders showed that 85.1% of the animals were raised in not disinfected facilities and 81.8% on properties with rodents in the environment, with 54.5% also found in food storage places. These reports indicate environmental contamination, mainly by the *Icterohaemorrhagiae* serogroup, since they were observed on the properties at harvest time. Also, the inadequate storage of food, sacks of open rations, food remains in the troughs, and the habit of taking the animals to graze and/or cutting the grass to supply them on the banks of streams used for sewage disposal.

The second most seroprevalent serogroup was *Australis* (14.94%). This serogroup is vital in the Northeast region because it is the most diagnosed in serological studies with horses from four states (Morais et al. 2019a) and reported in different regions of Brazil (Pinna et al. 2014). Considered adapted to the equine species, it is worrying from an epidemiological point of view, as these animals, when infected, become a source of dissemination and environmental contamination. The low production of antibodies during infection may result in underreporting its occurrence due to the manifestation in the subclinical or asymptomatic form of the disease (Divers et al. 2019), which may have occurred with some horses in the study.

A predominance of chronically produced antibodies is indicated by the rate of 78.97% of positive animals with titers ≤ 400 , which may show, in most cases, that there was no recent exposure to the agent. In this study, the most frequent serogroup was *Icterohaemorrhagiae* (72.88%), not adapted to the equine species, which usually leads to acute infection, followed by *Sejroe*, in third place (4.43%), which is adapted to the bovine species (Chiareli et al. 2008). This chronicity of the infection suggests that the agent was eliminated for long periods in the environment, contributing to the maintenance of the infection cycle (Hashimoto et al. 2010).

No factor related to the infection of horses by *Leptospira* spp. was identified. However, the high seropositive values verified in this study may have influenced the non-detection of variables already reported by other authors. The variables include age, sex (Morais et al. 2019a), the occurrence of abortions, stillbirth (Donahue et al. 1991), extensive rearing system and/or presence of other animal species (cattle and capybaras) (Farias et al. 2020). In cases where environmental contamination is very high, it is difficult to identify the relationship between the potential factors associated with infection (Figueiredo et al. 2009).

Vaccination against *Leptospira* spp. was not adopted on the properties of the study, which, as well as treatment with antibiotics, hygiene of the facilities, attention to the source of water provided, avoiding contact between horses and other species and rodent control, are important actions for disease prevention (Pinna et al. 2007). The leading vaccine marketed for horses in the country is manufactured from bacterial culture, has seven (94.8% of the reactions) of the ten serogroups identified in Rio Grande do Norte and has an excellent humoral response. However, it varies among the vaccine serogroups, with more intense responses against the most diagnosed serogroups in the State (*Icterohaemorrhagiae* and *Australis*). It is an essential tool to reduce the infection in herds and the zoonotic risk due to the decrease in the elimination of the agent in the environment and a consequent decrease in the intensity of symptoms (Martins et al. 2017).

The Shermani, Autumnalis and Hebdomadis serogroups are not present in commercial vaccines in Brazil. However, they were not relevant in this study, corresponding to only 5.17% (28/542) of the reactions.

Horses are vital beings in maintaining environmental and sanitary homeostasis of the environment in which they are inserted. They are also considered indispensable within the concept of one health since they are hosts of several zoonoses of sanitary relevance, including leptospirosis (Lönker et al. 2020). The Pan American Health Organization (PAHO) states that 70% of human public health emergencies of international relevance are linked to zoonoses. Within this group, outbreaks of leptospirosis in humans are essential, for it constitutes the third-highest risk of infection in the Americas. (Schneider et al. 2011, 2013).

CONCLUSION

The high prevalence rate identified in this survey indicates a potential risk to regional public health. We reinforce the importance of controlling potential natural hosts of *Leptospira* spp. in the equine breeding environment, especially the Icterohaemorrhagiae serogroup, and the periodic vaccination of this species, aiming to minimize the economic, sanitary and social impact of this zoonosis.

Conflict of interest statement.- The authors declare that they have no competing interests.

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