


Frequency of leptospirosis in horses in Manaus and metropolitan region in Amazonas State, Brazil

Frequência de leptospirose em equinos de Manaus e região metropolitana no estado do Amazonas, Brasil

Isadora Karolina Freitas de Sousa^{1,2}; Rebeca Larissa Castro Silva¹; Rejane dos Santos Sousa³; Cláudia Elisa Martins Vieira⁴; Sergio de Melo²; Geórgia Portella Quevedo⁵; Ana Eucares Von Laer⁵; Luciane Teresinha Lovato⁵; Alexandre Alberto Tonin^{1,4,5} 

¹ Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Manaus – AM, Brazil

² Universidade Federal do Oeste do Pará, Programa de Pós-graduação em Sociedade, Natureza e Desenvolvimento, Santarém – PA, Brazil

³ Universidade Federal do Sul e Sudeste do Pará, Instituto de Estudos do Trópico Úmido, Xinguara, PA – Brazil

⁴ Universidade Federal do Amazonas, Programa de Pós-graduação em Ciência Animal, Manaus, AM – Brazil

⁵ Universidade Federal de Santa Maria, Laboratório de Leptospirose, Santa Maria, RS – Brazil

ABSTRACT

The occurrence of leptospirosis is significantly higher in the tropical climate than in temperate regions, especially in periods with high rainfall levels. In horses, leptospirosis can range from subclinical to asymptomatic but maintains an important role in the transmission of leptospirosis by eliminating the agent in the environment. Regarding horses, the Amazonas herd increased by 30% between 2004 and 2013. Thus, the study aimed to determine the serological prevalence of leptospirosis in horses in Manaus and metropolitan regions, in the State of Amazonas, Brazil as well as to assess the main serogroups involved in the infections of these animals. For this purpose, from August 2018 to July 2019, serum from 198 horses was evaluated through the microscopic agglutination test with a panel of 10 serogroups. As a result, 92 horses (44.46%) were found to be positive for one or more *Leptospira* serovars, with the highest prevalence of serogroups Icterohaemorrhagiae and Pyrogenes. Therefore, with the increase in the equine herd in the region and, consequently, greater interaction between breeders and animals, the identification of reagents to at least one serovar of *Leptospira* spp. signals the possible existence of reservoirs of pathogenic strains for other animals and man.

Keywords: Amazonas. Metropolitan region. Epidemiology. Equine. *Leptospira*.

RESUMO

A ocorrência de leptospirose é significativamente maior no clima tropical do que nas regiões temperadas, especialmente em períodos com altos níveis de chuva. Em cavalos, a leptospirose pode variar de subclínica a assintomática, mas estes animais podem desempenhar um papel importante na transmissão da leptospirose por meio da eliminação do agente no ambiente. Em relação aos cavalos, entre os anos de 2004 e 2013, o rebanho Amazônico aumentou 30%. Assim, o objetivo do estudo foi determinar a prevalência sorológica de leptospirose em cavalos em Manaus e região metropolitana, estado do Amazonas, Brasil, bem como, determinar os principais sorogrupos envolvidos nas infecções desses animais. Para este propósito, entre agosto de 2018 e julho de 2019, foram colhidos soros de 198 cavalos para serem avaliados sorologicamente com o teste de microaglutinação microscópica, utilizando-se um painel de dez sorogrupos. Como resultado, observou-se que 92 cavalos (44,46%) foram positivos para um ou mais sorovares de *Leptospira*, com maior prevalência dos sorogrupos Icterohaemorrhagiae e Pyrogenes. Portanto, com o aumento do rebanho equino na região e, conseqüentemente, maior interação entre criadores e animais, a identificação de reagentes para pelo menos um sorovar de *Leptospira* spp. alerta para a possível existência de reservatórios de cepas patogênicas para outros animais e seres humanos.

Palavras-chave: Amazonas. Região metropolitana. Epidemiologia. Equino. *Leptospira*.

Correspondence to:

Alexandre Alberto Tonin
 Universidade Federal de Santa Maria, Laboratório de
 Leptospirose
 Avenida Roraima, 1000, Camobi, Prédio 20, sala 4235
 CEP: 97110-105, Santa Maria – RS, Brazil
 e-mail: alexandre.tonin@ifam.edu.br

Received: July 20, 2020

Approved: September 22, 2020

How to cite: Sousa IKF, Silva RLC, Sousa RS, Vieira CEM, Melo S, Quevedo GP, Von Laer AE, Lovato LT, Tonin AA. Frequency of leptospirosis in horses in Manaus and metropolitan region in Amazonas State, Brazil. *Braz J Vet Res Anim Sci.* 2020;57(4):e172607. <https://doi.org/10.11606/issn.1678-4456.bjvras.2020.172607>

Introduction

Between the years of 2004 and 2013, the herd of horses in the State of Amazonas increased by 30%, resulting in a herd of 15,479 equines at the end of the evaluated decade. In the same period, growth occurred in all states in the North region of Brazil, with rates between 0.57% in Pará and 98% in Acre. However, this rate differs from traditional states in equine breeding, such as Minas Gerais, Bahia, and São Paulo, which, in the same period, showed a decrease in the effective number of animals in their herds (Brasil, 2016).

The occurrence of leptospirosis is significantly higher in the tropical climate than in temperate regions (such as the Amazonas), mainly due to the higher survival of leptospire in a warm and humid environment, with elevated levels of rainfall and neutral or slightly alkaline soil. These characteristics can result in epidemic outbreaks due to increased exposure to water contaminated with urine or tissues from infected animals (Levett, 2001).

In horses, leptospirosis can be subclinical or asymptomatic, and fever, anorexia, jaundice, uveitis, abortions, or premature births can also be observed (Hong et al., 1993; Hunter & Herr, 1994; Timoney et al., 2011; Yan et al., 2010). Besides, horses have an important role in the transmission of leptospirosis by eliminating the agent in the environment. Even with a high concentration of antibodies in the host, *Leptospira* spp. can survive and multiply in kidney tubules, being eliminated in the urine by the species for at least 30 days post-inoculation or even for 2-3 months (Hamond et al., 2013).

The presence of susceptible animal species in regions with favorable environmental conditions for leptospirosis justifies the need for studies of seroprevalence in human and animal populations (Jesus et al., 2012; Silva et al., 2016).

Besides, there was an increase in the number of horses living in urban areas of Manaus and metropolitan regions, animals that are being used for leisure, work, and sports activities. As a consequence, frequent contact between people and animals, whether in handling, training, or riding therapy sessions, increases the risk of exposure and contamination of humans and other susceptible animals.

In this context, it is essential to know the occurrence of infectious diseases such as leptospirosis, which causes a negative economic impact on equine production systems, such as reproductive losses, and public health risk, due to its zoonotic character. Thus, the present investigation aimed to assess the serological frequency of leptospirosis in horses in Manaus and metropolitan region, in the State of Amazonas, Brazil, as well as to determine the main serogroups involved in the infections of these animals.

Material and Methods

Sampling

The study was carried out in 17 horse properties located in Manaus, capital of the state of Amazonas, and its metropolitan region, belonging to the North region of Brazil. Our statistical modeling for sampling was performed according to Miot (2011), which is considered a prevalence of 50%, with a confidence level of 95%. There is no predominance of the breed, sex, age, or purpose of use. All animals were studs, raised in a mixed breeding system (at the field during the day and housed at night). The option for not selecting breed, sex, or mean age was to have wider coverage and representative sampling of the location. From August 2018 to July 2019, blood samples were collected from 198 horses by external jugular venipuncture in vacutainer®. None of the animals were ever vaccinated against leptospirosis. For hematological analyses, samples were placed in individual test tubes containing diethylene diamino tetraacetic acid (EDTA), cooled to 4 °C until the analysis was carried out, within a maximum of 12 hours after collection. For serum separation, blood samples (in test tubes without anticoagulant) were centrifuged at 1,000 g x 15 min, followed by aliquotation into individual microtubes and frozen at 20 °C for subsequent serology.

Serologic assay

The serological diagnosis of leptospirosis was carried out in the Leptospirosis Diagnostic Laboratory at the Federal University of Santa Maria (UFSM). Serum samples were tested for anti-*Leptospira* antibodies by microscopic agglutination test (MAT) (Cole et al., 1973;

Galton et al., 1965), using live antigens grown in liquid medium Ellinghausen-McCullough-Johnson-Harris (EMJH) free from contamination or self-agglutination, as recommended by the World Health Organization (2012). A complete panel of ten serogroups (including 13 reference serovars) was used as test antigens, namely: 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 (serovars Australis and Bratislava), serogroup Pomona (serovar Pomona) and serogroup Autumnalis (serovar Butembo), serogroup Pyrogenes (serovar Pyrogenes), serogroup Ballum (serovar Ballum) and serogroup Tarassovi (serovar Tarassovi). Briefly, live suspensions of leptospire representing the 13 serovars were added to serum samples diluted in series in a microtiter plate (96 wells), incubated at room temperature for 2-4 h. The presence or absence of agglutination was examined using dark field microscopy at 100× magnification. Titers were obtained in a sequence of double dilutions and expressed as the reciprocal of the highest serum dilution that agglutinated at least 50% of leptospire (Cole et al., 1973; Galton et al., 1965).

Hematological analysis

To determine fibrinogen, the thermal precipitation technique described by Schalm (1970) was used, while hematocrit was assessed through the microcentrifugation technique. Based on hematocrit values and fibrinogen concentration, animals were classified as anemic (hematocrit <32%), borderline (hematocrit 32-33%) and non-anemic (hematocrit > 32%), with hyperfibrinogenemia (fibrinogen > 400 g/dL) and normal fibrinogen (fibrinogen <400 g/dL).

Data analysis

The data were analyzed using Excel® 2016 software for the preparation of spreadsheets, being presented with their absolute and relative distribution.

Results

The obtained results demonstrated that from 198 evaluated horses, 92 were positive for one or more *Leptospira* serovars (46.46%). There is a high frequency of animals reacting to serogroups Icterohaemorrhagiae and Pyrogenes, as shown in Table 1. Table 2 brings the results of hematocrit and fibrinogen, where 19 (20.65%) seropositive animals presented anemia, while 14 (15.22%) had borderline hematocrit. Still, 15 (16.30%) of the seropositive animals had hyperfibrinogenesis.

Table 1 – Antibody titers for serogroup and serovars of *Leptospira* spp. in horses in the State of Amazonas, Brazil. Blood collection was performed from August 2018 to July 2019

Serogroup	Serovar	Titration				N. of Animals
		100	200	400	800	
ICT	ICT	35	10	1	-	46
	COP	9	9	1	-	19
PYR	PYR	20	18	5	1	44
BAL	BAL	10	7	2	-	19
TAR	TAR	5	8	3	-	16
CAN	CAN	10	3	1	-	14
AUS	AUS	3	2	-	-	5
	BRA	1	-	-	-	1
SEJ	HAR	-	2	1	-	3
	WOL	1	-	1	-	2
GRI	GRI	1	1	-	-	2
Total		95	60	15	1	-

Obs. AUS: Australis; BAL: Ballum; BRA: Bratislava; CAN: Canicola; COP: Copenhageni; GRI: Grippotyphosa; HAR: Hardjo-prajitno; ICT: Icterohaemorrhagiae; PYR: Pyrogenes; SEJ: Sejroe; TAR: Tarassovi; WOL: Wolffi.

Table 2 – Hematocrit values and fibrinogen assessment of horses with positive serum agglutination and negative values for *Leptospira* spp. in Manaus - AM

Animals	Serum agglutination*	Serum agglutination	Total
	Positive	Negative	
Anemic (<32%)	19	25	44
Borderline (32-33%)	14	18	32
Non-Anemic (≥34%)	59	63	122
Hyperfibrinogenemia	15	17	32
Normal fibrinogen	77	89	166

*Cutoff titer: 100.

Discussion

Research conducted in the last decade in Brazil has shown great variation in the seroprevalence of leptospirosis in horses. The prevalence in the different regions of Brazil varies between 45 and 74.1% (Central West), 8 and 62.5% (Northeast), 79.3 and 100% (North), 17.9 and 71.9% (Southeast), and 60 and 87.1% (South) (Ribeiro, 2015). Of the 198 animals tested, 40 showed positive anti-leptospira agglutinins for only one serovar, 16 for Icterohaemorrhagiae (40%), 15 for Pyrogenes (37.5%), two for Copenhageni (5%), two for Ballum (5%), three for Tarassovi (7.5%) and two for Canicola (5%), while the other positive animals (52) presented coagglutination that could be infection by more than a serovar or crossed reactions between serovars (Levett, 2001). In a study carried out in the state of Pará of the 37 serum samples evaluated, 100% (37/37) showed a reaction against one or more serovars of *Leptospira* spp. Only the Copenhageni serovar did not react with any of the tested samples (Morais et al., 2010).

A survey and serological analysis study carried out with horses in the Rio Grande do Sul (2003) found that in

1,169 analyzed samples, 871 (74.51%) were reactive and 298 (25.49%) did not react with the tested serovars. The most prevalent serovar was: Bratislava (19.92%), followed by Copenhageni (15.06%), (Pires et al., 2005), while in the state of Pernambuco, the analysis of 100 equine sera showed the prevalence of serovars Patoc (35.71%), Butembo (32.14%) and Sentot (14.20%) (Alves et al., 2016). These studies partially corroborate the results reached by Pires et al. (2005), since the serological predominance of serogroup Icterohaemorrhagiae (where it is included the serovar Copenhageni) was observed. However, another main serological result was linked to serogroup Pyrogenes, which is completely different when compared to the other studies mentioned above. This draws attention to the epidemiological variation of leptospirosis across Brazilian territory.

L. interrogans serotype Icterohaemorrhagiae, belonging to Icterohaemorrhagiae serogroup, have synanthropic rodents as their main maintenance host and other non-synanthropic wild animals such as Brazilian guinea pig (Monte et al., 2013) and capybaras (Langoni et al., 2016). The Icterohaemorrhagiae serovar has been described as the most prevalent in horses in several seroepidemiological studies in Brazil, such as Lilenbaum (1998) in the State of Rio de Janeiro; Fávero et al. (2002) in animals from different Brazilian regions; Langoni et al. (2004) in the States of São Paulo, Goiás, and Mato Grosso do Sul; and Hashimoto et al. (2007) in the State of Paraná, which corroborates our results. It is important to emphasize that Icterohaemorrhagiae is the most frequent serovar found in horses and one of the main serovars responsible for the majority of infections in humans observed in tropical urban areas worldwide (Faria et al., 2008). It should be noted that the increase in the occurrence of this serovar can justify the settlement of special practices for rodent control in horse farms (Megid et al., 2016).

Serovar Pyrogenes, belonging to serogroup Pyrogenes, has a low prevalence in horses from other regions of Brazil. However, in Pará State (Morais et al., 2010), as in this study, a high prevalence of Pyrogenes was observed, which may suggest the maintenance of this serovar by other domestic or wild animals living in the Amazon region. The Pyrogenes serovar was isolated for the first time in Brazil from wild mammals (*Nectomys squamipes*) in areas close to São Paulo (Santa Rosa et al., 1980) and later detected in dogs from several states (Aguiar et al., 2007; Magalhães et al., 2006; Querino et al., 2003). In the Amazon region, a serological study of dogs in the municipality of Monte Negro (RO) showed that the Pyrogenes serovar was the second most prevalent in dogs (Aguiar et al., 2007), which may suggest

the transmission of this serovar between dogs and horses in this region.

In addition to participation in the transmission chain, it is important to correlate seroreactivity with clinical signs, or changes in the animals' physiological patterns. In this sense, within the seropositive group (92 animals), 19 presented anemia, with hematocrit between 27-31%, 14 with borderline hematocrit between 32-33%, and 59 animals with normal hematocrit with values ranging from 34-52%. Although the animals' seropositivity for *Leptospira* does not indicate that the animal has an acute clinical picture of the disease, but that at some point in his life he had contact with the bacteria, in the most severe forms, anemia, hyperfibrinogenemia, hemorrhagic petechiae in the mucosa, jaundice, liver damage, hematuria, hemoglobinuria, leukocytosis with neutrophilia or lymphocytosis, azotemia, isotenuria, general depression, neurological signs, renal failure (mainly in foals) and death (Donahue, 1995; Lilenbaum, 1998; Pinheiro et al., 1985; Radostits et al., 2007). Based on this, the presence of anemia and acute-phase protein (fibrinogen) was evaluated to verify the health of the horses studied. Leptospirosis provokes clinical abnormalities that can lead to significant changes in hematological and other laboratory findings. Decreased erythrocyte count, low Hb concentration, PCV, and lymphocytes are common lab findings (Tonin et al., 2012). Leucocytosis, neutrophilia, eosinopenia, and lymphopenia, along with high serum bilirubin concentration have also been reported in equine leptospirosis (Pinna et al., 2010). Laboratory evaluation of animals affected by leptospirosis can reveal hyperfibrinogenemia. Concerning this parameter, among the animals positive for *Leptospira*, 15 had hyperfibrinogenemia, with 60% of the cases having the involvement of serovar Pyrogenes isolated or in coagglutination. Unlike our results, concerning anemia and fibrinogen levels, in a serological study in horses, Farias (2019) showed that there was no difference in the blood count, fibrinogen concentration, and biochemistry between seropositive and seronegative animals for *Leptospira*.

Conclusion

The results obtained in the study indicated a high frequency of seropositivity for *Leptospira* spp. in horses in the state of Amazonas, Brazil, indicating the existence of a high frequency of reaction to serogroups Icterohaemorrhagiae and Pyrogenes. With the increase in the equine herd in the region and, consequently, greater interaction between breeders and animals, the identification of reagents serogroups of

Leptospira spp. signals the possible existence of reservoirs of pathogenic strains for other animals and man.

Conflict of Interest

The authors declare they have no conflicts of interest.

Ethics Statement

The procedures involving animals were approved by the Ethics committee on the use of animals at the Federal Institute of Education, Science, and Technology of Amazonas, under the identification number CEUA.008.02.1417.2404/2020.

References

Aguiar DM, Cavalcante GT, Marvulo MFV, Silva JCR, Pinter A, Vasconcellos SA, Morais ZM, Labruna MB, Camargo LMA, Gennari SM. Fatores de risco associados à ocorrência de anticorpos anti-*Leptospira* spp. em cães do município de Monte Negro, Rondônia, Amazônia Ocidental Brasileira. *Arq Bras Med Vet Zootec.* 2007;59(1):70. <http://dx.doi.org/10.1590/S0102-09352007000100012>.

Alves JRA, Oliveira KDS, Costa DE, Fernandes LG, Higino SSS, Alves CJ, Santos CSAB, Azevedo SS. Caracterização epidemiológica da leptospirose em equinos do estado de Pernambuco, nordeste do Brasil. *Arq Inst Biol.* 2016;83:1-5. <http://dx.doi.org/10.1590/1808-1657001032014>.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Revisão do estudo do complexo do agronegócio do cavalo. Brasília: MAPA; 2016. 54 p.

Cole JR Jr, Sulzer CR, Pursell AR. Improved microtechnique for the leptospiral microscopic agglutination test. *Appl Microbiol.* 1973;25(6):976-80. <http://dx.doi.org/10.1128/AEM.25.6.976-980.1973>. PMID:4736794.

Donahue M. Equine leptospirosis. *Equine Dis Quarterl.* 1995;3(4):4-5.

Faria MT, Calderwood MS, Athanzio DA, McBride AJA, Hartskeerl RA, Pereira MM, Ko AI, Reis MG. Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. *Acta Trop.* 2008;108(1):1-5. <http://dx.doi.org/10.1016/j.actatropica.2008.07.005>. PMID:18721789.

Farias DK. Aspectos soroepidemiológicos e laboratoriais da leptospirose em equinos [tese]. Lages: Programa de Pós-graduação em Ciência Animal, Universidade do Estado de Santa Catarina; 2019.

Acknowledgements

We thank the Pro-Rector of Research, Graduate Studies, and Technological Innovation of the Federal Institute of Education, Science, and Technology of Amazonas (IFAM). We also thank the Institutional Program of Scientific Initiation Scholarships – PIBIC (PPGI/IFAM). Funding to conduct the study was provided by IFAM by Scientific Development and Technological Innovation Support Program (PADCIT) - Edict No. 001/2018/PR-PPGI/IFAM.

Favero ACM, Pinheiro SR, Vasconcellos AS, Morais ZM, Ferreira F, Ferreira Neto JS. Sorovares de leptospiroses predominantes em exames sorológicos de bubalinos, ovinos, caprinos, eqüinos, suínos e cães de diversos estados brasileiros. *Cienc Rur.* 2002;32:613-619. <https://doi.org/10.1590/S0103-84782002000400011>

Galton MM, Sulzer CR, Santa Rosa CA, Fields MJ. Application of a microtechnique to the agglutination test for leptospiral antibodies. *Appl Microbiol.* 1965;13(1):81-5. <http://dx.doi.org/10.1128/AEM.13.1.81-85.1965>. PMID:14264852.

Hamond C, Martins G, Lawson-Ferreira R, Medeiros MA, Lilenbaum W. The role of horses in the transmission of leptospirosis in an urban tropical area. *Epidemiol Infect.* 2013;141(1):33-5. <http://dx.doi.org/10.1017/S0950268812000416>. PMID:22417781.

Hashimoto VY, Gonçalves DD, Silva FG, Oliveira RC, Alves LA, Reichmann P, Muller EE, Freitas JC. Occurrence of antibodies against *Leptospira* spp. in horses of the urban area of Londrina, Paraná, Brazil. *Rev Inst Med Trop São Paulo.* 2007;49(5):327-30. <http://dx.doi.org/10.1590/S0036-46652007000500010>. PMID:18026641.

Hong CB, Donahue JM, Giles JRRC Jr, Petrites-Murphy MB, Poonacha KB, Roberts AW, Smith BJ, Tramontin RR, Tuttle PA, Swerczek TW. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 following seasons. *J Vet Diagn Invest.* 1993;5(4):560-6. <http://dx.doi.org/10.1177/104063879300500410>. PMID:8286455.

Hunter P, Herr S. Leptospirosis. In: Cortez JAW, Thomson GR, Tustin RC, editors. *Infectious diseases of Livestock*. Oxford: Oxford University; 1994. p. 997-1008.

- Jesus MS, Silva LA, Lima KMS, Fernandes OCC. Cases distribution of leptospirosis in City of Manaus, State of Amazonas, Brazil, 2000-2010. *Rev Soc Bras Med Trop.* 2012;45(6):713-6. <http://dx.doi.org/10.1590/S0037-86822012000600011>. PMID:23295874.
- Langoni H, Kuribara IY, Correa APFL, Ullmann LS, Sánchez GP, Lucheis SB. Anti-leptospirosis agglutinins in Brazilian capybaras (*Hydrochoerus hydrochaeris*). *J Venom Anim Toxins Incl Trop Dis.* 2016;22(1):4. <http://dx.doi.org/10.1186/s40409-016-0059-6>. PMID:26819575.
- Langoni H, Silva AV, Pezerico SB, Lima VY. Anti-Leptospire agglutinins in equine sera, from São Paulo, Goiás and Mato Grosso do Sul, Brazil, 1996-2001. *J Venom Anim Toxins.* 2004;10(3):207-18. <http://dx.doi.org/10.1590/S1678-91992004000300003>.
- Levett PN. Leptospirosis. *Clin Microbiol Rev.* 2001;14(2):296-326. <http://dx.doi.org/10.1128/CMR.14.2.296-326.2001>. PMID:11292640.
- Lilenbaum W. Leptospirosis on animal reproduction: IV. Serological findings in mares from six farms in Rio de Janeiro, Brazil (1993-1996). *Braz J Vet Res Anim Sci.* 1998;35(2):61-3. <http://dx.doi.org/10.1590/S1413-95961998000200002>.
- Magalhães DF, Silva JA, Moreira EC, Wilke VML, Haddad JPA, Meneses JNC. Prevalência de aglutininas anti-*Leptospira interrogans* em cães de Belo Horizonte, Minas Gerais, 2001 a 2002. *Arq Bras Med Vet Zootec.* 2006;58(2):167-74. <http://dx.doi.org/10.1590/S0102-09352006000200004>.
- Megid M, Ribeiro MG, Paes AC. Doenças infecciosas em animais de produção e de companhia. Rio de Janeiro: Roca; 2016.
- Miot HA. Tamanho da amostra em estudos clínicos e experimentais. *J Vasc Bras.* 2011;10(4):275-8. <http://dx.doi.org/10.1590/S1677-54492011000400001>.
- Monte LG, Jorge S, Xavier MA, Leal FMA, Amaral MG, Seixas FK, Dellagostin AO, Hartleben CP. Molecular characterization of virulent *Leptospira interrogans* serogroup Icterohaemorrhagiae isolated from *Cavia aperea*. *Acta Trop.* 2013;126(2):164-6. <http://dx.doi.org/10.1016/j.actatropica.2013.02.009>. PMID:23435256.
- Morais CCG, Kuroda RBS, Pinho APVB, Ywasaki F, Meneses AMC, Martins AV, Amaral JM Jr. Pesquisa de anticorpos para sorovares de *Leptospira interrogans* patogênicas em equídeos criados na ilha de Algodão, Estado do Pará. *Rev Cienc Agrária.* 2010;53(2):188-94. <http://dx.doi.org/10.4322/rca.2011.027>.
- Pinheiro HH, Silveira W, Oliveira VC. Pesquisas de aglutininas anti- leptospirosas em soros equinos abatidos no frigorífico Xavante – Araguari, MG. *Hora Vet.* 1985;5(27):42-4.
- Pinna M, Martins G, Freire I, Lilenbaum W. Seropositivity to *Leptospira interrogans* serovar Bratislava associated to reproductive problems without significant biochemical or hematological alterations in horses. *Cienc Rural.* 2010;40(10):2214-7. <http://dx.doi.org/10.1590/S0103-84782010005000178>.
- Pires JAS No, Hesse F, Oliveira MAM. Leptospirose equina: aspectos clínicos, tratamento, prevenção e levantamento sorológico. *Vet Foco.* 2005;2(2):165.
- Querino AMV, Delbem ÁCB, Oliveira RC, Silva FG, Müller EE, Freire RL, Freitas JC. Fatores de risco associados à leptospirose em cães do município de Londrina-PR. *Semina.* 2003;24(1):27-34. <http://dx.doi.org/10.5433/1679-0359.2003v24n1p27>.
- Radostits OM, Gay CC, Hinchcliff KW, Constable PD. *Veterinary medicine.* 10th ed. London: Elsevier Saunders; 2007. 2156 p.
- Ribeiro TMP. Soroepidemiologia da infecção por *Leptospira* spp. em equinos do município de Rorainópolis, estado de Roraima, Brasil [dissertação]. Goiânia: Escola de Veterinária e Zootecnia, Universidade Federal de Goiás; 2015.
- Santa Rosa CA, Sulzer CR, Yanaguaita RM, Silva AS. Leptospirosis in wildlife in Brazil: Isolation of serovars Canicola, Pyrogenes and Grippotyphosa. *Int J Zoonoses.* 1980;7:40-3.
- Schalm OW. Clinical significance of plasma protein concentration. *J Am Vet Med Assoc.* 1970;157(11):1672-3. PMID:5530370.
- Silva LA, Lima KMS, Fernandes OCC, Balassiano IT, Avelar KE, Jesus MS. Seroprevalence of and risk factors for leptospirosis in the City of Manaus, State of Amazonas, Brazil. *Rev Soc Bras Med Trop.* 2016;49(5):628-31. <http://dx.doi.org/10.1590/0037-8682-0115-2016>. PMID:27812660.
- Timoney JF, Kalimuthusamy N, Velineni S, Donahue JM, Artiushin SC, Fetting M. A unique genotype of *Leptospira interrogans* serovar Pomona type Kennewicki is

associated with equine abortion. *Vet Microbiol.* 2011;150(3-4):349-53. <http://dx.doi.org/10.1016/j.vetmic.2011.02.049>. PMID:21450416.

Tonin AA, Silva ASD, Azevedo MID, França RT, Paim FC, Schaefer PC, Martins JLR, Badke MRT, Lopes STDA. Hematologic and biochemical alterations in Wistar rats experimentally infected by *Leptospira interrogans*. *Comp Clin Pathol.* 2012;21(5):833-8. <http://dx.doi.org/10.1007/s00580-011-1186-7>.

World Health Organization – WHO. Leptospirosis [Internet]. Geneva: WHO; 2012 [cited 2020 July 20]. Available

from: http://www.wpro.who.int/mediacentre/factsheets/fs_13082012_leptospirosis/en/

Yan W, Faisal SM, Divers T, McDonough SP, Akey B, Chang YF. Experimental *Leptospira interrogans* sorovar Kennewicki infection of horses. *J Vet Intern Med.* 2010;24(4):912-7. <http://dx.doi.org/10.1111/j.1939-1676.2010.00507.x>. PMID:20649749.

Financial Support: Funding to conduct the study was provided by IFAM by Scientific Development and Technological Innovation Support Program (PADCIT) - Edict No. 001/2018/PR-PPGI/IFAM.