Evidence of exposure to *Leptospira* spp. in dogs at a zoonosis control center in Brazil

Evidências de exposição à Leptospira *spp. em cães de um centro de controle de zoonoses no Brasil*

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Abstract

Evidence of exposure to *Leptospira* spp. in dogs housed in the kennel of the Zoonosis Control Center of Belém, Pará, Brazil, was investigated. Whole blood and serum samples from 145 dogs were investigated using the polymerase chain reaction (PCR) and microscopic agglutination test (MAT), respectively. A total of 64.14% of the dogs were found to be seropositive for *Leptospira* spp., with the most frequent serogroup being Djasiman (39.73%). However, PCR results revealed that all of the dogs were negative for *Leptospira* spp. DNA. Although the results of the study suggest the animals did not currently have leptospires in blood, they only show circulating anti-*Leptospira* spp. antibodies, implying prior contact with the bacteria.

Keywords: Dog. Animal shelter. Anti-*Leptospira* spp. antibodies. MAT. PCR.

Resumo

Evidências de exposição a Leptospira *spp. em cães abrigados no canil do Centro de Controle de Zoonoses de Belém, Pará, Brasil, foram investigadas. Amostras*

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Received: Jun 06 2020 | **Approved:** Aug 04 2020

Rev. Acad. Ciênc. Anim. 2020;18:e18301
DOI: 10.7213/2596-2868.2020.18301
ISSN: 2596-2868

de sangue total e soro de 145 cães foram investigadas utilizando a reação em cadeia da polimerase (PCR) e o teste de aglutinação microscópica (MAT), respectivamente. Um total de 64,14% dos cães foram sororreagentes para Leptospira *spp., sendo mais frequente o sorogrupo Djasiman (39,73%). No entanto, os resultados da PCR revelaram que todos os cães eram negativos para DNA de* Leptospira *spp. Embora os resultados do estudo sugiram que os animais não tinham atualmente leptospiras no sangue, eles evidenciam apenas anticorpos anti-*Leptospira *spp. circulantes, implicando contato prévio com a bactéria.*

Palavras-chave: *Cão. Abrigo de animais. Anticorpos anti-*Leptospira *spp. MAT. PCR.*

Introduction

Currently, zoonosis control centers aim to control the population of stray or abandoned dogs by collecting, recovering, and surgically sterilizing the dogs before making them available for responsible adoption (Miotto et al., 2018; Arruda et al., 2019).

Most of these institutions act as transit locations, with the dogs being sheltered temporarily and then adopted within a short timeframe. However, not all dogs have the privilege of being adopted, and the number of animals housed in these locations for a long time or until the end of their life is significant (Arruda et al., 2019).

These places are often lack of financial, technical, and human resources For this reason, effective health strategies are not regularly implemented (Miotto et al., 2018). This contributes to an increased risk of exposure of dogs to *Leptospira* spp. (Paz et al., 2015; Miotto et al., 2018).

Some studies have already shown evidence of exposure to *Leptospira* spp. in dogs from zoonosis control centers. Among them are the serological surveys of Oliveira et al. (2012), Paz et al. (2015) and Silva et al. (2017), which reported seroprevalences ranging from 17.41 to 53.8%, and the molecular genetic studies by Meira et al. (2011) and Oliveira et al. (2012) reporting 4% and 7.7% positive results for *Leptospira* spp. in blood samples, respectively.

Dogs can act as sentinels, i.e., they can signal the presence of *Leptospira* spp. in a given environment through evidence of exposure to the bacteria (Castro et al., 2011). Thus, they can even serve as a warning for people who may also be vulnerable to the risk of exposure (Azócar-Aedo et al., 2018).

In this context, further investigations are of great importance to improve the health of these dogs and curtail the spread of *Leptospira* spp. Thus, in this study, we investigated the evidence of exposure to *Leptospira* spp. in dogs housed in the kennel of the Zoonosis Control Center of Belém (ZCC), Pará, Brazil.

Material and methods

To calculate the sample size, the mean number of adult dogs collected by the ZCC in 2016 and 2017 (n = 230) was considered as the target population. The Statcalc tool of the EpiInfoTM7 software (CDC - Atlanta, Georgia, USA) was used. We took into consideration an expected frequency of 50%, acceptable error of 5%, and confidence interval of 95% to obtain a sample size of 144 animals. However, to prevent sample loss, 145 animals were analyzed. Convenience sampling was used in the collection of blood samples.

This study was conducted in accordance with ethical terms and conditions, and was approved by the Ethics Committee for the Use of Animals of the Federal University of Pará, Belém, Brazil (approval number: 4553100718, and publishing date: February 07, 2019).

The study was conducted at a kennel of the ZCC, Belém, Pará state, Brazil (118'58.2''S and 48°27'17.7''W). This is a government institution that among its other functions collects stray or abandoned animals that can potentially transmit zoonoses, cause traffic accidents, and attack people in different neighborhoods and districts of the municipality. Animals that are collected are dewormed, vaccinated against rabies, castrated, and if they are in good health conditions, are made available for responsible adoption by the community. All the dogs in this study were adults of unknown age, and any history of vaccination against *Leptospira* spp. was unknown.

From August to December 2018, blood samples were obtained by puncturing the cephalic vein with

a 25.0 × 0.7 mm needle and a 5 mL syringe. The samples were stored in tubes containing gel/clot activator and tubes containing K3-EDTA to obtain serum and whole blood, respectively.

Blood serum samples were subjected to the microscopic agglutination test (MAT) as per Moraes et al. (2010) to detect anti-*Leptospira* antibodies. Live *Leptospira* cultures representing 19 serogroups Australis (Aus), Autumnalis (Aut), Ballum (Bal), Bataviae (Bat), Canicola (Can), Celledoni (Cel), Cynopteri (Cyn), Djasiman (Dja), Grippotyphosa (Gry), Hebdomadis (Heb), Icterohaemorragiae (Ict), Panama (Pan), Pomona (Pom), Pyrogenes (Pyr), Sejroe (Sej), Shermani (She), Tarassovi (Tar), Andamana (And), and Seramanga (Ser) were used as antigens. The cultures were maintained by weekly subculture in liquid EMJH medium (Difco™ Laboratories) at 29 °C. The samples with the most evident antigen-antibody agglutination reaction, with at least 50% of the leptospires agglutinated and 50% free from a cut-off point of 100 in the screening stage, and ≥ 100 in the titration stage, were considered as reagents for the specific serogroup(s).

DNA from whole blood samples was extracted using the ReliaPrepTM Blood gDNA Miniprep System kit (Promega, Madison WI, USA), by following the manufacturer's recommendations.

For PCR, the Lep1 (5'GGCGGGCGCGTCTTA AACATG3') and Lep2 (5'TTCCCCCCATTGAGC AAGATT 3') primers described by Mérien et al. (1992) were used to amplify a 331 bp fragment of the 16S rRNA gene of *Leptospira* spp.

The protocol previously reported by Rocha (2016) was followed, and the PCR mix was composed of a total volume of 25 µL including 2.5 µL of 10 x buffer (500 mM KCl; 100 mM Tris-HCl pH 8.5); 1.0 µL of MgCl2 (50 mM); 1.0 µL of dNTP solution (1.0 mM); 0.3 µL of Taq DNA polymerase (5 U/µL Ludwig Biotec); 1 µL of each primer (2.5 pmol); 5 µL of extracted DNA, and 13.2 µL of sterile water.

The PCR reaction was processed in a thermal cycler (Veriti 96 Well Thermal Cycler, Applied Biosystems, Foster CA, USA) using the following conditions: an initial denaturation cycle at 94 °C for 5 min, followed by 40 amplification cycles that included denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with the reaction being completed by an extension at 72 °C for 5 min. The positive control was DNA (5 µL) extracted from *Leptospira* cultivated in liquid EMJH medium serovar Can and the negative control was sterile water (5 µL). The PCR products were subjected to agarose gel electrophoresis (1.5%) and stained with GelRed® (Biotium Inc., Fremont CA, USA) for visualization under ultraviolet light of a transilluminator with a photo-documentation system (Gel DOCTM XR+ Imaging System, BioRad, Hercules CA, USA).

Results and discussion

Of the 145 serum samples examined by MAT, 64.14% (93/145) were reactive with one or more *Leptospira* spp. serogroups, with titers ranging from 100 to 6400 (Table 1). Twenty co-agglutinations were observed in the analyses, i.e., equal titrations for two or more serovars that made it impossible to identify the predominant serological variant. For this reason, they were not included in the frequency of serogroups. The 145 whole blood samples tested by the PCR technique for *Leptospira* spp. were negative.

Lower seroprevalences than the current results were reported by Oliveira et al. (2012), Paz et al. (2015) and Silva et al. (2017), who found seroprevalences ranging from 17.41% to 53.8% in dogs from ZCC in Brazil.

In the current study, the observed prevalence of dogs seropositive for *Leptospira* spp. may be due to the fact that before their stay in the kennel of the ZCC, these dogs lived for long periods on the streets. This exposed the dogs to a situation of vulnerability characterized by ingestion of food waste from garbage and water contaminated with bacteria, in addition to having contact with other animals such as infected synanthropic rodents (Hafemann et al., 2018).

The seroprevalence values may also have resulted from the dogs being housed in the kennel of the ZCC, where exposure to *Leptospira* spp. may have been favored by the difficulty of maintaining sanitary conditions due to the high density of animals and lack of financial, technical, and human resources (Miotto et al., 2018).

Table 1 - Distribution of samples seropositive for *Leptospira* spp. based on the titers obtained by MAT in dogs from the Zoonosis Control Center of Belém, Pará, Brazil, 2018

		Antibody titers (UI)						
Serogroups	Frequency (%)	100	200	400	800	1600	3200	6400
Djasiman	29 (39.73)	13	10	-	03	-	03	-
Canicola	13 (17.80)	06	02	03	-	-	01	01
Cynopteri	09 (12.33)	06	01	01	01	-	-	-
Icterohaemorrhagiae	07 (9.59)	03	03	01	-	-	-	-
Seramanga	05 (6.85)	-	-	02	02	-	01	-
Sejroe	03 (4.11)	-	-	01	-	01	01	-
Pyrogenes	03 (4.11)	-	-	01	02	-	-	-
Australis	02 (2.74)	-	01	-	01	-	-	-
Shermani	01 (1.37)	-	01	-	-	-	-	-
Celledoni	01 (1.37)	01	-	-	-	-	-	-
Total	73 (100)	29	18	09	09	01	06	01
		(39.73%)	24.65%)	(12.33%)	(12.33%)	(1.37%)	(8.22%)	(1.37%)

In most cases, low serological titrations equal to 100 UI (39.73%) and 200 UI (24.65%), were observed (Table 1). Low antibody titers might be explained by either previous or recent exposure to the bacteria (Azócar-Aedo et al., 2018). High titers, 400 and 800 (12.33%), 1600 (1.37%), 3200 (8.22%), and 6400 (1.37%) are considered to be an indication of a response to recent exposure to bacteria (Jimenez-Coello et al., 2008).

However, to ensure a reliable interpretation of serological titers, it is recommended to not rely on the analysis of a single sample, but to perform paired serological analysis with an interval of 1–2 weeks between sampling, to show an increase of four-fold or more between the obtained titers (Goldstein, 2010). Unfortunately, this procedure was not possible due to the difficulty of collecting a second blood sample from the same dog because some animals were no longer housed at the study site.

Serological responses to the Dja serogroup were predominant in the dogs analyzed; however, these responses may have been related to contact with maintenance hosts of the Dja serogroup. These results need to be further examined because of the paucity of data available on the species that effectively play this role.

Serological evidence of the Can serogroup in the analyzed dogs mainly reflects contact of dogs housed at the ZCC with infected dogs, which plays an important role in the maintenance of this serogroup. This is frequently identified as the cause of clinical and asymptomatic cases (Paz et al., 2015).

To our knowledge, this is the first study to report serological responses to the Cyn serogroup in dogs from a zoonosis control center. These findings may be associated with the possibility of contact with maintenance hosts of this serogroup. However, little is known about the species which play this role and this needs to be clarified.

The occurrence of the Ict serogroup in the analyzed animals shows that they could be exposed to infected synanthropic rodents, which are considered to be the most important reservoirs of this serogroup (Paz et al., 2015).

Reactivity to the Ser serogroup is a common finding. Its occurrence in the analyzed dogs may be related to their exposure to the street environment or even the kennels of the ZCC, where the leptospires of this serogroup are generally present, as they are saprophytes and wild (Moraes et al., 2010).

None of the 145 whole blood samples tested positive for *Leptospira* spp. by PCR. In contrast, Meira et al. (2011) and Oliveira et al. (2012) found evidence of leptospiremia in 4% and 7.7%, respectively, of the dogs they tested by PCR at

zoonosis control centers in Brazil. The negative molecular analysis results in our study suggest the absence of the bacterial agent (leptospiremia) in blood at the time of sampling, i.e., the dogs were probably not actively infected (Hua et al., 2016).

Conclusion

We conclude that the dogs housed in the kennel of the Zoonosis Control Center of Belém, Pará, had no leptospires in the bloodstream at the time of sampling; they were previously exposed to the bacterial agent. It is extremely important to carry out further studies in this regard, as they are still incipient in canine populations housed in shelters in this region.

Acknowledgments

The authors are grateful to the Zoonosis Control Center of Belém, Pará, Brazil, for authorizing the study and for helping in the collection of biological samples from the dogs in their center.

References

Arruda EC, Noronha J, Molento CFM, Garcia RCM, Oliveira ST. Características relevantes das instalações e da gestão de abrigos públicos de animais no estado do Paraná, Brasil, para o bem-estar animal. Arq Bras Med Vet Zootec. 2019; 71(1):232-42.

Azócar-Aedo L, Monti G, Jara R. Serological conversion for anti-*Leptospira* antibodies among domestic dogs from southern Chile, a prospective study. J Vet Med Res. 2018;5(8):1154.

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.

Goldstein RE. Canine leptospirosis. Vet Clin North Am Small Anim Pract. 2010; 40(6):1091-101.

Hafemann DCM, Merlini LS, Gonçalves DD, Fortes MS, Navarro IT, Chiderolli RT, et al. Detection of anti-*Leptospira* spp., anti-*Brucella* spp., and anti-T*oxoplasma gondii* antibodies in stray dogs. Semina Cienc Agrar. 2018;39(1): 167-76.

Hua KK, Xian TW, Fong LS, Roslan MA, Radzi R, Bejo SK, et al. Seroprevalence and molecular detection of leptospirosis from a dog shelter. Trop Biomed. 2016; 33(2):276-84.

Jimenez-Coello M, Vado-Solis I, Cárdenas-Marrufo MF, Rodríguez-Buenfil JC, Ortega-Pacheco A. Serological survey of canine leptospirosis in the tropics of Yucatan Mexico using two different tests. Acta Trop. 2008;106(1):22-6.

Meira CD, Wenceslau AA, Carvalho FS, Albuquerque GR, Dias RC. Molecular diagnosis of Leptospirosis in blood of dogs naturally infected. Braz J Vet Med. 2011;33(1): 7-11.

Mérien F, Amouriaux P, Perolat P, Baranton G, Saint-Girons I. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. J Clin Microbiol. 1992;30(9):2219-24.

Miotto BA, Guilloux AGA, Tozzi BF, Moreno LZ, Hora AS, Dias RA, et al. Pro-spective study of canine leptospirosis in shelter and stray dog populations: identification of chronic carriers and different Leptospira species infecting dogs. PLoS One. 2018;13(7):e0200384.

Moraes CCG, Kuroda RBS, Pinho APVB, Ywasaki F, Meneses AMC, Martins AV, et al. Pesquisa de anticorpos para sorovares de *Leptospira interrogans* patogênicas em equídeos criados na ilha de Algodoal, Estado do Pará. Rev Ci Agra. 2010;53(2):188-94.

Oliveira ST, Messick JB, Biondo AW, Santos AP, Stedile R, Dalmolin ML, et al. Exposure to *Leptospira* spp. in sick dogs, shelter dogs and dogs from an endemic area: points to consider. Acta Sci Vet. 2012;40(3):1-7.

Paz GS, Rocha KS, Lima MS, Jorge EM, Pantoja JCF, Moraes CCG, et al. Seroprevalence for brucellosis and leptospirosis in dogs from Belém and Castanhal, State of Pará, Brazil. Acta Amaz. 2015;45(3):265-70.

Rocha KS. Pesquisa de *Leptospira* spp. em fragmentos de fígado e rim de marsupiais e roedores de vida livre em fragmentos florestais na Amazônia Oriental [dissertação]. Castanhal: Universidade Federal do Pará; 2016. 40 p.

Silva ERDFS, Castro V, Prianti MG, Gonçalves LMF, Sobrinho Jr EPC, Drumond KO, et al. Occurrence of antibodies against *Leptospira* spp. in dogs from Teresina, Piauí, Brazil. Braz J Vet Res Anim Sci. 2017;54(1):88-91.