FULL ARTICLE

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Canine leptospirosis in asymptomatic populations from the Southwest Region of São Paulo State, Brazil

Leptospirose canina em uma população assintomática da região sudoeste do estado de São Paulo, Brasil

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ABSTRACT

Leptospirosis is an infectious disease that can affect animals and humans. Distributed worldwide, the disease is more prevalent in tropical regions due to socioenvironmental characteristics. Dogs can serve as sentinels for environmental contamination due to their frequent contact with humans. This study investigated the frequency of occurrence of canine leptospirosis in asymptomatic populations from the Southwest Region of the State of São Paulo, Brazil. Thus, blood samples collected from 572 asymptomatic dogs from the cities of Apiaí, Cananeia, Itapeva, and Itu were tested with a microscopic agglutination test (MAT). A total of 40.5% of animals in Apiaí reacted to *Leptospira* spp., 42.6% in Itapeva, 7.1% in Cananeia, and 5.1% in Itu. The data from the present study demonstrate that at least one animal from the municipalities of Itapeva, Apiaí, and Cananeia had a titer equal to or higher than 800, indicating that *Leptospira* is circulating in these municipalities and that the teams working on castration campaigns need to be educated on the correct use of personal protective equipment, especially when mechanically emptying the bladder of these animals. This study also suggests that castration campaigns can strategically monitor zoonotic diseases and assist in establishing preventive strategies for human and animal health.

Keywords: *Leptospira* spp. Leptospirosis. Seroprevalence. Dog. Sentinel.

RESUMO

A leptospirose é uma enfermidade infectocontagiosa que pode acometer os animais e o homem. Nos países tropicais e em desenvolvimento ocorrem 70% dos casos humanos, com mortalidade variando entre 10 a 70%. Os cães podem se tornar portadores assintomáticos por um longo período, podendo transmitir a *Leptospira* para humanos. Devido ao intenso convívio com o ser humano, os cães podem servir como sentinelas da contaminação ambiental. Esse trabalho investigou a frequência de ocorrência da leptospirose canina em populações assintomáticas da região sudoeste do estado de São Paulo. Para isso foram examinadas pela técnica de soroaglutinação microscópica (MAT), amostras de sangue provenientes de 572 cães assintomáticos dos municípios de Apiaí, Cananeia, Itapeva e Itu por amostragem de conveniência, oriundos de campanhas de castração. Em Apiaí, foram encontrados 40,5% dos animais reagentes para *Leptospira* spp.; em Itapeva, 42,6%; em Cananeia, 7,7% e em Itu, 5,1%. Os dados encontrados demonstram que, pelo menos, um animal dos municípios de Itapeva, Apiaí e Cananeia apresentaram título igual ou maior que 800, indicando a circulação da bactéria nessas localidades e que a equipe envolvida nas campanhas de castração precisam ser alertadas sobre o correto uso de equipamento de proteção individual, principalmente no esvaziamento mecânico da bexiga antes do procedimento cirúrgico. O estudo também sugere que as campanhas de castração podem ser estratégicas no monitoramento de doenças zoonóticas e poderiam auxiliar no estabelecimento de ações preventivas para a saúde humana e animal.

Palavras-chave: *Leptospira* spp. Leptospirose. Soroprevalência. Cães. Sentinela.

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Introduction

Leptospirosis is a disease that is distributed worldwide and can affect several species of mammals. Tropical and developing countries account for approximately 70% of global human cases (Costa et al., 2015), with the mortality ranging between 10 and 70% (McBride et al., 2015). This pattern is attributed to socioeconomic, climatic, environmental factors, and the abundance of reservoirs that favor the survival of *Leptospira* spp., increasing the risk of transmission to humans and animals (Ganoza et al., 2006; Reis et al., 2008).

Human infection generally occurs through contact with water or soil contaminated with the bacteria, especially during floods, in which *Rattus norvegicus* are expelled from burrows and contaminate the water. As a consequence, humans who have prolonged contact with floods are infected. *Rattus norvegicus* is the maintenance host for the Copenhageni and Icterohaemorrhagiae serovars (Sakata et al., 1992). For this reason, most human infections in Brazil and worldwide are attributed to these serovars, both of which belong to the serogroup Icterohaemorrhagiae (Acha & Szyfres, 1989; Guernier et al., 2016; Pagès et al., 2016), but there are reports of human infection due to recreational contact with water (Abb, 2002; Grobusch et al., 2003; Pagès et al., 2016). Humans can also become infected with other serovars, such as Canicola (Sakata et al., 1992).

Dogs are accidental hosts for most of the described serovars, except for the Canicola serovar, for which dogs are considered a maintenance host (Schuller et al., 2015). Transmission to dogs occurs mainly through contact with soil and/or water contaminated with urine or through direct contact of susceptible individuals with infected dogs (Picardeau, 2013). Once infected, dogs can become chronic carriers, excreting leptospires in urine, putting other animals, and humans at risk (Miotto et al., 2018a).

Dogs are good sentinels to detect the presence of leptospires in an environment with high population density because they are more exposed to risk factors and environmental contamination (Jorge et al., 2017). Also, dogs are predators that can ingest prey (rats) that may be infected and thus probably have more contact with *Leptospira*. Surveillance in dogs is also important, as they can alert local health authorities of the introduction of a new serovar in each population or geographic area (Bier et al., 2013; Blazius et al., 2005).

For this reason, the objective of this work was to investigate the frequency of the presence of anti-*Leptospira* agglutinins in asymptomatic canine populations from the Southwest Region of the State of São Paulo, Brazil.

Materials and Methods

Characterization of study samples

Serum samples collected from 572 healthy dogs were analyzed for *Leptospira* antibodies. Conveniently available samples were collected from animals treated during neutering campaigns in Itapeva (202), Apiaí (41), and Cananeia (52) municipalities and from presurgical evaluations for castration in Itu (277). Samples were collected between 2014 and 2016 in municipalities, and at the time of the procedure, all animals were healthy. Samples were collected in July in the cities of Itapeva, Apiaí, and Cananeia, and in Itu, the collection took place between March and November. The dogs' vaccination history was unknown.

The study was performed as a part of the Rondon® SP Project and the university extension of Santo Amaro University and was approved by the Ethics Commission of Animal Use (CEUA) of the Santo Amaro University (UNISA) under protocol number CEUA 35/2012, 19/2014, 28.1/2016.

Characterization of the study area

The municipalities of Itu, Itapeva, and Apiaí are classified as subtropical and temperate (Cwa) regions, with hot summers and summer rains, by the Koppen-Geiger scale and Cananéia county (Af) has a hot and humid climate without a defined dry season (Dubreuil et al., 2018). Cananeia, Itu and Apiaí municipalities contain the Atlantic Forest as a biome, and Itapeva contains a savanna

biome (Cerrado) (Instituto Brasileiro de Geografia e Estatística, 2020)

Itu is a city with an estimated population and population density of 241.01 inhabitants/km. A total of 97% of households have adequate sanitation, 93% of urban households are on public roads with afforestation, and 52% of urban households are on public roads with adequate urbanization (Instituto Brasileiro de Geografia e Estatística, 2020). Apiaí is a municipality in the state of São Paulo, located in the Vale do Ribeira region, Brazil. It has a population of approximately 25,191 and a population density of 25.85 inhabitants/km^2. The area of the territorial unit in 2017 was 974.322 km^2. In the municipality, 54.4% of houses have basic sanitation, 32% are urbanized, and 72.3% are on public roads with afforestation (Instituto Brasileiro de Geografia e Estatística, 2020).

Itapeva is a municipality in the state of São Paulo that is in the southwest region of the state of São Paulo and has a territory of 1,826,258 km^2. Approximately 84.4% of the houses have basic sanitation, 24.7% of the public roads are urbanized, and 66.8% have afforestation (Instituto Brasileiro de Geografia e Estatística, 2020). Cananeia is a municipality in the state of São Paulo, located in the far south of the São Paulo coast in Brazil. It had an estimated population in 2018 of 12,539 people, with a demographic density of 9.86 inhabitants/km^2. Its territorial area in 2017 was 1,237,357 km^2; in 2010, approximately 76% of houses had basic sanitation, with 4.9% of public roads were urbanized and 43.4% were afforested (Instituto Brasileiro de Geografia e Estatística, 2020).

Microscopic Agglutination Technique (MAT)

For anti-*Leptospira* spp., the microscopic agglutination test (MAT) was used, applying a collection with 24 serological variants of live antigens among reference samples and native strains from Brazil (Fávero et al., 2002).

Serum screening was performed with 24 antigens (Castellonis (Ballum, *L. borgpetersenii)*, Hardjo-prajitno (Sejroe, *L. interrogans*), Hardjobovis (Sejroe, *L. borgpetersenii*), Javanica (Javanica, *L. borgpetersenii*), Tarassovi (Tarassovi, *L. borgpetersenii*), Whitcombi (Whitcombi, *L. borgpetersenii*), Australis (Australis, *L. interrogans*), Autumnalis (Autumnalis, *L. interrogans*), Bataviae (Bataviae, *L. interrogans*), Bratislava (Australis, *L. interrogans*), Canicola (Canicola, *L. interrogans*), Copenhageni (Icterohaemorrhagiae, *L. interrogans*), Hebdomadis (Hebdomadis, *L. interrogans*), Pomona (Pomona, *L. interrogans*), Pomona (GR6) (Pomona, *L. interrogans*), Pyrogenes (Pyrogenes, *L. interrogans*), Icterohaemorrhagiae (Icterohaemorrhagiae, *L. interrogans*), Sentot (Djasiman, *L. interrogans*), Grippotyphosa (Grippotyphosa, *L. kirschneri*), Butembo (Autumnalis, *L. kirschneri*), Cynopteri (Cynopteri, *L. kirschneri*), Panama (Panama, *L. noguchi*), Shermani (Shermani, *L. santarosai*), and Guaricura (Sejroe, *L. santarosai*)).

Serum samples with a titer equal to 100 were subjected to titration with reagent antigens using a serial dilution with ratio 2. The final titer was the reciprocal of the highest dilution that generated 50% of agglutination. The most likely serovar was the one with the highest titration at the highest frequency. Animals with serovars with equal titers were considered seroreactive for *Leptospira* spp., but serovar identifications were disregarded (Fávero et al., 2002). For analysis, animals with a titer equal to or above 800 were not considered to be vaccinated (Reagan & Sykes, 2019) or have an active infection.

Spatial localization

To perform georeferencing, geographic coordinates were obtained from the address provided by the dog's owner when filling out the consent form. The longitude and latitude were obtained using the global positioning system (GPS) and map ccordinates.net, a service of Vivid Planet Software GmbH.

The QGIS version 2.18 program was used to plot results obtained from animals (reactive and nonreactive) in each city analyzed.

Results

Sera samples from 572 dogs were analyzed for *Leptospira* spp. One hundred twenty-one (21.15%) were reagents for at least one serological variant investigated. The dogs were healthy at sample collection.

In Apiaí, 40.5% (17/41) of the animals reacted to *Leptospira* spp. The most frequent serovar was Canicola, with a mode of 1,600, followed by Castellonis (Table 1). The most likely serovar was Canicola (14/16). Seven animals had a

Table 1 – Absolute, relative frequency, mode, and variation of serovar titers found in seroreagent dogs regardless of whether it is single or coagglutination, in the city of Apiaí, SP, 2014-2016

Serovar	Absolute frequency	Relative frequency (%)	Titer (variation)	Mode
Bratislava	1	3.22	100	100
Castellonis	9	29.04	100-400	400
Canicola	14	45.16	100-2500	1600
Copenhageni	1	3.23	800	800
Pyrogenes	6	19.35	100-400	200
Total	31	100		

titer equal to or greater than 800 for the serovar Canicola and one for the serovar Copenhageni.

In the municipality of Itapeva, 42.6% (86/202) of the animals were seroreactive for *Leptospira* spp., with the predominance of the Copenhageni serovar (29.07%). The titers ranged from 100 to 12,800, followed by the Icterohaemorrhagiae serovar (20.54%) (Table 2). The most common serovar was Copenhageni (48/73), followed by Cynopteri (7/73). Thirty-three animals had titers equal to or greater than 800, 79% for the serovar Copenhageni, 9% for Canicola, 6% for Cynopteri, and 3% for the serovars Icterohaemorrhagiae and Castellonis.

The municipalities of Cananeia and Itu had a lower occurrence of animal seroreactivity for *Leptospira* spp., 7.7% (4/52), and 5.05% (14/277), respectively (Tables 3 and 4). In Itu, an animal presented an 800 titer for Icterohaemorrhagiae and in Cananeia, one for Copenhageni.

The spatial distribution of seroreactive and nonreactive animals in the municipalities of Itu, Itapeva, and Cananeia by the municipality can be seen in Figure 1.

Table 2 – Absolute, relative frequency, mode, and variation of serovar titers found in seroreagent dogs, regardless of being single or coagglutination, in the municipality of Itapeva, SP, 2014-2016

Serovar	Absolute frequency	Relative frequency (%)	Titer (variation)	Mode
Australis	18	6.98	100-800	200
Bratislava	1	0.39	100	100
Autumnalis	6	2.32	200-800	200
Butembo	1	0.39	400	400
Castellonis	9	3.49	100-1600	200 and 400
Canicola	35	13.56	100-25600	200
Cynopteri	37	14.34	100-6400	200
Hebdomadis	2	0.77	100-1600	100 and 1600
Copenhageni	75	29.07	100-12800	400
Icterohaemorrhagiae	53	20.54	100-1600	200
Panama	1	0.39	400	400
Pomona	2	0.77	200	200
Pyrogenes	13	5.04	100-800	100 e 200
Wolffi	3	1.16	100-400	100, 200 and 400
Andamane	2	0.77	100-400	100 and 400
Total	258	100		

Table 3 – Absolute, relative frequency, mode, and variation of serovar titers found in seroreagent dogs, regardless of being single or coagglutination, in the municipality of Cananeia, SP, 2014-2016

Serovar	Absolute frequency	Relative frequency (%)	Titer (variation)
Canicola	2	33.33	100-400
Hebdomadis	1	16.66	200
Copenhageni	1	16.66	800
Icterohaemorrhagiae	1	16.66	100
Cynopteri	1	16.66	100
Total	6	100	

Table 4 – Absolute, relative frequency, mode, and variation of serovar titers found in seroreagent dogs, regardless of being single or coagglutination, in the municipality of Itu, SP, 2014-2016

Serovar	Absolute frequency	Relative frequency (%)	Titer (variation)	Mode
Australis	1	3.85	100	100
Bratislava	1	3.85	100	100
Autumnalis	1	3.85	100	100
Canicola	5	15.38	100 and 200	100 and 200
Cynopteri	2	7.69	100	100
Grippotyphosa	3	11.54	200	200

Discussion

In Brazil, the prevalence of canine leptospirosis varies greatly depending on the region, the sample used and the presence or absence of clinical manifestations (Favero et al., 2002; Fonzar & Langoni, 2012; Jorge et al., 2017; Latosinski et al., 2018; Miotto et al., 2018b; Morikawa et al., 2015; Oliveira, 2010; Rodrigues et al., 2007; Vasconcellos, 1997). In a systematic review by Pinto et al. (2017), the most prevalent serogroups found in dogs were Canicola and Icterohaemorrhagiae, the same ones found in the cities studied, showing direct or indirect contact with the sources of infection.

Of the 572 animals examined in the four municipalities, 121 were reagents for *Leptospira* spp. The municipality of Itapeva (42.6%) presented the highest frequency of reactive animals, followed by Apiaí (40.5%) and, finally, Cananeia (7.79%) and Itu (2.4%). The four cities have different biomes and population densities, and these characteristics inherent to each municipality, together with the management that these animals are subjected to (domiciliation, vaccination, origin, and age), may influence the distribution of the agent (Bier et al., 2013).

Of the 109 animals positive for *Leptospira* spp., 71 (65.14%) had a titer above 800, 65.14% came from animals in the municipality of Itapeva, 15.5% from Apiaí, and 1.4% from Cananeia. Despite the similar occurrence of animal seroreactivity for *Leptospira* spp. in the municipalities of Apiaí (40.5%) and Itapeva (46.6%), this result should be analyzed with caution.

In Apiaí county, blood samples came from dogs from a single shelter, and the most common serological serovar

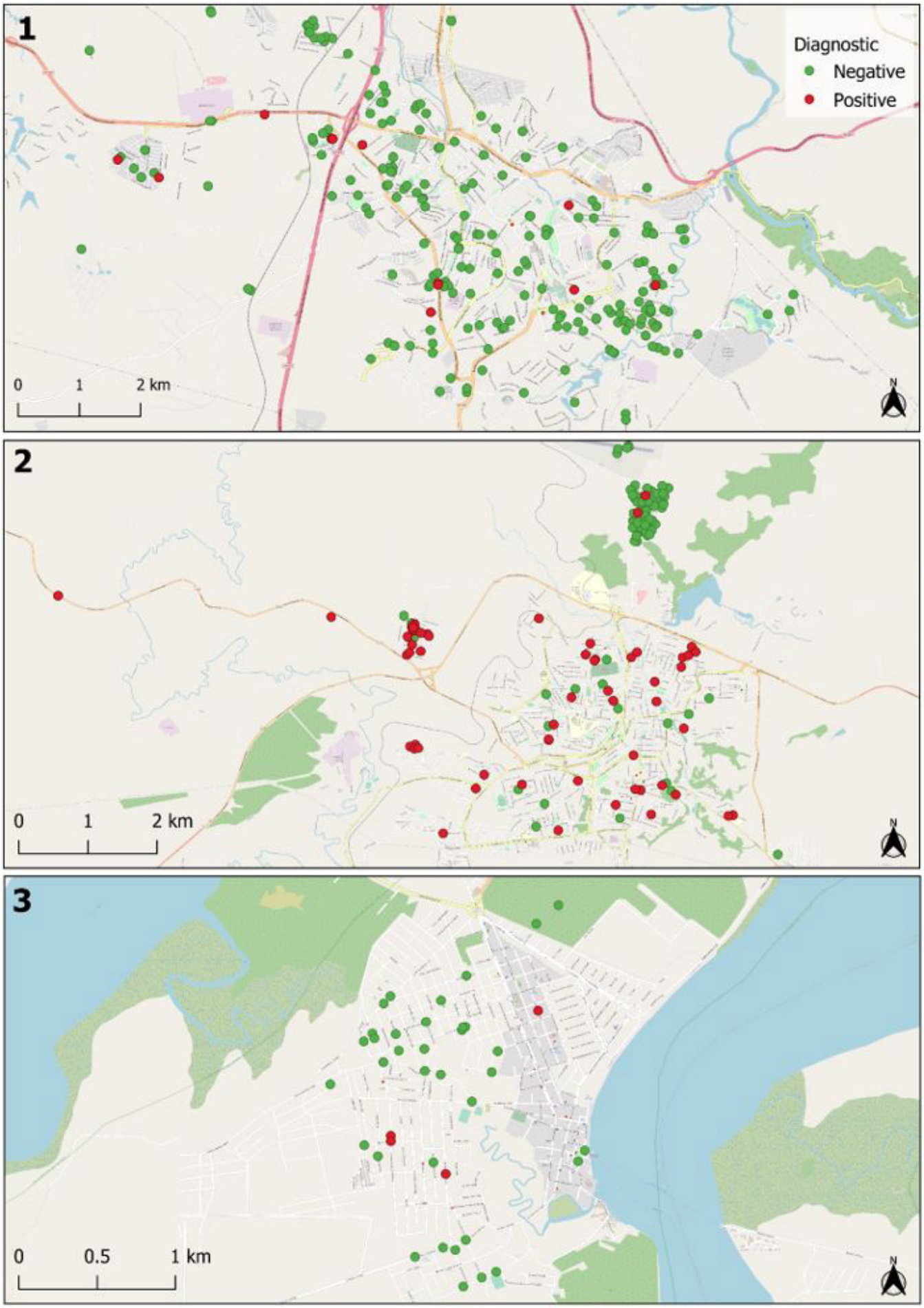

Figure 1 – Distribution of seroreagent (red) and non-reactive (green) dogs for leptospirosis in the municipality of Itu (1), Itapeva (2), and Cananeia (3) municipality of São Paulo State, 2014-2016.

was Canicola. The finding that the Canicola serogroup was the most common and animals had titers greater than 800 indicates that the Canicola serogroup is being maintained in the population (Table 2). Dogs without symptoms of leptospirosis have already been reported, showing the risk of transmission of the agent to potential adopters and employees (Adler & Moctezuma, 2010; Miotto et al., 2018b). Measures such as the use of personal protective equipment (EPIS), quarantine, vaccination, and treatment of dogs are important to reduce the circulation of the agent among animals in the shelter. Shelter dog treatment to eliminate the carrier status of dogs and, consequently, minimize possible transmission to humans was supported by Sakata et al. (1992) and Damião (2015).

In Itapeva, the dispersion of seroreactive animals was observed in 29 districts, the most common serogroup being Copenhageni, whose maintenance host is the rat (*Rattus norvegicus*). It is noteworthy that 26 animals had titers greater than or equal to 800. In the map of the municipality (Figure 1), there was a cluster of samples from the Vila Santa Maria and Bela Vista neighborhoods. This agglomeration occurred because the castration campaigns were carried out in both neighborhoods.

The Vila Santa Maria district (Itapeva) was in front of a landfill that was on the other side of the highway, and many families worked in recycling at the time of sample collection. Currently, the dump has been deactivated. Of the 87 sera analyzed in Vila Santa Maria, only two were reactive for *Leptospira* spp., one for the serological variant Autumnalis, with a titer of 100, and another for Wolffi, with a titer of 400. Autumnalis has already been described by other authors (Aguiar et al., 2007; Batista et al., 2004; Castro et al., 2011; Pinto et al., 2017), and together with the serovariant Wolffi, indicates that the environment is shared by different animal species.

In the Bela Vista district, the Copenhageni serological variant had the highest occurrence (12/17) among seroreactive animals, indicating that measures of deratization, anti-ratification, and health education are necessary to reduce environmental contamination and decrease the likelihood of human infection. Most human infections in Brazil and the world occur through the serovars Copenhageni and Icterohaemorrhagiae, both belonging to the serogroup Icterohaemorrhagiae. (Acha & Szyfres, 1989; Guernier et al., 2016; Pagès et al., 2016). Six out of 12 dogs seroreactive to the Copenhageni serological variant had a titer equal to or greater than 800, which, according to the criterion used, is characteristic of active infection.

In Cananeia, 7.7% (4/52) of the animals were reactive to *Leptospira* spp. (Table 3), and only one animal had a titer equal to 800 for serovar Copenhageni. The others presented titers for Hebdomadis (one animal with a titer of 200), another had a titer of 100 for Canicola, one had a titer of 100 for Cynopteri, and the fourth presented coagglutination for the serological variants Canicola, Copenhageni, and Icterohaemorrhagiae. Animals that had titers for a single serovar of Canicola or Hebdomadis lived on the same street in the neighborhood of Acaraú, suggesting contact with different hosts, probably due to greater contact between forest areas and the municipality (Carvalho, 2016). The other animals were from the Rocio and Carijo neighborhoods (Figure 1). It is interesting to note that the presence of the spotted fever agent was demonstrated (Carvalho, 2016) in these same neighborhoods, showing that both the human and canine populations are susceptible to various infectious agents, highlighting the importance of using dogs from sterilization campaigns as sentinel animals.

In Itu, the 14 samples positive for *Leptospira* spp. were found in nine different neighborhoods (Figure 1), with Canicola the most common serological variant. Only one sample presented a titer of 800 for the Canicola serovar. This was the city with the largest number of samples. They came from screening performed before surgical sterilization carried out by the Zoonosis Control Center. In the cities of Apiaí, Itapeva, and Cananeia, samples were collected during campaigns in partnership with the municipality and university extension.

Considering that samples were obtained in July in Apiaí, Cananeia, and Itapeva, it can be seen that even during drier periods of the year, great environmental contamination and infection by *Leptospira* cannot be ruled out. And, as can be seen mainly in the municipality of Itapeva, this should alert the agents that promote human and animal health.

Given the spread of *Leptospira* spp. infection in animals treated by surgical sterilization programs for the population control of dogs and cats, biosafety measures, such as the use of personal protective equipment to minimize the transmission of the agent through the mucous membranes, should be adopted by the castration team. The monitoring, quarantine, diagnosis, vaccination, and treatment of asymptomatic shelter dogs should be instituted as routine measures to minimize infection risks for employees, volunteers, and adopters.

Conclusions

The data from the present study demonstrated that at least one animal from the municipalities of Itapeva, Apiaí, and

Cananeia had a titer equal to or greater than 800, indicating that *Leptospira* spp. is circulating in these municipalities and that the teams working in the neutering campaigns need to be educated on the correct use of personal protective equipment, especially when mechanically emptying the bladder of these animals before a surgical procedure.

This study also suggests that castration campaigns can strategically monitor zoonotic diseases and assist in establishing preventive strategies for human and animal health.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

This study was approved by the Ethics Committee on the Universidade Santo Amaro - UNISA (CEUA 35/2012, 19/2014, 28.1/2016).

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References

Abb J. Acute leptospirosis in a triathlete. Wilderness Environ Med. 2002;13(1):45-7. http://dx.doi.org/10.1580/1080-6032(2002)013[0045:ALIAT]2.0.CO;2. PMid:11929061.

Acha PN, Szyfres B. Zoonosis y enfermidades transmisibles comunes al hombre y a los animales. 2nd ed. Washington: Organizacion Panamericana de la Salud; 1989. (no. 503).

Adler B, Moctezuma AP. Leptospirosis. In: Gyles CL, Prescott JF, Songer JG, Thoen CO. Pathogenesis of bacterial infections in animals. 4th ed. Ames: Blackwell Publishing; 2010. p. 527-47.

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):70-6. http://dx.doi.org/10.1590/S0102-09352007000100012.

Batista CSA, Azevedo SS, Alves CJ, Vasconcellos AS, Morais M, Clementino IJ, Lima FS, Araújo JO No. Soroprevalência de leptospirose em cães errantes da cidade de Patos, Estado da Paraíba, Brasil. Braz J Vet Res Anim Sci. 2004;41(2):131-6. http://dx.doi.org/10.1590/S1413-95962004000200009.

Bier D, Shimakura SE, Morikawa VM, Ullmann LS, Kikuti M, Langoni H, Biondo AW, Molento MB. Análise espacial do risco de leptospirose canina na Vila Pantanal, Curitiba, Paraná. Pesq Vet Bras. 2013;33(1):74-9. http://dx.doi.org/10.1590/S0100-736X2013000100013.

Blazius RD, Romão PRT, Blazius EMCG, Silva OS. Ocorrência de cães errantes soropositivos para *Leptospira* spp. na cidade de Itapema, Santa Catarina, Brasil. Cad Saude Publica. 2005;21(6):1952-6. http://dx.doi.org/10.1590/S0102-311X2005000600046. PMid:16410883.

Carvalho AS. Inquérito Sorológico de anticorpos anti-rickettsia em cães do município de Cananeia e Itapeva, estado de São Paulo, Brasil [dissertação]. Santo Amaro: Universidade Santo Amaro; 2016.

Castro JR, Salaberry SRS, Souza MA, Lima-Ribeiro AMC. Sorovares de *Leptospira* spp. predominantes em exames sorológicos de caninos e humanos no município de Uberlândia, Estado de Minas Gerais. Rev Soc Bras Med Trop. 2011;44(2):217-22. http://dx.doi.org/10.1590/S0037-86822011005000012. PMid:21468478.

Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, Stein C, Abela-Ridder B, Ko AI. Global morbidity and mortality of Leptospirosis: a systematic review. PLoS Negl Trop Dis. 2015;9(9):e0003898. http://dx.doi.org/10.1371/journal.pntd.0003898. PMid:26379143.

Damião AO. Caracterização das leptospiras isoladas dos pacientes atendidos no Hospital Couto Maia [dissertação]. Salvador: Universidade Federal da Bahia; 2015.

Dubreuil V, Fante KP, Planchon O, Sant'Anna JL No. Os tipos de climas anuais no Brasil: uma aplicação da classificação de Köppen de 1961 a 2015. Confins. 2018;(37). https://doi.org/10.4000/confins.15738.

Favero ACM, Pinheiro SR, Vasconcellos SA, Morais ZM, Ferreira F, Ferreira JS No. Sorovares de leptospiras predominantes em exames sorológicos de bubalinos, ovinos, caprinos, eqüinos, suínos e cães de diversos estados brasileiros. Cienc Rural. 2002;32(4):613-9. http://dx.doi.org/10.1590/S0103-84782002000400011.

Fonzar UJV, Langoni H. Geographic analysis on the occurrence of human and canine leptospirosis in the City of Maringá, State of Paraná, Brazil. Rev Soc Bras Med Trop. 2012;45(1):100-5. http://dx.doi.org/10.1590/S0037-86822012000100019. PMid:22370837.

Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, Segura ER, Gilman RH, Gotuzzo E, Vinetz JM. Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira.* PLoS Med. 2006;3(8):e308. http://dx.doi.org/10.1371/journal.pmed.0030308. PMid:16933963.

Grobusch MP, Bollmann R, Schönberg A, Slevogt H, Garcia V, Teichmann D, Jelinek T, Flick H, Bergmann F, Rosseau S, Temmesfeld-Wollbrück B, Suttorp N. Leptospirosis in travelers returning from the Dominican Republic. J Travel Med. 2003;10(1):55-8. http://dx.doi.org/10.2310/7060.2003.30683. PMid:12729515.

Guernier V, Lagadec E, Cordonin C, Le Minter G, Gomard Y, Pagès F, Jaffar-Bandjee MC, Michault A, Tortosa P, Dellagi K. Human leptospirosis on reunion island, indian ocean: are rodents the (only) ones to blame? PLoS Negl Trop Dis. 2016;10(6):e0004733. http://dx.doi.org/10.1371/journal.pntd.0004733. PMid:27294677.

Instituto Brasileiro de Geografia e Estatística – IBGE [Internet]. 2020 [cited 2020 Sep 4]. Available from: https://cidades.ibge.gov.br/

Jorge S, Schuch RA, Oliveira NR, Cunha CEP, Gomes CK, Oliveira TL, Rizzi C, Qadan AF, Pacce VD, Coelho Recuero AL, Soares Brod C, Dellagostin OA. Human and animal leptospirosis in Southern Brazil: a five-year retrospective study. Travel Med Infect Dis. 2017;18:46-52. http://dx.doi.org/10.1016/j.tmaid.2017.07.010.

Latosinski GS, Fornazari F, Babboni SD, Caffaro K, Paes AC, Langoni H. Serological and molecular detection of *Leptospira* spp in dogs. Rev Soc Bras Med Trop. 2018;51(3):364-7. http://dx.doi.org/10.1590/0037-8682-0276-2017. PMid:29972570.

McBride AJ, Athanazio DA, Reis MG, Ko AI. Leptospirosis. Curr Opin Infect Dis. 2015;18(5):376-86. http://dx.doi.org/10.1097/01.qco.0000178824.05715.2c. PMid:16148523.

Miotto BA, Hora AS, Taniwaki SA, Brandão PE, Heinemann MB, Hagiwara MK. Development and validation of a modified TaqMan based real-time PCR assay targeting the lipl32 gene for detection of pathogenic *Leptospira* in canine urine samples. Braz J Microbiol. 2018a;49(3):584-90. http://dx.doi.org/10.1016/j.bjm.2017.09.004. PMid:29233483.

Miotto BA, Guilloux AGA, Tozzi BF, Moreno LZ, da Hora AS, Dias RA, Heinemann MB, Moreno AM, Souza AF Fo, Lilenbaum W, Hagiwara MK. Prospective study of canine leptospirosis in shelter and stray dog populations: identification of chronic carriers and different *Leptospira* species infecting dogs. PLoS One. 2018b;13(7):e0200384. http://dx.doi.org/10.1371/journal.pone.0200384. PMid:29995963.

Morikawa VM, Bier D, Pellizzaro M, Ullmann LS, Paploski IAD, Kikuti M, Langoni H, Biondo AW, Molento MB. Seroprevalence and seroincidence of *Leptospira* infection in dogs during a one-year period in an endemic urban area in Southern Brazil. Rev Soc Bras Med Trop. 2015;48(1):50-5. http://dx.doi.org/10.1590/0037-8682-0213-2014.

Oliveira ST. Leptospirose canina: Dados clínicos, laboratoriais e terapêuticos em cães naturalmente infectados [tese]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2010.

Pagès F, Larrieu S, Simoes J, Lenabat P, Kurtkowiak B, Guernier V, Le Minter G, Lagadec E, Gomard Y, Michault A, Jaffar-Bandjee MC, Dellagi K, Picardeau M, Tortosa P, Filleul L. Investigation of a leptospirosis outbreak in triathlon participants, Réunion Island, 2013. Epidemiol Infect. 2016;144(3):661-9. http://dx.doi.org/10.1017/S0950268815001740. PMid:26211921.

Picardeau M. Diagnosis and epidemiology of leptospirosis. Med Mal Infect. 2013;43(1):1-9. http://dx.doi.org/10.1016/j.medmal.2012.11.005. PMid:23337900.

Pinto PS, Libonati H, Lilenbaum W. A systematic review of leptospirosis on dogs, pigs, and horses in Latin America. Trop Anim Health Prod. 2017;49(2):231-8. http://dx.doi.org/10.1007/s11250-016-1201-8. PMid:27909915.

Reagan KL, Sykes JE. Diagnosis of Canine Leptospirosis. Vet Clin North Am Small Anim Pract. 2019;49(4):719-31. http://dx.doi.org/10.1016/j.cvsm.2019.02.008. PMid:30961998.

Reis RB, Ribeiro GS, Felzemburgh RDM, Santana FS, Mohr S, Melendez AX, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI. Impact of environment and social gradient on *Leptospira* infection in urban slums. PLoS Negl Trop Dis. 2008;2(4):e228. http://dx.doi.org/10.1371/journal.pntd.0000228. PMid:18431445.

Rodrigues AMA, Vasconcellos AS, Moraes ZM, Hagiwara MK. Isolamento de *Leptospira* spp. de cães com diagnóstico clínico de leptospirose em São Paulo (Brasil). Acta Sci Vet. 2007;35:s705-6.

Sakata EE, Yasuda PH, Romero EC, Silva MV, Lomar AV. Sorovares de *Leptospira interrogans* isolados de casos de leptospirose humana em São Paulo, Brasil. Rev Inst Med Trop São Paulo. 1992;34(3):217-21. http://dx.doi.org/10.1590/S0036-46651992000300006. PMid:1342073.

Schuller S, Francey T, Hartmann K, Hugonnard M, Kohn B, Nally JE, Sykes J. European consensus statement on leptospirosis in dogs and cats. J Small Anim Pract. 2015;56(3):159-79. http://dx.doi.org/10.1111/jsap.12328. PMid:25754092.

Vasconcellos SA. Leptospirose. Biologico. 1997;59:29-32.

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