



## Serological survey of leptospirosis in horses with historical displacement through different geographic regions in Brazil

[Investigação sorológica de leptospirose em cavalos com histórico de deslocamento através de diferentes regiões geográficas no Brasil]

### "Scientific Article/Artigo Científico"

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### Abstract

Leptospirosis has a worldwide occurrence and shows higher incidence in tropical areas. Equines can present several serovars according to geographic region and it is important to analyze the possible contribution of animal displacement on the *Leptospira* spp. strains occurrence. This research aimed to conduct a serological investigation in an equine regiment belonging to the Brazilian Army Guard Cavalry, resident in an urban area of Rio de Janeiro municipality, but with a history of displacement between different regions in Brazil. A microscopic agglutination test was performed in a serum bank of 197 horses using 29 *Leptospira* spp. strains. The test showed 94.92% positivity, with 89.84% of the animals presenting a positive result for two or more serovars. Higher titers ( $\geq 1:400$ ) could be detected in 57.22% of horses for at least one serovar, whereas seropositivity for more than one bacterial type occurred mostly at lower titers ( $< 1:400$ ). All animals were clinically healthy. The occurrence of serogroup Icterohaemorrhagiae in urban areas was confirmed; however, equine serovars Bratislava and Australis presented low frequencies. The serovars Hardjo, Wolffi, and Canicola were detected and indicated the presence of ruminants and dogs as typical reservoirs in proximity to the equines. It is suggested that travel history has contributed to the high frequency found and possible greater contact with different types of *Leptospira* spp. Therefore, veterinarians should consider the history of geographic displacement and other animal species' coexistence with horses when conducting their clinical investigation.

**Keywords:** equine; *Leptospira* spp.; serological diagnosis; Microscopic Agglutination Test.

### Resumo

A leptospirose é uma doença de ocorrência mundial, apresentando maior incidência em áreas tropicais. Equinos podem apresentar diferentes sorovares de acordo com a região geográfica, sendo importante analisar a possível contribuição do deslocamento animal sobre a ocorrência das cepas de *Leptospira* spp. Esta pesquisa objetivou realizar uma investigação sorológica em um regimento de equinos pertencente à Cavalaria de Guarda do Exército Brasileiro, residente em área urbana do município do Rio de Janeiro, mas com histórico de deslocamento entre diferentes regiões do Brasil. O teste de aglutinação microscópica foi realizado em banco de soro oriundo de 197 cavalos, utilizando 29 sorovares do patógeno. O teste evidenciou 94,92% de positividade, com 89,84% dos animais apresentando resultado positivo para dois ou mais sorovares. Títulos mais altos ( $\geq 1:400$ ) foram detectados em 57,22% dos cavalos para pelo menos um sorovar, enquanto a soropositividade para mais de um tipo bacteriano ocorreu principalmente junto a títulos mais baixos ( $< 1:400$ ).

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Todos os animais estavam clinicamente saudáveis. A ocorrência do sorogrupo Icterohaemorrhagiae em áreas urbanas foi confirmada; entretanto, os sorovares equinos Bratislava e Australis apresentaram baixas frequências. Hardjo, Wolffii e Canicola foram detectados e indicaram a presença de ruminantes e cães, que são seus reservatórios típicos, em proximidade junto aos equinos. Sugere-se que o histórico de viagens tenha contribuído para a alta frequência encontrada e um possível maior contato com diferentes tipos de *Leptospira* spp. Médicos-veterinários devem considerar o histórico de deslocamento geográfico e a convivência de outras espécies animais com cavalos ao realizar sua investigação clínico-epidemiológica.

**Palavras-chave:** equino; *Leptospira* spp.; diagnóstico sorológico; Teste de Aglutinação Microscópico.

## Introduction

Leptospirosis is a worldwide bacterial disease caused by *Leptospira* spp. (WHO, 2010). This bacterium causes a transmissible disease between animals and humans (WOAH, 2021), and its incidence is higher in tropical areas than temperate zones (Desvars et al., 2011), occurring in both urban and rural areas and having different characteristics (Martins and Lilenbaum, 2013).

In horses, the infection may present systemic and ocular manifestations and be recognized as an essential disease of the reproductive system (Hamond et al., 2014). It can also generate acute dyspnea (Verma et al., 2013) or be a subclinical disease associated with a performance decrease in racehorses (Hamond et al., 2012a).

The occurrence of the serogroup Icterohaemorrhagiae predominantly characterizes urban cycles. To this end, serovars are maintained by rodents, mainly *Rattus norvegicus*, and the clinical presentation in horses is acute, including fever, lung disease, and renal failure (Martins and Lilenbaum, 2013). This serogroup also composes the most widespread zoonosis in the world (Desvars et al., 2011).

Serological tests are acceptable as epidemiological investigation tools in herds (Loureiro et al., 2013), being the most widely used tests today (WOAH, 2021). Among these, Microscopic Agglutination Test (MAT) is considered standard (WOAH, 2021), and is recommended by both the animal and human world health organizations (WHO, 2010; WOA, 2021).

Horse infection by *Leptospira* spp. is widely diffused with serovars varying considerably according to the affected geographical region (Verma et al., 2013; Hamond et al., 2014). Because of this, this study aimed to conduct a serological investigation for equine leptospirosis in a regiment of the Brazilian Army Guard Cavalry, composed of animals with a history of displacement between different regions in Brazil.

## Material and Methods

### Study design

The study consisted of a serological survey using MAT in a database of serum samples previously obtained for health surveillance actions, specifically for the diagnosis of glanders, from 197 horses belonging to the Brazilian Army Guard Cavalry, located in an urban area of Rio de Janeiro municipality, Brazil. The samples were ceded from the Army and stocked at the Research Institution at -20°C until analysis.

### Characterization of the evaluated group

The animals evaluated are mainly characterized as Brazilian Equestrian Horses and are employed in military activities and participate in equestrian competitions. These animals belonged to the Brazilian Army or to military people (in a private guard), being all of these used in military actions. The private animals could be seen in cohabitation with other animal species, such as ruminants and they have traveled throughout Brazil, a country of continental dimensions, between the following regions: the southeast (in the municipalities of Rio de Janeiro and Resende), midwest (Brasília and Ponta-Porã), and south (Porto Alegre and Osório-Tramandaí).

When they are not purchased in the market, the animals are bred in the Brazilian Army's facilities, after artificial insemination, in a unit located in the south region, municipality of São Borja, Rio Grande do Sul state.

Vaccination against *Leptospira interrogans* serovars Pomona, Canicola, Icterohaemorrhagiae, Wolffii, and Hardjo, as well as *Leptospira kirschneri* serovar Grippotyphosa (Lepto-Bac-6, Fortdodge), occurs every six months, being the animals submitted to this protocol.

### Serum Microscopic Agglutination Test (MAT)

For MAT confection, live cultures from 29 *Leptospira* spp. strains were used as antigens. All

of them were grown in EMJH (Ellinghausen-McCullough-Johnson-Harris) medium, free of previous contamination or agglutination.

The following strains were used: *L. interrogans* serovars Australis, Bratislava, Autumnalis, Bataviae, Canicola, Djasiman, Sentot, Copenhageni, Icterohaemorrhagiae, Pomona, Wolffi, Hardjo, Hardjoprajitno, Hardjobovis, HardjoCTG, and Hardjominiswajezak; *L. kirschneri* serovars Butembo, Cynopteri and Gryppotyphosa; *L. borgpetersenii* serovars Castellonis, Whitcombi, Hebdomadis, Javanica, Pyrogenes, and Tarassovi; *L. noguchii* serovar Panama; *L. santarosai* serovar Shermani; the environmental strains *L. biflexa* serovars Andamana and Patoc.

Agglutinations from 1:100 serum dilutions were considered positive.

## Results

Among the 197 sera evaluated, 94.92% (187/197) were positive for antibodies against *Leptospira* spp. In the positive group, only 10.16% (19/187) were reagents for only one serovar, whereas 89.84% (168/187) tested positive for two or more serovars.

Higher titers ( $\geq 1:400$ ) were found in 57.22% (107/187) of the positive samples for at least one serovar. Seropositivity to more than one serovar occurred mostly in lower titers ( $< 1:400$ ), with only 21 samples sustaining seropositivity for at least two serovars when higher titers ( $\geq 1:400$ ) were analyzed.

The highest frequencies found and their titration variations were 77.00% (144/187) for Patoc (from 1:100 to 1:3200); 55.08% (103/187) for Icterohaemorrhagiae (from 1:100 to 1:800); 52.40% (98/187) for HardjoCTG (from 1:100 to 1:3200); 49.20% (92/187) for Gryppotyphosa (from 1:100 to 1:800); 42.77% (80/187) for Wolffi (from 1:100 to 1:800); 30.48% (57/187) for Hardjoprajitno (from 1:100 to 1:400); 28.88% (54/187) for Canicola (from 1:100 to 1:800), and 25.66% (48/187) for Copenhageni (from 1:100 to 1:800).

Other frequencies and titers found were as follows: 16.57% (31/187) for Hardjo (from 1:100 to 1:800); 13.90% (26/187) for Pyrogenes (from 1:100 to 1:400); 13.90% (26/187) for Djasiman (from 1:100 to 1:800); 10.69% (20/187) for Hardjobovis (from 1:100 to 1:200); 8.55% (16/187) for Sentot (from 1:100 to 1:400); 4.80% (09/187) for Australis (1:100); 3.74% (07/187) for

Bratislava (from 1:100 to 1:400); 2.67% (05/187) for Hebdomadis (from 1:100 to 1:200); 2.14% (04/187) for Castellonis (from 1:100 to 1:200); 1.60% (03/187) for Bataviae (from 1:100 to 1:200); 1.60% (03/187) for Hardjominiswajezak (from 1:100 to 1:800); 1.06% (02/187) for Autumnalis (1:100); 1.06% (02/187) for Shermani (1:100); 0.53% (01/187) for Pomona (1:100), and 0.53% (01/187) for Andamana (1:100).

There was no detection for the following serovars: Butembo, Whitcombi, Cynopteri, Javanica, Panama, and Tarassovi.

All seropositive or seronegative animals were clinically healthy, and the place underwent the application of rodent control measures, but the presence of rodents was reported.

Since a serum bank was used, an active infection could not be confirmed by molecular methods.

## Discussion

The seroreactivity found was higher than those evidenced in other studies conducted in tropical areas (Langoni et al., 2004; Hamond et al., 2011; Hamond et al., 2012a; Martins and Lilenbaum, 2013; Pinna et al., 2014; Bastiani et al., 2021). It's suggested that the endemicity of the Rio de Janeiro region (Martins and Lilenbaum, 2013) and the history of travel to different areas of the Brazilian territory may have contributed to greater contact of the animals with *Leptospira* spp. Despite the lack of specific animal leptospirosis data from each place where animals had been traveled, all cities cited are located in a Tropical region, being these areas endowed with many particularities that could affect the occurrence of the infection, including geographical conditions and aspects such as climate or topography (Martins and Lilenbaum, 2013), which suggests the contribution of displacement in the increasing seroreactivity among these horses. It is important to highlight that, in some horses, the absence of seropositivity maintenance for more than one serovar at high titers suggests the occurrence of cross-reactivity, as reported by Hamond et al. (2012a) and Pinna et al. (2014). Furthermore, there is the possibility of vaccine response contributed to the serovars used in immunization, because MAT does not distinguish it from natural infection response (Loureiro et al., 2013). Nonetheless, because the detection of these vaccine serovars does not cover the majority of the assessed group, including low

detection of the Pomona serovar, the results presented herein remain relevant.

The high levels of seroreactivity associated with higher titers for the Icterohaemorrhagiae and Copenhageni serovars, both belonging to the Icterohaemorrhagiae serogroup, confirm its high occurrence in urban areas (Martins and Lilenbaum, 2013). This highlight for such a group agrees with the studies by Langoni et al. (2004), Hashimoto et al. (2007), Hamond et al. (2012b), and Hamond et al. (2012a); and it is consistent with the reported presence of rodents, which indicates maintenance of *Leptospira* spp. (Martins and Lilenbaum, 2013). However, there is evidence that horses may also play a role in the transmission of *Leptospira* spp. in urban areas (Hamond et al., 2012b).

The presence of antibodies against the Grippotyphosa serovar in equine species agrees with previous reports (Hashimoto et al., 2007; De Souza et al., 2016). However, interestingly, although Bratislava is a host-adapted serovar in horses, it presented low frequency, differing from other studies (Pinna et al., 2008; Pinna et al., 2014), as well as Australis, previously highlighted among the main serovars found by Martins and Lilenbaum (2013) in these animals. Since all these papers had applied the Serum Microscopic Agglutination technique, it is suggested other factors to explain the difference between the research articles, such as animal management, husbandry practices, and frequency of veterinary assistance, that applied in different levels probably may have affected overall seroprevalence and the serovar distribution (Martins and Lilenbaum, 2013).

The occurrence of Hardjo serovars and their variations, as well as of Wolffi serovar, demonstrates the presence of the Sejroe serogroup and suggests proximity to infected ruminants in some of the places frequented by the horses. Also in this sense, the high frequency of Canicola serovar indicates the proximity of dogs as reservoirs of *Leptospira* spp. (Martins and Lilenbaum 2013). Concerning Wolffi, it is also worth noting that this serovar has been identified as a potential pathogen in humans, dogs, and sheep (Zakeri et al., 2010). Therefore, its finding in equines, with high titers and frequency, indicates a need for research on this subject in horses.

Despite the possibilities of Icterohemorrhagic syndrome (Martins and Lilenbaum 2013) or ophthalmologic

abnormalities, such as post-infection recurrent uveitis in horses (Verma et al., 2013), these no reports in the evaluated group with high titers found to suggest a subclinical leptospirosis status. This condition may be considered an alert since this feature can cause a performance decrease in racehorses according to previous research (Hamond et al., 2012a).

Finally, taking into account the higher titers and the suggestions of seropositivity increment due to displacement and other animal species in proximity, the following control actions are suggested (Verma et al., 2013): application of hygiene measures, minimization of rodent contact, periodic vaccination of horses, and, where possible, vaccination of production and company animals nearby. These measures aim at minimizing the flow of pathogens.

### Conclusion

A high frequency of seropositivity was obtained in the equines evaluated. These findings reinforce the need for animal and environmental preventive measures in all places where horses are maintained. In this same way, the low frequencies of serovars Bratislava and Australis point out the possible application of different efficient management.

Besides, the detection of serovars Hardjo, Wolffi, and Canicola indicates the presence of ruminants and dogs in proximity to some of the places frequented by the horses and reinforce the necessity of preventive measures related to other animal species nearby. Concerning Wolffi, it is also worth noting that this serovar had been identified as a potential pathogen in humans, dogs, and sheep. Therefore, the high titers and frequency of this serovar suggest, a need for research on this subject in horses, including serological surveys with risk factors analysis; research of active cases through molecular methods and investigation about the role of horses in the spread of this pathogen; besides clinical studies related to the Wolffi infection impact on the health of equine species.

### Conflicts of Interest

The authors declare no conflict of interest.

The statements in this article must be considered as the author's own opinion and do not necessarily reflect the official position of the Brazilian Ministry of Agriculture and Livestock.

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## Ethics Committee

This research did not require ethical approval. The study consisted of a serological survey using a database of serum samples previously obtained for health surveillance actions. The samples were ceded from the Brazilian Army Guard Cavalry and stocked at the Research Institution. The study team had no contact with the animals, so there was no need for authorization from the Animal Ethics Committee.

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