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Seroepidemiology of leptospirosis in horses from Santarém, Pará

Soroepidemiologia de leptospirose em equinos da região de Santarém, Pará

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Abstract
Leptospirosis is an endemic zoonotic disease that is distributed worldwide, which has the potential to have health and economic impacts. *Leptospira* spp. is spiral-shaped and capable of infecting mammals, including horses, which may result in asymptomatic or clinical forms. Therefore, the current study aimed to analyze the frequency of anti-*Leptospira* antibodies in serum samples from horses from Santarém, Pará, Brazil. For that purpose, 88 blood samples from horses without a history of leptospirosis vaccination were serologically evaluated through the microscopic agglutination technique (MAT) with a 13 serovars, belonging to ten different serogroups. There were 58 samples that were seropositive (65.90%), which included 28 samples seropositive for Pyrogenes (48.3%), 24 for Autumnalis (41.4%), 18 for Icterohaemorrhagiae (31.0%), and 16 for Grippotyphosa (27.6%). Even without clinical suspicion of leptospirosis or a history of vaccination, the horses showed different frequency of seropositivity. Considering the well-known impact of leptospirosis in human and animal health, our results are important to establish preventive measures to reduce the economic loss in equine production as well as a reduction in public health risk.
Keywords: *Leptospira* spp.; horses; epidemiology; public health; Pará.

Resumo
Leptospira spp. é a bactéria causadora da leptospirose, uma doença endêmica, distribuída mundialmente, de caráter zoonótico responsável por gerar impacto sanitário e também econômico. Esse microrganismo, com característica espiralada, infecta mamíferos, dentre eles, os equinos. Estes animais, podem apresentar a doença na forma assintomáticos ou clínica. Desta forma, o atual estudo objetivou analisar amostras equinas na região de Santarém- Pará. Analisou-se 88 amostras de animais que não apresentavam histórico de vacinação contra a leptospirose, através da técnica da Microaglutinação Microscópica (MAT), utilizando um painel de13 sorovares, pertencentes a dez diferentes sorogrupos. Desses animais, 58 foram soropositivos (65,90%), distribuídos em 28 (48,3%) amostras soro reagentes para Pyrogenes, 24 (41,4%) para Autumnalis, 18 (31%) para Icterohaemorrhagiae e 16 (27,6%) para Grippotyphosa. Mesmo não havendo suspeita de leptospirose, os animais apresentaram diferentes frequências sorológicas. Considerando-se o conhecido impacto da leptospirose na saúde humana e animal, nossos resultados apontam que é de suma importância estabelecer medidas de prevenção para reduzir perdas econômicas e não oferecer riscos à saúde pública.
Palavras-chave: *Leptospira* spp.; equídeos; epidemiologia; saúde pública; Pará.

1. Introduction

Leptospirosis is an infectious zoonotic bacterial disease that affects domestic and wild mammals and humans[1,2]. It presents with a high worldwide distribution and has considerable economic and public health impact[3]. There are more than 260 identified serovars, each with their preferred host(s) which can harbor one or more serovars[4].

Leptospira spp. can be found in different epidemiological contexts, such as urban, rural, and wild[5]. Among mammals, horses can develop the disease as a clinical or asymptomatic form; its evolution may be an acute or chronic disease[6], and clinical signs such as fever, anorexia, and breathing difficulty may be observed. In addition, jaundice, hematuria, miscarriage, stillbirth/premature foals, uveitis, and liver and kidney dysfunction can be present[7,8].

In horses, *Leptospira* spp. is mostly caused by the serovars Bratislava and Icterohaemorrhagiae[8], however, the prevalence may vary according to region[9]. Accordingly, in some studies that were conducted in

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northern Brazil the seroprevalence of other serovars such as Australis, Autumnalis, and Pyrogenes, in addition to Bratislava and Icterohaemorrhagiae, were observed[10-12]. However, in northern Brazil, there are fewer studies regarding the current prevalence of leptospirosis in horses. Therefore, our study aimed to perform a seroepidemiological survey of leptospirosis in horses from the region of Santarém-Pará, Northern Brazil.

2. Materials and methods

The samples of this study were obtained from 88 horses raised in 15 rural farms of Santarém-Pará, Northern Brazil. Blood samples were drawn via venipuncture using sterile tubes without anticoagulant. Among the 88 animals, 51 were males and 37 females, aged between 2 and 16 years, with no recent history of leptospirosis vaccination.

Serum samples were tested for anti-*Leptospira* antibodies by microscopic agglutination test (MAT)[13,14], using live antigens grown in a liquid Ellinghausen–McCullough-Johnson–Harris medium free from contamination or self-agglutination, as recommended by the World Health Organization[15]. A 1:100 dilution was used as the cutoff point.

A complete panel of 10 serogroups (including 13 reference serovars) was used as the test antigens. This panel included: serogroup Sejroe (serovars Hardjo [subtype Hardjo-prajitno] and Wolffi), serogroup Grippotyphosa (serovar Grippotyphosa), serogroup Canicola (serovar Canicola), serogroup Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni), serogroup Australis (serovar Bratislava), serogroup Pomona (serovar Pomona), serogroup Autumnalis (serovar Butembo), serogroup Pyrogenes (serovar Pyrogenes), serogroup Ballum (serovar Ballum), and serogroup Tarassovi (serovar Tarassovi).

Briefly, live suspensions of leptospires representing the 13 serovars were added to series dilution of the serum samples in a microtiter plate (96 wells), incubated at temperature of 36 degrees celsius for 2–4 h. The presence or absence of agglutination was determined using dark field microscopy at 100× magnification. Titers were obtained from the sequence of double dilutions and expressed as the reciprocal of the highest serum dilution that agglutinated at least 50% of the *Leptospira* spp.[13,14].

Data were recorded in Excel spreadsheets and subsequently used for descriptive data analysis through the use of frequency tables. Means were compared using the Chi-Square test, with a statistically significant level of 5% ($P > 0.05$). Experimental procedures were approved by the Ethics Committee on Animal Use at the Federal Institute of Education, Science, and Technology of Amazonas (Brazil), with the approval reference number CEUA.008.02.1417.2404/2020.

3. Results

From the 88 serum samples, 58 (65.9%) were seropositive and 30 (34.1%) were seronegative. The seroprevalence for single infections (considering only 1 reagent serogroup) is shown in Table 1, and Table 2 presents the results of mixed infections, i.e., seropositivity for more than 1 serogroup.

In the single infection results (Table 1), it is observed that the seroprevalence included the serogroup Pyrogenes [28 horses (48.3%)], followed by Autumnalis serogroup [24 horses (41.4%)], Icterohaemorrhagiae [18 horses (31.0%)], and Grippotyphosa [16 horses (27.6%)]. In the same manner for the mixed infections, the serogroups for Pyrogenes, Atumunalis, and Icterohaemorrhagiae were predominant and notably three horses (14.3%) were seropositive for the serogroup Icterohaemorrhagiae and serovar Copenhageni. No statistically significant differences were obtained regarding infection by sex or age, with a mean age of 8,5 years.

Table 1. Seroprevalence of *Leptospira* spp. serogroups in single infections (considering only in one serogroup) in horse serum samples from Santarém, Pará.

SEROGROUPS	SEROPOSITIVE (n)	SEROPOSITIVE (%)
Pyrogenes	28	48,3
Autumnalis	24	41,4
Icterohaemorrhagiae	18	31,0
Grippotyphosa	16	27,6
Ballum	13	22,4
Sejroe	12	20,7
Canicola	12	20,7
Tarassovi	9	15,5
Pomona	1	1,72
Australis	0	0

Table 2. Seroprevalence of *Leptospira* spp. serogroups in mixed infections (considering more than one serogroup) in horse serum samples from Santarém, Pará.

SEROGROUPS	SEROPOSITIVE (n)	SEROPOSITIVE (%)
Pyrogenes	6	28,6
Autumnalis	10	47,6
Icterohaemorrhagiae	3 (all of them to Copenhageni)	14,3
Grippotyphosa	0	0
Ballum	0	0
Sejroe	1 (Hardjo)	4,76
Canicola	0	0
Tarassovi	1	4,76
Pomona	0	0
Australis	0	0

4. Discussion

Our results demonstrated that 58 horses (65.9%) were seropositive, indicating that these horses had direct or indirect contact with *Leptospira* spp. Research conducted in the last decade in Brazil showed considerable variation regarding the leptospirosis seroprevalence in horses[9,12]. The observed frequency in this study (65.9%) was similar to that found in studies performed in the State of Paraná (Brazil), which reported a 66.88% seropositivity[16]. However, another survey performed in Maranhão, northern Brazil, showed an 85% of serological frequency[6].

A higher seroprevalence was observed for the serogroups Pyrogenes (48.3%) and Autumnalis (41.4%), which differs from what is generally observed in serologic studies of horses in Brazil, where a high frequency of the serogroups Bratislava and Icterohaemorrhagiae is observed[8]. In a study performed in Bahia State, northeast Brazil by Gomes et al.[17] also observed a higher prevalence of the serogroup Pyrogenes (24.0%), demonstrating the equal importance of this serogroup in serologic surveys of horses in different Brazilian regions. Moraes et al.[11], in Pará, and Sousa et al.[12], in Amazonas, also observed, as in this study, a high prevalence of the serogroup Pyrogenes in horses, which may suggest the maintenance of this serogroup in some domestic or wild animals of the Amazonic region. Abroad, the Pyrogenes serogroup has also been identified, with the highest seroprevalence in horses from Switzerland[18].

Within the Icterohaemorrhagiae serogroup, the serovar Copenhageni showed predominance when compared with the serovar Icterohaemorrhagiae, which is generally not the most frequently observed result in horses. However, a study by Hamond et al.[8], in Rio de Janeiro, also reported a higher prevalence of the serovar Copenhageni in horses. In the Netherlands, Houwers et al.[19] also found a major predominance of the Icterohaemorrhagiae serogroup in horses, which suggests that seropositivity in these animals was not the result of a vaccine, but rather as a result of a greater exposure to the serovars, and the horses developing subclinical leptospirosis. Considering the public health issues in Brazil, the serovars Icterohaemorraghiae and Copenhageni are related to the most severe cases of leptospirosis in humans[20], with approximately 1% of patients developing the most severe form and being fatal in 1–5% of these patients[21]. Considering that infection in humans usually occurs after direct or indirect contact with the urine of an infected animal[5], the high prevalence of the Icterohaemorrhagiae serogroup in our study may require attention as a public health concern.

Our results, for a single infection, showed that 16 horses (27.5%) were seropositive for Grippotyphosa serogroup, which is usually related to environmental contamination, as this group is harbored by wild animals[22], and affect horses in an incidental manner[23]. The serological reaction for this serogroup stresses the probable infection in horses through contact with wild animals, since the region where our study was conducted had suitable environments for wild animals and favorable conditions for the survival and spread of the infectious agent.

Ballum seropositivity was observed in 13 samples (22.4%), while the serogroups Sejroe and Canicola each had 12 seropositive results (20.7%). Seroprevalence results for the Ballum serovar are less frequent, and it has rarely identified as the most prevalent serovar in equine studies[24]. In Brazil, there is a study showing its seroprevalence in 11.76% of horses in Rio Grande do Sul[25]. Equine infections with Sejroe or Canicola serogroups are not considered common in Latin America[26], but regional and international sero-reactivity for these serogroups have been reported[27-29]. High titers, with a titer equal to or greater than 400, is correlated with acute leptospirosis infection in horses; however, low titers from 100 to 200 may represent a chronic infection due to the curve after seroconversion, or an early acute infection with an ascending antibody curve (seroconversion)[30].

It is worth noting that there are risk factors of leptospirosis epidemiology[24] and that these risk factors may are different in the various regions of Brazil. Due to the wide territory of northern Brazil and the vast animal diversity of this region may provide particular conditions, especially regarding the reservoirs and environmental conditions for *Leptospira* spp. maintenance. Thus, it is necessary to establish, evaluate, monitor, and attend to these risk factors, as well as performing field studies on the reservoirs to be able to provide preventive measures and, thus reduce the risks to humans and horses.

5. Conclusion

Our study reported on horses from Santarém-Pará, Brazil. A higher seroprevalence for the serogroups Pyrogenes and Autumnalis was observed, which is different from the generally observed serology in horses from Brazil. Considering the environmental peculiarity of northern Brazil, the results may occur due to the maintenance of these serogroups by some domestic or wild animals from the Amazonic region.

Declaration of Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

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