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Anti-*Leptospira* spp. seroreactivity and associated factors in equids from northern Tocantins, Brazilian Legal Amazon[1]

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ABSTRACT.- Silva BV, Ribeiro APD, Rodrigues FMPS, Santos HD, De Carvalho CA, Dos Santos AJF, Pereira WLA, Silva MAG. **Anti-*Leptospira* spp. seroreactivity and associated factors in equids from northern Tocantins, Brazilian Legal Amazon.** *Pesquisa Veterinária Brasileira 47:e07893, 2027.* Graduate Program in Animal Health and Public Health in the Tropics, Universidade Federal do Norte do Tocantins, BR-153 Km 112, Setor Rural, Araguaína, TO 77804-970, Brazil. E-mail: marco.silva@ufnt.edu.br

Leptospirosis is a globally distributed zoonosis with a significant impact on animal and public health, and tropical regions are particularly favorable for the environmental persistence of *Leptospira* spp. This study aimed to determine the seroprevalence, identify the most frequent serovars, and investigate factors associated with seroreactivity to *Leptospira* spp. in equids from the municipality of Araguaína, Tocantins, Brazil. A total of 200 serum samples were collected from equids belonging to 81 properties (77 rural and four urban). Serological diagnosis was performed using the Microscopic Agglutination Test (MAT) using a panel of 24 live serovars. The overall seroprevalence was 92.5% (185/200), and 97.5% (79/81) of the properties had at least one seroreactive equid. Among the seropositive animals, 83.8% (155/185) showed coagglutination with two or more serovars. A total of 733 serological reactions were detected, distributed among 21 of the 24 serovars tested, with *Icterohaemorrhagiae* (19.10%) being the most frequent, followed by *Shermani* (7.91%), *Wolffi* (7.78%), *Pyrogenes* (7.50%), Whitcombi (6.68%), Canicola (6.68%), and Copenhageni (6.55%). No reactivity was detected against the *Andamana*, *Cynopteri*, or *Patoc* serovars. In the univariate analysis, seroreactivity was associated with grazing in

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flooded areas (OR = 8.32; 95% CI = 1.07–64.76; p = 0.021), age group (p = 0.020), and production system (p = 0.035). In conclusion, equids from the municipality of Araguaína showed high seroreactivity to *Leptospira* spp., widely distributed among the evaluated properties, with flooded grazing areas, age group, and production system associated with seroreactivity in the univariate analysis.

INDEX TERMS: Equine leptospirosis, microscopic agglutination test, seroprevalence, risk factors, Legal Amazon, One Health.

RESUMO.- [**Sororreatividade anti-*Leptospira* spp. e fatores associados em equídeos do norte do Tocantins, Amazônia Legal.**] A leptospirose é uma zoonose de distribuição global com importante impacto na saúde animal e pública, sendo as regiões tropicais particularmente favoráveis à persistência ambiental de *Leptospira* spp. O objetivo deste estudo foi determinar a soroprevalência, identificar os sorovares mais frequentes e identificar os fatores associados à sororreatividade para *Leptospira* spp. em equídeos do município de Araguaína, Tocantins. Foram analisadas 200 amostras de soro provenientes de equídeos de 81 propriedades (77 rurais e 4 urbanas). O diagnóstico foi realizado pela técnica de Soroaglutinação Microscópica (SAM), utilizando painel de 24 sorovares. A soroprevalência foi de 92,5% (185/200), e 97,5% (79/81) das propriedades apresentaram pelo menos um equídeo sororreagente. Entre os animais positivos, 83,8% (155/185) apresentaram coaglutinações para dois ou mais sorovares. Foram registradas 733 reações sorológicas distribuídas entre 21 dos 24 sorovares avaliados, com predomínio de *Icterohaemorrhagiae* (19,10%), seguido por *Shermani* (7,91%), *Wolffi* (7,78%), *Pyrogenes* (7,50%), *Whitcombi* (6,68%), *Canicola* (6,68%) e *Copenhageni* (6,55%). Os sorovares *Andamana*, *Cynopteri* e *Patoc* não apresentaram reatividade. Na análise univariada, a sororreatividade esteve associada ao pastejo em áreas alagadas (OR = 8,32; IC 95% = 1,07 – 64,76; p = 0,021), ao grupo etário (p = 0,020) e ao sistema de criação (p = 0,035). Conclui-se que os equídeos do município de Araguaína apresentaram elevada sororreatividade para *Leptospira* spp., amplamente distribuída entre as propriedades avaliadas, estando o pastejo em áreas alagadas, o grupo etário e o sistema de criação associados à sororreatividade na análise univariada.

TERMOS DE INDEXAÇÃO: Leptospirose equina, soroaglutinação microscópica, soroprevalência, fatores de risco, Amazônia Legal, Saúde Única.

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INTRODUCTION

Leptospirosis is a globally distributed zoonosis responsible for more than one million human cases annually (Costa et al. 2015) and is considered an important public health problem in tropical and subtropical countries (Hartskeerl et al. 2011, Díaz et al. 2023). Caused by bacteria of the genus *Leptospira*, it is relevant to both human and veterinary medicine because of its reproductive and productive impacts on different domestic animal species (Ellis 2015), as well as its epidemiological importance at the interface among animals, the environment, and humans (Romanowski et al. 2023, Morais et al. 2024).

In Brazil, leptospirosis occurs in both urban and rural environments and is favored by sanitary and environmental conditions such as inadequate waste management, poor sanitation, and high densities of synanthropic rodents (Barcellos & Sabroza 2000, Antoniolli et al. 2025). In rural areas, the interaction among different domestic species, wildlife, and humans increases opportunities for exposure to the pathogen. In equids, infection may present as an acute disease, characterized by fever, jaundice, and reproductive disorders, or remain subclinical, making its recognition and diagnosis more difficult (Ellis 2015). This profile highlights the importance of serological studies for understanding the epidemiology of the disease in this species.

Serological surveys in Brazilian equids have reported considerable variation in seroprevalence among regions. In the Brazilian Amazon, Aguiar et al. (2008) reported a seroprevalence of 91.4% in equids from Monte Negro, Rondônia, whereas Ulsenheimer et al. (2023) found 65.9% seropositivity in horses from the Santarém region, Pará. In Central Brazil, Romanowski et al. (2023) reported 61.6% seropositivity in horses from Goiás State. These findings indicate substantial regional variation in exposure to *Leptospira* spp. and reinforce the need for epidemiological data from areas that remain poorly characterized.

Environmental conditions such as high humidity, elevated temperatures, and prolonged rainfall favor the survival of *Leptospira* spp. in the environment and increase the likelihood of exposure of both animals and humans (Hartskeerl et al. 2011, WOAH 2021). Tocantins State, located in the transition zone between the Amazon and Cerrado biomes, presents favorable environmental conditions for *Leptospira* persistence during part of the year. In addition, confirmed human cases of leptospirosis have been reported in Tocantins State in recent years (Brasil 2026). This scenario highlights the need to expand knowledge on the circulation of *Leptospira* spp. in animal populations and reinforces the relevance of integrated surveillance within a One Health framework.

Despite the importance of leptospirosis to animal and public health, information regarding exposure of equids to *Leptospira* spp. in northern Tocantins remains scarce. Considering the epidemiological context of the region and the limited number of studies involving equids in northern Tocantins, investigations of this nature are essential to improve knowledge of pathogen circulation and to support surveillance strategies within the One Health framework. Therefore, the present study aimed to determine the seroprevalence of antibodies against *Leptospira* spp. and to identify factors associated with seroreactivity in equids from northern Tocantins State, Brazilian Legal Amazon.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Animal Use Ethics Committee of the Federal University of Tocantins (CEUA/UFT) under protocol No. 23.101.001.297/22-13, in accordance with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

The study was conducted in the municipality of Araguaína, in the northernmost region of Tocantins State, Brazil, located in the transition zone between the Amazon and Cerrado biomes (Fig. 1). From August 2022 to July 2023, the mean air temperature was 25.6°C, mean relative humidity was 76.5%, and cumulative rainfall was 1,859.2 mm, according to data from the Araguaína automatic weather station (A021) of the Brazilian National Institute of Meteorology (INMET 2026).

Population sampling. Sample size was calculated using Epi Info version 7.2, considering an estimated population of 6.000 equids, a 95% confidence level, a 5% sampling error, and an expected prevalence of 90%, based on the high seroprevalence previously reported in equids from the Brazilian Amazon (Aguiar et al. 2008, Moraes et al. 2010). A total of 200 equids from 81 properties were included, comprising 77 rural and four urban properties. The properties were randomly selected from the database of properties with registered equids maintained by the Agricultural Defense Agency of the State of Tocantins (ADAPEC/TO). Sampling within each property was proportional to herd size, with one animal sampled from properties with up to 10 equids, three animals from properties with 11 to 70 equids, and five animals from properties with more than 70 equids. Only equids aged ≥1 year, not vaccinated against leptospirosis, and randomly selected from lists provided by the property owners were included.

The properties were georeferenced using a Global Positioning System (GPS) receiver, with coordinates recorded in the WGS 84 datum, UTM projection, Zone 22S. The spatial distribution of the properties was mapped using the 2025 Digital Municipal Boundary database of the Brazilian Institute of Geography and Statistics (IBGE). Properties were classified as positive when at least one equid tested positive by the MAT (titer ≥1:100) and as negative when all examined animals were non-reactive.

Blood sampling and epidemiological questionnaire. Blood samples were collected by jugular venipuncture using sterile vacuum tubes without anticoagulant. After clot formation, the samples were centrifuged, and the serum was transferred to sterile microtubes and stored at −20°C until serological testing. Simultaneously with blood collection, a structured epidemiological questionnaire, adapted from Ribeiro (2015), was administered to obtain information on property characteristics, production system, herd composition, contact with other animal species, rodent occurrence, reproductive history, management practices, water supply, access to flooded grazing areas, disposal of biological waste, and participation in animal events.

For the epidemiological analyses, equid age was categorized into four groups: Group 1 (<4 years), Group 2 (4 to <8 years), Group 3 (8 to <12 years), and Group 4 (≥12 years).

Microscopic Agglutination Test (MAT). Anti-*Leptospira* spp. antibodies were detected using the MAT, according to Faine et al. (1999) and the recommendations of the World Organisation for Animal Health (WOAH 2021), using a panel of 24 live serovars: *Andamana*, *Australis*, *Autumnalis*, *Bataviae*, *Bratislava*, *Butembo*, *Canicola*, *Castellonis*, *Copenhageni*, *Cynopteri*, *Grippotyphosa*, *Hardjoprajitno*, *Hebdomadis*, *Icterohaemorrhagiae*, *Javanica*, *Panama*, *Patoc*, *Pomona*, *Pyrogenes, Sentot, Shermani, Tarassovi, Whitcombi,* and *Wolffi*. Samples showing ≥50% agglutination at the initial dilution of 1:100, based on comparison with the corresponding antigen control, were considered reactive and were subsequently titrated in serial two-fold dilutions of 1:200, 1:400, 1:800, 1:1600, and 1:3200. The endpoint was defined as the highest serum dilution showing approximately 50% agglutination.

Statistical analysis. Statistical analyses were performed using Epi Info™ version 7.2. Seroprevalence was expressed as absolute and relative frequencies. Associations between seroreactivity to *Leptospira* spp. and the epidemiological variables were investigated at the individual level, considering each equid as a sampling unit, using Pearson's chi-square (χ^2) test, with Yates' correction when applicable, or Fisher's exact test when expected frequencies were less than five. Odds ratios (OR) and their respective 95% confidence intervals (95% CI) were calculated for dichotomous variables. Variables with more than two categories were analyzed using overall association tests, without calculation of OR. Statistical significance was set at $p < 0.05$. Multivariable logistic regression was not performed because the high overall seroprevalence resulted in only 15 seronegative animals,

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limiting the number of observations in the less frequent outcome category available for reliable multivariable modeling and increasing the risk of unstable parameter estimates and model overfitting.

RESULTS

The seroprevalence of antibodies against *Leptospira* spp. in the evaluated equids was 92.5% (185/200). Among the 81 properties included in the study, 79 (97.5%) had at least one seroreactive equid (Fig. 1). The main characteristics of the study population are presented in Table 1.

Among the 185 seroreactive animals, 155 (83.8%) showed coagglutination with two or more serovars, whereas 30 (16.2%) reacted to only one serovar. A total of 733 serological reactions were recorded, distributed among 21 of the 24 serovars evaluated. No reactivity was detected for the *Andamana*, *Cynopteri*, and *Patoc* serovars. *Icterohaemorrhagiae* was the most frequently detected serovar (19.10%), followed by *Shermani* (7.91%), *Wolffi* (7.78%), *Pyrogenes* (7.50%), *Whitcombi* (6.68%), *Canicola* (6.68%), and *Copenhageni* (6.55%). The remaining serovars occurred at frequencies below 6% (Table 2).

In the univariate analysis of the epidemiological variables (Table 3), significant associations were observed between seroreactivity to *Leptospira* spp. and grazing in flooded areas (OR = 8.32; 95% CI = 1.07–64.76; p = 0.021), age group (p = 0.020), and production system (p = 0.035). The remaining variables were not significantly associated with seroreactivity (p > 0.05).

The lowest seroreactivity was observed in Group 1 (22/28; 78.6%), whereas higher frequencies were observed in Group 2 (71/74; 95.9%), Group 3 (45/47; 95.7%), and Group 4 (47/51; 92.2%).

DISCUSSION

The seroprevalence of 92.5% observed in the present study is among the highest reported in equids in Brazil and indicates widespread exposure to *Leptospira* spp. in the municipality of Araguaína, Tocantins. Considerable variation in seroreactivity has been reported among Brazilian equine populations, including studies conducted in the Brazilian Amazon. Aguiar et al. (2008) reported 91.4% seropositivity in equids from Monte Negro, Rondônia, whereas Moraes et al. (2010) found antibodies against *Leptospira* spp. in all 37 equids evaluated on Algodoal Island, Pará. More recently, seropositivity rates of 44.5% in Manaus and its metropolitan region (Sousa et al. 2020) and 65.9% in Santarém, Pará (Ulsenheimer et al. 2023) have been reported. Outside the Amazon region, Rizzo et al. (2022) found a similarly high frequency (97.2%) in horses from Rio Grande do Norte. Differences among studies may reflect variation in environmental conditions, management practices, sampled populations, and the composition of the antigen panels used in the MAT.

In addition to the high seroprevalence observed among the animals, the occurrence of at least one seroreactive equid in 97.5% (79/81) of the evaluated properties demonstrates the widespread distribution of exposure to *Leptospira* spp. in the municipality of Araguaína. In tropical environments, high temperatures, rainfall, humidity, and the presence of surface water may favor the persistence of leptospires in the environment and increase opportunities for exposure of susceptible animals (Levett 2001, Hartskeerl et al. 2011, WOAH 2021).

Grazing in flooded areas was associated with seroreactivity, with equids having access to these areas showing higher odds of being seroreactive (OR = 8.32; 95% CI = 1.07–64.76; p = 0.021). This association is biologically plausible because *Leptospira* spp. can persist for prolonged periods in moist environments and surface water, particularly under favorable temperature and humidity conditions, increasing opportunities for indirect exposure through contaminated water and soil (Ko et al. 2009, WOAH 2021). Similar findings have been reported in horses exposed to environments subject to flooding, supporting the importance of water accumulation and environmental contamination in leptospiral transmission (Finger et al. 2014). In the study region, where seasonal rainfall may result in temporary flooding of grazing areas, these conditions may facilitate environmental exposure of equids to *Leptospira* spp.

From a One Health perspective, the high seroreactivity observed in equids should also be considered in the context of human leptospirosis in the region. According to official surveillance data, confirmed human cases of leptospirosis have been reported in Tocantins State in recent years (Brasil 2026). Although these surveillance data cannot establish a direct epidemiological link between seroreactivity in equids and human infection, they demonstrate the occurrence of leptospirosis in the human population within the same regional context. The shared exposure of humans and animals to environmental sources contaminated with pathogenic *Leptospira*, particularly in areas favorable to bacterial persistence, reinforces the relevance of integrated animal, human, and environmental surveillance.

Age group was also associated with seroreactivity (p = 0.020). The lowest frequency was observed in the youngest animals (22/28; 78.6%), whereas frequencies above 90% were observed in the three older age groups. This pattern may reflect cumulative exposure to *Leptospira* spp. over time, as older animals have had longer opportunities for contact with contaminated environments. An association between increasing age and seroreactivity has also been reported in other Brazilian equine populations (Langoni et al. 2004, Santos et al. 2012,

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Ribeiro et al. 2018, Siqueira et al. 2020). Therefore, the age-related pattern observed in the present study is consistent with repeated environmental exposure throughout life rather than necessarily indicating greater individual susceptibility in older animals.

Production system was also associated with seroreactivity ($p = 0.035$). Seroreactivity was frequent among extensively managed equids; however, this association should be interpreted cautiously because production system comprises a set of interrelated management and environmental characteristics. Equids maintained extensively may have greater contact with natural water sources, flooded areas, other domestic species, wildlife, and environments contaminated with urine from infected animals. Previous studies in equids have likewise identified management and environmental characteristics as factors associated with seroreactivity to *Leptospira* spp. (Siqueira et al. 2020, Ribeiro et al. 2018). Because the present analysis was univariate, the association with production system cannot be interpreted independently from other correlated exposures, particularly access to flooded grazing areas.

The predominance of serological reactions to Icterohaemorrhagiae (19.10%), together with those to Copenhageni (6.55%), indicates substantial seroreactivity to antigens of the Icterohaemorrhagiae serogroup in the studied population. A high frequency of reactions to this serogroup has also been reported in Brazilian equids, including horses from Rio Grande do Norte, where Icterohaemorrhagiae was the predominant serovar (Rizzo et al. 2022), and from Manaus and its metropolitan region, where the Icterohaemorrhagiae serogroup was among the most frequently detected (Sousa et al. 2020). Rodents are recognized as important maintenance hosts and sources of environmental contamination for leptospires of this serogroup (Levett 2001, Ellis 2015). However, rodent occurrence on the properties was not associated with equine seroreactivity in the present study. This finding does not exclude their epidemiological relevance, because visual reports of rodent occurrence may not accurately represent the intensity of environmental contamination or previous exposure of the animals.

Serological reactions to Wolffi (7.78%) and Canicola (6.68%) further demonstrate the diversity of leptospiral antigens recognized by the equids evaluated. Wolffi belongs to the Sejroe serogroup, which is frequently associated with ruminants, particularly cattle, whereas dogs are recognized as the principal maintenance hosts for Canicola (Ellis 2015). Considering the frequent coexistence of different domestic animal species in rural environments, these findings are compatible with opportunities for shared environmental exposure. Nevertheless, because MAT seroreactivity does not identify the source of exposure or reliably establish the infecting serovar, the participation of particular animal species in transmission to the equids evaluated cannot be inferred from these results alone.

Shermani (7.91%), Pyrogenes (7.50%), and Whitcombi (6.68%) were also among the most frequent serological reactions. Although Shermani has been less frequently reported in Brazilian equids, seroreactivity to this serovar has been documented in different animal species, indicating that its epidemiological distribution is not restricted to a single host species (Chadsuthi et al. 2017). Pyrogenes, in contrast, has been reported in equine surveys conducted in the Brazilian Amazon, including studies from Pará and Amazonas (Moraes et al. 2010, Sousa et al. 2020, Ulsenheimer et al. 2023). In Santarém, Pará, Pyrogenes was the most frequently detected serogroup among seroreactive horses (Ulsenheimer et al. 2023). These regional findings demonstrate that reactions to Pyrogenes are not uncommon in equine populations from the Brazilian Amazon, although serological data alone do not allow identification of the maintenance host or source of exposure.

The high frequency of coagglutination observed in 83.8% of the seroreactive animals should be interpreted considering the inherent characteristics of the MAT. Reactions to multiple serovars may result from exposure to different leptospiral antigens, but may also reflect cross-reactivity among serovars, particularly within related serogroups (Faine et al. 1999, Zilch et al. 2021, WOAH 2021). Therefore, MAT is useful for demonstrating serological exposure but has limitations for identifying the infecting serovar based solely on the pattern or magnitude of antibody titers. This limitation is particularly relevant in the present study, in which most seroreactive animals reacted to more than one serovar. Accordingly, the frequencies observed for individual serovars should be interpreted as patterns of seroreactivity rather than evidence of circulation or infection by specific serovars.

This study has some limitations. The high seroprevalence resulted in a small number of seronegative animals (15/200), limiting the reliability of conventional multivariable modeling; consequently, the associations identified in the univariate analysis should be interpreted as unadjusted associations rather than independent risk factors. In addition, the cross-sectional design does not allow temporal or causal relationships to be established. Serological testing by MAT provides evidence of exposure to *Leptospira* spp. but does not demonstrate active infection or bacterial shedding, and no bacterial isolation or molecular detection was performed. Furthermore, cross-reactions and coagglutination among serovars may limit the attribution of seroreactivity to a specific infecting serovar.

CONCLUSION

Under Review

The high seroreactivity to *Leptospira* spp. observed in equids from northern Tocantins indicates widespread exposure to the agent in the studied population. Access to flooded grazing areas, age group, and production system were associated with seroreactivity in the univariate analysis, highlighting environmental and management characteristics that warrant further investigation. The diversity of serological reactions observed reinforces the potential importance of environmental exposure and interactions among animal species in the epidemiology of leptospirosis. These findings support integrated surveillance and preventive measures, particularly those aimed at reducing environmental exposure in areas favorable to Leptospira persistence, within a One Health framework.

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Credit author statement.- Bruna V. Silva and Fernanda Mírian P. dos Santos - Project writing, field visits to rural properties, and sample collection. Ana Paula D. Ribeiro, Helcileia D. S. Rodrigues and Cristiane A. de Carvalho – Article review. Alessandro José F. dos Santos - Study design and statistical analysis. Washington Luiz A. Pereira - Study conception, study design, data interpretation, and article review. Marco Augusto G. da Silva - Study conception, study design, field investigation, statistical analysis, data interpretation, article writing, and article review.

Data availability statement.- The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Aguiar D.M., Cavalcante G.T., Lara M.C.C.S.H., Villalobos E.M.C., Cunha E.M.S., Okuda L.H., Stéfano E., Nassar A.F.C., Souza G.O., Vasconcellos S.A., Labruna M.B., Camargo L.M.A. & Gennari S.M. 2008. Prevalência de anticorpos contra agentes virais e bacterianos em eqüídeos do Município de Monte Negro, Rondônia, Amazônia Ocidental Brasileira. Braz. J. Vet. Res. Anim. Sci. 45(4):269-276. DOI: 10.11606/issn.1678-4456.bjvras.2008.26685.

Antoniolli A., Guis H., Picardeau M., Goarant C. & Flamand C. 2025. One Health field approach applied to leptospirosis: a systematic review and meta-analysis across humans, animals and the environment. Open Forum Infect. Dis. 12(1):ofae757. DOI: 10.1093/ofid/ofae757.

Barcellos C. & Sabroza P.C. 2000. Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: a geographical approach. Int. J. Environ. Health Res. 10(4):301-313. DOI: 10.1080/0960312002001500.

Brasil. 2026. Casos e óbitos confirmados de leptospirose, 2000 a 2026. Ministério da Saúde, Brasília, DF. Atualizado em 29 jan. 2026. Atualizado em 29 jan. 2026. Available at https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/l/leptospirose/situacao-epidemiologica/casos-e-obitos-2000-a-2026.pdf/view?utm_source=chatgpt.com. Accessed on Jul. 5, 2026.

Chadsuthi S., Bicout D.J., Wiratsudakul A., Suwancharoen D., Petkanchanapong W., Modchang C., Triampo W., Ratanakorn P. & Chalvet-Monfray K. 2017. Investigation on predominant *Leptospira* serovars and its distribution in humans and livestock in Thailand, 2010-2015. PLoS Negl. Trop. Dis. 11(2):e0005228. DOI: 10.1371/journal.pntd.0005228.

Costa F., Hagan J.E., Calcagno J., Kane M., Torgerson P., Martinez-Silveira M.S., Stein C., Abela-Ridder B. & Ko A.I. 2015. Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl. Trop. Dis. 9(9):e0003898. DOI: 10.1371/journal.pntd.0003898.

Díaz E.A., Arroyo G., Sáenz C., Mena L. & Barragán V. 2023. Leptospirosis in horses: sentinels for a neglected zoonosis? A systematic review. Vet. World 16(10):2110-2119. DOI: 10.14202/vetworld.2023.2110-2119.

Ellis W.A. 2015. Animal leptospirosis. Curr. Top. Microbiol. Immunol. 387:99-137. DOI: 10.1007/978-3-662-45059-8_6.

Faine S., Adler B., Bolin C. & Perolat P. 1999. *Leptospira and Leptospirosis*. 2nd ed. MediSci, Melbourne.

Finger M.A., Barros Filho I.R., Leutenegger C., Estrada M., Ullmann L.S., Langoni H., Kikuti M., Dornbush P.T., Deconto I. & Biondo A.W. 2014. Serological and molecular survey of *Leptospira* spp. among cart horses from an endemic area of human leptospirosis in Curitiba, southern Brazil. Rev. Inst. Med. Trop. São Paulo 56(6):473-476. DOI: 10.1590/S0036-46652014000600003.

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Hartskeerl R.A., Collares-Pereira M. & Ellis W.A. 2011. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin. Microbiol. Infect. 17(4):494-501. DOI: 10.1111/j.1469-0691.2011.03474.x.

INMET. 2026. Banco de Dados Meteorológicos do Instituto Nacional de Meteorologia: Estação Meteorológica Automática de Araguaína (A021), dados de agosto de 2022 a julho de 2023. Instituto Nacional de Meteorologia, Brasília, DF. Available at https://bdmep.inmet.gov.br/ Accessed on Oct. 5, 2026.

Ko A.I., Goarant C. & Picardeau M. 2009. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nat. Rev. Microbiol.* 7(10):736–747. DOI: 10.1038/nrmicro2208.

Langoni H., Da Silva A.V., Pezerico S.B. & De Lima V.Y. 2004. Anti-leptospire agglutinins in equine sera, from São Paulo, Goias, and Mato Grosso do Sul, Brazil, 1996-2001. J. Venom. Anim. Toxins incl. Trop. Dis. 10(3):207-218. DOI: 10.1590/S1678-91992004000300003.

Levett P.N. 2001. Leptospirosis. Clin. Microbiol. Rev. 14(2):296-326. DOI: 10.1128/CMR.14.2.296-326.2001.

Moraes C.C.G., Kuroda R.B.S., Pinho A.P.V.B., Ywasaki F., Meneses A.M.C., Martins A.V., Amaral Júnior J.M., Dias H.L.T. & Vasconcellos S.A. 2010. Pesquisa de anticorpos para sorovares de *Leptospira interrogans* patogênicas em equídeos criados na ilha de Algodoal, Estado do Pará. Rev. Ciênc. Agrár. 53(2):188-194. DOI: 10.4322/rca.2011.027.

Morais D.A., Nunes B.C., Barnabé N.N.C., Anjos D.M., Bezerra C.S., Costa D.F., Santos C.S.A.B., Azevedo S.S. & Alves C.J. 2024. Spatial and seasonal distribution of *Leptospira* spp. seropositive horses in the Northeast region of Brazil. Prev. Vet. Med. 231:106301. DOI: 10.1016/j.prevetmed.2024.106301.

Ribeiro T.M.P. 2015. *Soroepidemiologia da infecção por Leptospira spp. em equinos do município de Rorainópolis, Estado de Roraima, Brasil*. Dissertação de Mestrado em Ciência Animal, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, GO.

Ribeiro T.M.P., Correia L., Spohr K.A.H., Aguiar D.M., Martins G. & Jayme V.S. 2018. Risk factors associated with seroreactivity against *Leptospira* sp. in horses from Brazilian Amazon. J. Equine Vet. Sci. 68:59-62. DOI: 10.1016/j.jevs.2018.05.197.

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. Pesq. Vet. Bras. 42:e06784. DOI: 10.1590/1678-5150-PVB-6784.

Romanowski T.N.A., Dias R.A., Heinemann M.B., Carvalho S.F., Silva T.A., Martins A.S., Caetano G.D.C., Ferreira Júnior A., Santos J.P. & Borsanelli A.C. 2023. Seroprevalence of equine leptospirosis in the State of Goiás, Brazil. Vet. Sci. 10(10):590. DOI: 10.3390/vetsci10100590.

Santos C.S., Guedes Júnior D.S., Pereira R.C.G., Santos C.C.A., Castro V. & Jesus V.L.T. 2012. Inquérito sorológico da leptospirose equina na microrregião de Itaguaí, Estado do Rio de Janeiro. Braz. J. Vet. Med. 34(2):96-100.

Siqueira C.C., Fraga D.B.M., Chagas-Junior A.D., Athanazio D.A., Silva M.M.N., Cerqueira R.B., McBride F.W.C., Pinna M.H. & Ayres M.C.C. 2020. Seroprevalence and risk factors associated with equine leptospirosis in the metropolitan region of Salvador and Recôncavo Baiano region, Bahia state (NE Brazil). Trop. Anim. Health Prod. 52(1):31-39. DOI: 10.1007/s11250-019-01956-5.

Sousa I.K.F., Silva R.L.C., Sousa R.S., Vieira C.E.M., Melo S., Quevedo G.P., Von Laer A.E., Lovato L.T. & Tonin A.A. 2020. Frequência de leptospirose em equinos de Manaus e região metropolitana no estado do Amazonas, Brasil. Braz. J. Vet. Res. Anim. Sci. 57(4):e172607. DOI: 10.11606/issn.1678-4456.bjvras.2020.172607.

Ulsenheimer R.C., Barboza C.L., Oberbeck E., Silva R.L.C., Sousa I.K.F., Von Laer A.E. & Tonin A.A. 2023. Soroepidemiologia de leptospirose em equinos da região de Santarém, Pará. Cienc. Anim. Bras. 24:e-74800E. DOI: 10.1590/1809-6891v24e-74800E.

WOAH. 2021. Leptospirosis. Chapter 3.1.12. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. World Organisation for Animal Health, Paris.

Zilch T.J., Lee J.J., Bressan G.C., McDonough S.P., Mohammed H.O., Divers T.J. & Chang Y.F. 2021. Evaluation of new leptospiral antigens for the diagnosis of equine leptospirosis: an approach using pan-genomic analysis, reverse vaccinology and antigenic selection. Equine Vet. J. 53(5):1025-1035. DOI: 10.1111/evj.13380.

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Figure

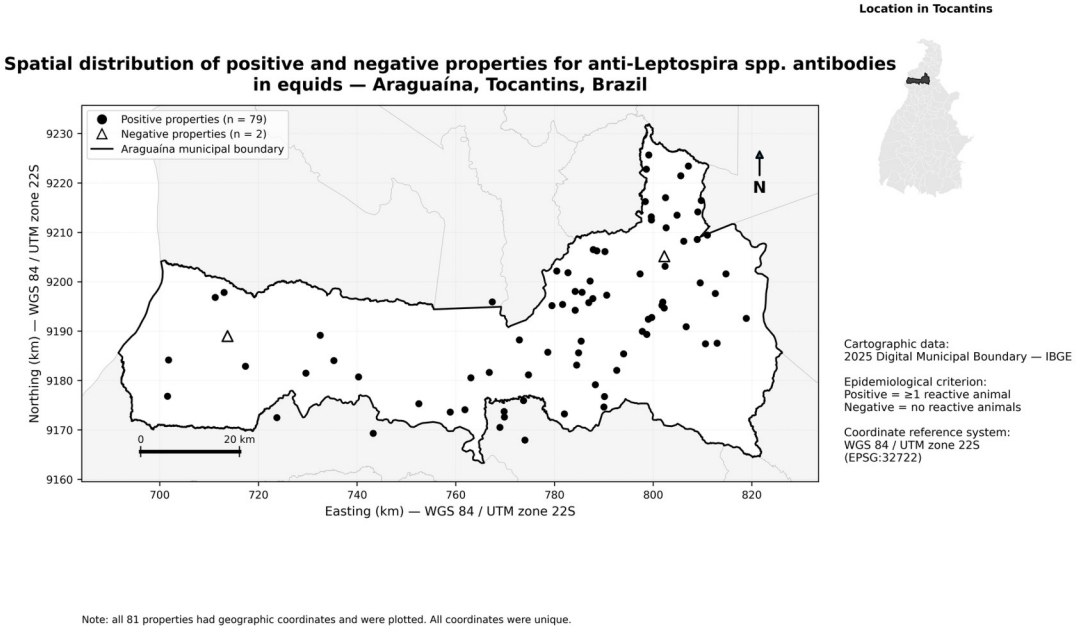


Fig. 1. Spatial distribution of properties positive and negative for antibodies against *Leptospira* spp. in equids from the municipality of Araguaína, Tocantins, Brazil. Properties were classified as positive when at least one animal tested positive by the Microscopic Agglutination Test (MAT) and as negative when none of the examined animals showed seroreactivity. The map includes 81 georeferenced properties, of which 79 were positive and two were negative. Municipal boundaries were obtained from the 2025 Digital Municipal Boundary database of the Brazilian Institute of Geography and Statistics (IBGE). Coordinate reference system: WGS 84, UTM Zone 22S.

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Table 1. Characteristics of the equid population and evaluated properties in the municipality of Araguaína, Tocantins, Brazil (2022–2023)

Variable	Value
Evaluated equids	200
Evaluated properties	81
Positive properties	79 (97.5%)
Seroreactive equids	185 (92.5%)
Horses	136 (68.0%)
Donkeys	56 (28%)
Mules	08 (4.0%)
Rural property	77 (95.1%)
Urban property	04 (4.9%)

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Table 2. Distribution of antibody titers and frequency of seroreactivity to *Leptospira* spp. serovars in equids from the municipality of Araguaína, Tocantins, Brazil (2022–2023)

Serovar[2]	Antibody titer (UI)						Serological reactions, n	%
	1:100	1:200	1:400	1:800	1:1600	1:3200		
Australis	8	4	1	1	-	-	14	1.91
Autumnalis	5	1	-	-	-	-	06	0.82
Bataviae	7	1	-	-	-	-	08	1.09
Bratislava	14	16	2	1	-	-	33	4.50
Butembo	24	9	3	1	-	-	37	5.05
Canicola	31	16	2	-	-	-	49	6.68
Castellonis	5	6	-	-	-	-	11	1.50
Copenhageni	19	21	5	1	1	1	48	6.55
Grippotyphosa	12	12	-	2	-	-	26	3.55
Hardjoprajitno	6	4	1	-	-	-	11	1.50
Hebdomadis	4	3	-	1	-	-	08	1.09
Icterohaemorrhagiae	68	58	11	2	-	-	140	19.10
Javanica	6	2	-	-	-	-	08	1.09
Panama	29	9	-	-	-	-	38	5.18
Pomona	21	11	5	5	-	-	42	5.73
Pyrogenes	26	4	22	3	-	-	55	7.50
Sentot	7	1	-	-	-	-	08	1.09
Shermani	28	20	7	1	1	1	58	7.91
Tarassovi	18	5	4	-	-	-	27	3.68
Whitcombi	32	13	3	1	-	-	49	6.68
Wolffi	37	11	5	3	1	-	57	7.78
Total							733[1]	100%

[1]Percentages were calculated using the total number of serological reactions (n=733) as the denominator. Because the same animal could react to more than one serovar (coagglutination), the total number of reactions exceeded the number of unique seroreactive animals (n=185); [2]The *Andamana, Cynopteri,* and *Patoc* serovars, although included in the panel of 24 serovars tested, showed no seroreactivity in any of the evaluated samples (titer <1:100).

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Table 3. Univariate analysis of epidemiological factors associated with seroreactivity to *Leptospira* spp. in equids from the municipality of Araguaína, Tocantins, Brazil (2022–2023)

Variable	Category	OR	95% CI	*p*-value
Grazing in flooded areas	Yes vs No	8.32	1.07 – 64.76	0.021
Age group	<4 y/ 4 to < 8 y / 8 to <12 y/ ≥12 y	-	-	0.020
Production system	Extensive/Intensive/Mixed	-	-	0.035

OR = Odds Ratio; 95% CI = Confidence interval.

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