SEROPREVALENCE AND FACTORS ASSOCIATED WITH Leptospira SPP. INFECTION IN DOGS AN AMAZON-CERRADO ECOTONE OF BRAZIL

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ABSTRACT

The present study aimed to investigate the prevalence of antibodies against *Leptospira* spp., identify the serovars and to evaluate possible factors associated with antibodies serological prevalence in urban dogs of a municipality in the Amazon-Cerrado-ecotone. We analyzed 275 sera samples, representative of the canine population of the urban area of the municipality. Samples were collected from random families. Antibodies research was performed by microscopic agglutination test (MAT), golden standard for the diagnosis of leptospirosis. Those responsible for the animals were given a structured questionnaire, elaborated with variables related to the animals, owners and the environment, to evaluate possible associated factors. Data were submitted to univariate analysis, using chi-squared test with Yates correction or Fisher's exact test. The seroprevalence was 13.8%, with a predominance of Castellonis (26.3%), Pyrogenes (10.5%). and Pomona (10.5%) serovars. We observed statistically significant association ($p<0.05$) between *Leptospira* spp. infection and rainy season, temporarily flooded areas, presence of native vegetation and solid waste accumulation. The results showed that, in the studied area, canine infection had an epidemiological profile partially different from the profiles reported in most Brazilian regions, especially regarding the potential role of wild reservoirs as a source of infection.
KEYWORDS: Dogs, *Leptospira* spp., epidemiology, leptospirosis, risk.

SOROPREVALÊNCIA E FATORES ASSOCIADOS À INFECÇÃO EM CÃES POR *Leptospira* spp. EM UM ECÓTONO AMAZÔNICO-CERRADO DO BRASIL

RESUMO

Este estudo teve como objetivo investigar a prevalência de anticorpos anti-*Leptospira* spp., identificar os sorovares e avaliar possíveis fatores associados à prevalência sorológica antileptospírica em cães urbanos de um município localizado em uma zona do ecótono Cerrado-Amazônia. Foram analisadas 275 amostras de soro, representativas da população canina da zona urbana do município. As amostras foram colhidas em domicílios sorteados aleatoriamente. A pesquisa de

anticorpos foi realizada através da técnica de soroaglutinação microscópica (SAM), padrão-ouro para diagnóstico da leptospirose. Aos responsáveis pelos animais foi aplicado um questionário estruturado, elaborado com variáveis relativas aos animais, aos proprietários e ao ambiente, para avaliação de possíveis fatores associados. Os dados foram submetidos a análise univariada, utilizando o teste Qui-Quadrado com correção de Yates ou teste exato de Fisher. A soroprevalência detectada foi de 13,8%, predominando os sorovares Castellonis (26,3%), Pyrogenes (10,5%). e Pomona (10,5%). Associação estatisticamente significativa (P<0,05) foi detectada entre infecção por *Leptospira* spp. e as variáveis estação chuvosa, localidade com áreas de alagamento, presença de vegetação nativa e acúmulo de resíduos sólidos. Os resultados demonstraram que, na área estudada, a infecção canina apresentou um perfil epidemiológico parcialmente distinto do relatado na maioria das regiões brasileiras, em especial quanto ao potencial papel dos reservatórios silvestres como fonte de infecção.
PALAVRAS-CHAVE: Cães, epidemiologia, *Leptospira* spp., leptospirose, risco

INTRODUCTION

Leptospirosis is an infectious contagious zoonotic disease with strong social and economic relevance. This disease is caused by bacteria of the genus *Leptospira*, which is divided in 64 species with different serovars defined after cross-agglutination absorption with homolog antigens (VINCENT *et al.,* 2019). *Leptospira* spp. are excreted in the urine of reservoir hosts, also called maintenance hosts.

Dogs play an important role in the transmission of leptospirosis to humans. These animals can retain bacteria for a long time in the kidney, as elimination may occur through urine without any clinical sign. Most common serovars associated with canine leptospirosis are Canicola and Icterohaemorrhagiae. Both serovars are highly pathogenic to humans and have been detected in several regions of Brazil (SAMIR *et al*., 2015; MIOTTO *et al.,* 2018). The habits of dogs and their strict coexistence with humans may ease dissemination of leptospirosis to human species, but are uncommon when standard hygiene protocols are followed (FERNANDES *et al.*, 2018).

Few studies have been performed on the prevalence of *Leptospira* infection among dogs in the northern region of Brazil. This region has favorable environmental conditions for the occurrence of leptospirosis, especially in urban areas (GALAN *et al.,* 2021). Epidemiologic studies developed in other biomes are currently used to conduct control strategies in the northern region, such as vaccine production and reservoirs control. It is important to understand if risk factors for canine leptospirosis are equal or similar to those of human infection, or if there are variables that are uniquely associated with dog's habits and handling.Considering the relevance of dogs for the transmission of *Leptospira* spp. to humans, the present study aimed to investigate the serological prevalence, the *Leptospira* serovars present in dogs of an urban area in the Amazon-Cerrado ecotone, and the variables associated with canine infection.

MATERIALS AND METHODS

The research was performed in the municipality of Araguaína, in the northern region of Brazil, State of Tocantins, included in the Amazon-Cerrado ecotone. The municipality covers an area of 4,000.4 km^2 at a 7°11'26" south, 48°12'28" west, 236 m above sea level. The climate of the region is wet and tropical, average maximum temperature 32°C. There are two defined seasons: rainy season, between November

and May, and dry season, between June and October. Average annual rainfall is above 1,700-1,800 mm (PENEREIRO *et al*., 2016).

As a representative of the entire canine population of Araguaína, 275 dogs have been sampled. For the definition of sampling size, confidence level of 95% and expected prevalence of 20% have been considered, basing on the results of other studies that had been performed in Brazil. On the basis of these considerations, minimal sample size should have been of 246 blood sera. To this number we added a safety margin of 10% and we established a minimum of three samples per district of the municipality.

Stratified sampling was used as allocation method for sampling. We divided the municipality in nine layers, according to geographic region. Layers were classified according to the codes used in the dengue control program (n=91 localities) (ARAGUAÍNA, 2008). Later we selected 20% of these, totaling 19 localities.

The nine layers were selected by proportional stratified sampling. Each layer corresponded to a geographic region. Each region participated in the investigation with 1/5 (20%) of its localities. The number of samples to be collected from each locality was proportional to the estimation of dog population. Where the presence of dogs was estimated to be higher, more samples were collected. The calculus of the proportion of dogs/locality was performed considering the estimation of dog population provided by Araguaína (2008).

We used random systematic sampling to select the addresses where to collect the samples (THRUSFIELD; CHRISTLEY, 2018). If there was no dog at the selected address, we substituted it by the closest address, where dogs were present.

The blood samples were collected by jugular or cephalic venipuncture, then they were centrifuged, and the sera were stored in microtubes at -20°C for the serologic tests. Dog owners were interviewed following a structured, closed and pre-coded questionnaire, according to Thrusfield e Christley (2018). Questions were related to the animal, the owner and the environment. Dog owners receivedexplanation about the study's aim. Animals were included in the study only after their owner's acceptance.

For the detection of antibodies against *Leptospira* spp. we used the microscopic agglutination test (MAT). We used a battery of 24 serovars, composing the collection of live cultures of Leptospirosis Laboratory of the School of Veterinary and Zootechnics of the Federal University of Goiás State (Brazil). Sera were tested against serovars Andamana, Australis, Bataviae, Brasiliensis, Bratislava, Butembo, Canicola, Castellonis, Copenhageni, Cynopteri, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Panama, Patoc, Pomona, Pyrogenes, Shermani, Tarassovi, Whitcombi e Wolffi. Sera were tested at a 1:100 dilution. Sera presenting 50% or more agglutination were titrated in doubling dilutions. Serum titer was the reciprocal of the endpoint serum dilution.

The serovar presented at the highest titer was considered the probable causative agent of infection. If two or more serovars presented the same high titers, the sample was considered as a co-agglutination. As a single animal could agglutinate several tested antigens, we considered the highest antibodies titer for each animal, independently from the serovar.

The present study was elaborated and performed according to the directions and norms of the Resolution 196/1996 of the National Council of Health of Brazil and was endorsed by the Ethical committee of the Tropical Medicine Foundation of Tocantins, under protocol number 137.

A database of animal serology and interviews was built using the software Epi Info version 3.5.4. Data were later analyzed using descriptive statistics.

Univariate analysis was performed for the study of associated factors. We considered MAT results as dependent variable and the variables associated with animal, owner and environment as independent. Statistical significance of associations was calculated through the Chi-square test (χ^2). If the sample was small, we applied Yates correction or Fisher's exact test. Confidence was set at 95%. All data were analyzed using Epi Info version 3.5.4.

RESULTS AND DISCUSION

Thirty-eight among 275 canine sera reacted against *Leptospira* antibodies (Table 1). The prevalence was, therefore, 13.82% (38/275, 95% CI 10.0-18.5%).

TABLE 1 - Distribution of antibodies titers of dog sera collected in Araguaína, Tocantins, Brazil, 2008, against 24 *Leptospira* serovars tested

Serovar	Antibody titer (UI)				Total	%
	1:100	1:200	1:400	1:800		
Co-agglutinations	3	1	-	2	6	15.8
Castellonis	7	2	1	-	10	26.3
Pyrogenes		2	1	1	4	10.5
Pomona	1	1	-	2	4	10.5
Gryppotyphosa	2	-	-	1	3	7.9
Butembo		-	1	2	3	7.9
Patoc	1	-	2	-	3	7.9
Andamana		-	-	1	1	2.6
Australis	1	-	-	-	1	2.6
Cynopteri	-	-	1	-	1	2.6
Panamá	1	-	-	-	1	2.6
Shermani	-	-	1	-	1	2.6
TOTAL	**16**	**6**	**7**	**9**	**38**	**100.0**

Table 1 shows the frequency of agglutinations against tested sera. Castellonis was the most frequent serovar, followed by serovars Pyrogenes and Pomona. Positive reactions were also detected against serovars Gryppotyphosa, Butembo and Patoc. Co-agglutination occurred in 15.8% of samples.

Other studies in Brazil, for example Paz *et al.* (2015) in Belém-Pará, Felix *et al.* (2020) in Pelotas-Rio Grande do Sul, and Benitez *et al.* (2021) in Londrina-Paraná identified higher prevalence of *Leptospira* than we did. The prevalence detected in the present study, although low, is alarming, as leptospirosis is a zoonosis and it is widespread in the municipality.

Serovar Castellonis has seldom been described as the most prevalent in sampled animals. In Southeast Brazil, Silva *et al.* (2009) indicated a similar situation. This finding suggests the coexistence of reactive dogs positive for this serovar, with wild rodents, which are the main reservoirs of the bacteria. The serovar Castellonis has also been isolated from the urine of white-eared opossum (*Didelphis albiventris*) in Brazil (JORGE *et al.,* 2012).

Serovars Pyrogenes and Pomona are commons among dogs in Brazil, as described by Moraes *et al.* (2020) and Benitez *et al.* (2021). The Pomona serovar has swine and possibly some marsupials as its reservoir, which is plausible, since we find large green areas and clandestine pig farming in the urban space of the

municipality of Araguaína (BHARTI *et al.,* 2003; LEITE *et al.,* 2018). Serovar Pyrogenes was isolated from water rats (*Nectomys squamipes*) and bovines in Brazil (MANZINI *et al.,* 2021).

Positive serologic reaction to accidental serovars in dogs such as Australis*,* Shermani and Cynopteri may be associated with the presence of other host species in urban environment, especially synanthropic ones, such as opossum (*Didelphis albiventris*) (WARD *et al.,* 2002). Opossums are often captured or observed in the urban area of the municipality. We highlight the absence of reactions to the serovar Canicola, one of the most common in canine species (MIOTTO *et al.,* 2018). Concomitant infection by several serovars of *Leptospira* spp. or cross reactions among serovars of the same serogroup may explain the co-agglutinations we detected.

Environmental variables were significantly associated with positive serological reaction to *Leptospira*. Rainy season and high temperatures facilitate bacterial survival (BARRAGAN *et al.,* 2017) and dog's infection. Heavy rain and local lack of water drainage (sewage system), as is in some studied areas, facilitate the accumulation of stagnant water and the contact between dog and the infective agent (MD-LASIM *et al.* 2021).

Studying the correlation between the positivity for *Leptospira* and the different studied variables, only the locality *"Sonhos Dourados"* and rainy season showed significant association with *Leptospira* ($p<0.05$) (Table 2).

TABLE 2 - Bivariate analysis of microscopic agglutination test (MAT) against *Leptospira* spp. and locality of dogs' residence in the urban area of Araguaína, Tocantins, Brazil, 2008

Locality	MAT for *Leptospira* spp.				*Odds Ratio* (OR)	CI (95%)	P value
	Reacting		Not reacting				
	N	%	N	%			
Anhanguera	6	15.0	34	85.0	0.89	0.36 - 2.51	0.99
Barros	2	28.6	5	71.4	2.56	0.33 - 13.53	0.25
Centro	5	11.1	40	88.9	1.33	0.52 - 4.08	0.73
Jardim Filadélfia	1	33.3	2	66.7	0.32	0.02 - 9.52	0.36
Jose Ferreira	2	40.0	3	60.0	4.29	0.49 - 29.80	0.14
Monte Sinai	3	42.9	4	57.1	4.94	0.89 - 24.90	0.06
Santa.Terezinha	1	9.1	11	81.8	1.80	0.29 - 40.11	0.48
São João	12	18.2	54	81.1	0.64	0.30 - 1.39	0.33
Setor Couto	1	5.6	17	94.4	2.85	0.49 - 61.90	0.26
Sonhos Dourados	3	60.0	2	40.0	9.92	1.43 - 85.91	0.02
Tiuba	1	12.5	7	87.5	1.12	0.17 - 26.22	0.69
Universitário	1	12.5	7	87.5	1.12	0.17 - 26.22	0.69

The locality "*Sonhos Dourados*" presented the highest probability of infection in dogs. The association between the locality and the infection may be caused by

several environmental and social aspects. *"Sonhos Dourados"* is a downtown area with two riverbeds that cause flooding during the rainy season. In the location there are several unoccupied areas, with garbage and vegetation acting as covert and feeding areas for synanthropic animals.

Dry season was a protective factor against the risk of canine infection with *Leptospira* in Araguaína (Table 3).

TABLE 3 - Bivariate analysis of microscopic agglutination test (MAT) against *Leptospira* spp. in dogs and univariate analysis according to the season in the urban area of Araguaína, Tocantins, Brazil, 2008

Season	MAT for *Leptospira* spp.				*Odds Ratio* (OR)	CI (95%)	P value
	Reacting		Not reacting				
	N	%	N	%			
Dry	33	86.8	228	96.2	0.26	0.08 - 0.91	0.03
Rainy	05	13.1	09	3.8	3.81	1.09 - 12.1	0.03

No variable associated with social or economic situation of dog owners, such as educational level or family income, showed significant association with *Leptospira* infection (Table 4).

TABLE 4 - Bivariate analysis of microscopic agglutination test (MAT) against *Leptospira* spp. in dogs and dogs features in the urban area of Araguaína, Tocantins, Brazil, 2008

Feature	MAT for *Leptospira* spp.				*Odds Ratio* (OR)	CI (95%)	P value
	Reacting		Not reacting				
	N	%	N	%			
Age							
< 1 year	07	18.4	45	19.0			
1 to 7 years	27	71.1	169	71.3			
> 7 years	04	10.5	23	9.7			0.98
Sex							
Male	19	50.0	120	50.6			
Female	19	50.0	117	49.4	0.97	0.49 - 1.95	0.92
Breed							
Pure breed	10	26.3	65	27.4			
Cross-breed	28	73.7	172	72.6	1.05	0.40 - 2.40	0.96
Vaccination against *Leptospira*							
Yes	20	52.6	94	39.7			
No	18	47.4	143	60.3	1.68	0.84 – 3.37	0.19
Public waste management							
Yes	34	89.5	215	90.7			
No	4	10.5	22	10.5	0.87	0.30–3.11	0.50
Rodents presence inside the house or around							
Yes	35	92.1	221	93.2	0.84	0.25– 3.80	0.50
No	3	7.9	16	6.8			

Other variables did not confirm their association with the disease. Several authors have already associated the presence of rodents or garbage close to or in the dog's residence with leptospirosis and question the role of dogs as sentinels for disease (SILVA *et al.* 2009; BENITEZ *et al.,* 2021).

We must consider that the studied area has been recently colonized. Rapid and sometimes confusing expansion of urban area with incorporation of rural and/or sylvan localities increased the relevance of synanthropic rodents as important epidemiologic factor for the persistence of *Leptospira* in the environment and dog infection. The high prevalence of serovar Castellonis corroborate this hypothesis, as the main reservoirs of this serovar are wild rodents or opossum.

The present study indicates that the infection of dogs with *Leptospira* in Araguaína municipality has a peculiar epidemiological profile.

The elaboration of vaccines for the prevention of canine leptospirosis in this region should be performed considering the most prevalent serovars. Most national vaccines do not consider serovar Castellonis and do not protect completely local animals.

CONCLUSIONS

The prevalence of antibodies against *Leptospira* spp. was not high in the studied area. Several serovars were detected in the region, with Castellonis being the most common. We identified an association between the presence of leptospirosis in dogs in the Cerrado-Amazon ecotone and rainy season, stagnant water accumulation and solid waste disposal areas. Wild rodents possibly play a relevant role as reservoirs of *Leptospira* spp. infection in dogs in the municipality of Araguaína, state of Tocantins, Brazil.

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