

## SEROEPIDEMIOLOGICAL SURVEY FOR CANINE LEPTOSPIROSIS IN THE COAST OF SÃO PAULO STATE, BRAZIL

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### ABSTRACT

Leptospirosis is a worldwide anthroponosis, mainly distributed in coastal and tropical regions, and caused by the bacterium *Leptospira* spp. In urban areas, synanthropic rodents are the main transmitters to humans and other animals through the urine. Dogs can participate as domestic reservoirs to the human infection due to the renal persistence and elimination of the bacterium through the urine. Thus, the present study aimed to determine the presence of antibodies to *Leptospira* spp. in serum samples from dogs in Ubatuba municipality, São Paulo State, Brazil. Associations between the serological results and epidemiological variables such as breed, age, sex, access to the street, access to untreated water, previous vaccination against leptospirosis, and presence of rodents, were assessed. *Leptospira* spp. antibodies were detected in 30/205 (14.6%) serum samples, and the prevalent serovars were Pyrogenes (30%), Autumnalis (23.3%), and Canicola (20%). No coagglutination was observed, as well as no significant association was observed between the studied epidemiological variables and the serology (p-value > 0.05). Thus, leptospirosis is active and circulating the studied city, which has high rainfall index; however, factors like access of dogs to the street, accumulated water and presence of rodents did not present the expected importance. Further studies should be conducted in the studied region focusing the detection of antibodies in stray dogs, which are more exposed to the bacterium in coastal and tropical areas, as well as live close to the production and herbivorous animals.

**Keywords:** *Leptospira* spp., dogs, antibodies, infection, public health, coast.

## INQUÉRITO SOROEPIDEMIOLÓGICO PARA LEPTOSPIROSE CANINA NO LITORAL DO ESTADO DE SÃO PAULO, BRASIL

### RESUMO

A leptospirose é uma antropozoonose mundialmente distribuída, principalmente em regiões litorâneas e tropicais, e causada pela bactéria *Leptospira* spp. Em áreas urbanas, os roedores sinantrópicos são os principais transmissores aos seres humanos e outros animais pela urina. Os cães podem atuar como reservatórios domésticos para a infecção no homem devido a persistência renal da bactéria e eliminação pela urina. Deste modo, o presente trabalho teve como objetivo determinar a presença de anticorpos para *Leptospira* spp. em amostras de soro de cães do município de Ubatuba, SP, Brasil. As associações entre os resultados sorológicos e variáveis epidemiológicas como raça, idade, sexo, acesso a rua, acesso a água não tratada,

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vacinação prévia contra sorovares de *Leptospira* spp., e presença de roedores, foram analisadas. Anticorpos para *Leptospira* spp. foram detectados em 30/205 (14,6%) amostras de soro, e os sorovares prevalentes foram Pyrogenes (30%), Autumnalis (23,3%) e Canicola (20%). Nenhum sorovar co-aglutinante foi observado, e também não foi observada associação significativa entre as variáveis epidemiológicas estudadas e os resultados da sorologia ( $p > 0,05$ ). Assim, conclui-se que a leptospirose está circulante no município estudado, município que apresenta alto índice pluviométrico, porém fatores como acesso de cães à rua, água acumulada e presença de roedores não apresentaram importância esperada. Futuros estudos devem ser realizados na região estudada focando a pesquisa de anticorpos também em cães errantes, que se encontram mais expostos à bactéria em áreas litorâneas e tropicais, e o convívio com animais herbívoros e de produção.

**Palavras-chave:** *Leptospira* spp., cães, anticorpos, infecção, saúde pública, litoral.

## ESTUDIO SEROEPIDEMIOLÓGICO PARA LEPTOSPIROSIS CANINA EN LA COSTA DEL ESTADO DE SÃO PAULO, BRASIL

### RESUMEN

La leptospirosis es una antropozoonosis distribuida por todo el mundo principalmente en las regiones costeras y tropicales, y es causada por la bacteria *Leptospira* spp. En las zonas urbanas, los roedores sinantrópicos son los principales transmisores a los seres humanos y otros animales por la orina. Perros pueden actuar como los principales reservorios domésticos para la infección en el hombre debido a la persistencia renal de la bacteria y la eliminación en la orina. Así, el presente estudio tuvo como objetivo determinar la presencia de anticuerpos para *Leptospira* spp. en muestras de suero de perros de la ciudad de Ubatuba, SP, Brasil. Las asociaciones entre los resultados serológicos y variables epidemiológicas como la raza, edad, género, acceso a la calle, acceso al agua no tratada, vacunación previa contra *Leptospira* spp. y presencia de roedores fueron analizadas. Anticuerpos para *Leptospira* spp. fueron detectados en 30/205 (14,6%) muestras de suero, y los serovares prevalentes fueron Pyrogenes (30%), Autumnalis (23,3%) y Canicola (20%). No se observó serovar co-aglutinante, ni asociación significativa entre las variables epidemiológicas estudiadas y los resultados de la serología ( $p > 0,05$ ). Así, concluyese que la leptospirosis está circulante en el municipio estudiado, municipio que presenta alta precipitación, pero factores como el acceso a los perros de la calle, agua acumulada y presencia de roedores no se mostraron importantes como esperado. Futuros estudios deben ser realizados en la región estudiada, centrándose también en la pesquisa de anticuerpos en perros callejeros, los cuales están más expuestos a la bacteria en las zonas costeras y tropicales, y que viven junto con los animales herbívoros y de producción.

**Palabras clave:** *Leptospira* spp., perros, anticuerpos, infección, salud pública, costa.

### INTRODUCTION

Leptospirosis is a worldwide anthroponosis, caused by the bacterium, spirochete, *Leptospira* spp. that affects several wild and domestic animal species, and humans (1,2). This infection presents a high social, economical and cultural importance. It is spread by factors like disordered growth of urban centers, migrations, deficiencies in basic sanitary conditions, high rainfall indexes, and uncontrolled garbage accumulation. The persistence of the agent in the nature and its high infectious potential rate are assured by different serovars, multiple host

species and relative degree of survival in the environment (3,4). The environmental resistance increases the importance of animals considered healthy carriers, which can excrete leptospira and are the major responsible for the persistence of the disease foci, responsables for the maintenance of the agent in the environment (1). In this context, dogs have great importance in the epidemiology of leptospirosis due to their close relationship o the humans (5,6). Dogs eliminate some serovars through the urine, intermittently, for months and years. Rodents, specially *Rattus norvegicus* (*R. norvegicus*), are the main reservoirs of the serovar Icterohaemorrhagiae (6,7). *L. interrogans* serovar Icterohaemorrhagiae and serovar Canicola are the most common serovars isolated from dogs with leptospirosis (4,5,8-10).

Leptospirosis can be transmitted among the animals by direct contact, venereal and placentar transmission, and wounds. Indirect transmission occurs by the exposure of susceptible animals to contaminated vegetation, soil, food and water, as well as fomites (1).

In this way, the present study aimed to determine the frequency of antibodies to *Leptospira* spp. in dogs from Ubatuba, a coastal municipality in São Paulo State characterized by a high rainfall index in the tropical region of Brazil, as well as the most frequent epidemiological variables associated to the occurrence of the infections in the studied population.

## MATERIAL AND METHODS

### *Experimental design and sampling*

This study was carried out in Ubatuba, a coastal municipality located in the north of the São Paulo State (23°26'S; 45°04'W), Southeastern region of Brazil. Ubatuba is crossed by the Tropic of Capricorn (11) and presents rainfall index ranging approximately from 1,600 to 3,000 mm/year (12); it is located at 250 km away from the capital of the State, and surrounded by "Serra do Mar" and Atlantic Forest (712 Km<sup>2</sup> total area), and mean altitude of 835 m above the sea level, with a range from 800 to 1670 m in the highest parts. The climate is humid tropical, and present dominant winds from the South and Southeast. The population is estimated in 78,801 habitants (13) (105.33 inhabitants/km<sup>2</sup>). The urbanization rate is 97.8%, with 22.8% receiving sanitary treatment and 76.2% treated water.

This study was designed as a cross-sectional study. The sample size was determined considering a human population from the studied city, obtained with the Brazilian government by the Censo 2010, and using a 1:10 (dog:man) proportion (14), which resulted in 7,501 dogs. Considering a prevalence of 50%, standard-error of 7%, the minimum number of animals calculated was 191, but increased to 205 due to the availability and safety reasons (15). Sample size was calculated in EpiInfo 3.1 (16). All animals had an owner, and they were randomly selected with no preferences for indoor or stray dogs, sex, age or breed. All dog owners signed a consent and authorization term for the utilization of samples in this research. Blood samples (4-5 mL) were drawn by cephalic or jugular vein puncture, and serum samples obtained by the centrifugation at 1,600 x g, 10 min. Serum samples were transferred to microtubes, and stored at -20°C.

### *Questionnaire*

An epidemiological questionnaire was applied to the owners or those who fed the animals used in this study. It was directed to the risk factors related to the leptospirosis. The collected data included breed, age, sex, access to the street, previous vaccination against *Leptospira* spp. serovars, access to untreated water (both in the house and on the street), and presence of rodents in the house and surrounding areas.

### *Microscopic agglutination test (MAT)*

Serum samples were tested for *Leptospira* spp. antibodies using the microscopic agglutination test (MAT) (17). A panel of 25 serovars were tested and represented ten *Leptospira* spp. serogroups, as follows: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Gryppotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, and Patoc. Samples were two-ratio diluted, starting at 1:100. The titer was considered the reciprocal of the highest dilution with less than 50% leptospira agglutination. The cut-off titer was considered as 100.

### *Statistical analysis*

All data were entered in a Excel spreadsheet. The association between the epidemiological variables and the serological results for *Leptospira* spp. antibodies was assessed using the Chi-square ( $\chi^2$ ), or Fisher's exact test, considering  $\alpha = 0.05$ . All tests were run using InStat 3 (GraphPad Software, USA).

## RESULTS

*Leptospira* spp. antibodies were detected in 30/205 (14.6%; CI95% 10.5-20.1%) canine serum samples, with higher occurrence in animals living close to beach areas. The prevalent serovars were Pyrogenes, Autumnalis and Canicola (Table 1). No coagglutination was observed. None of the positive animals had been previously vaccinated against *Leptospira* spp. serovars according to the questionnaire. Also, no significant association was observed between the serology and the studied epidemiological variables (p-value > 0.05) (Table 2).

Table 1. Frequency of *Leptospira* spp. serovars in 205 studied dogs.

Serovar	Positive samples	Percentage (%)	CI95% <sup>a</sup>
Pyrogenes	9	30.0	16.7-48.0
Autumnalis	7	23.3	11.9-41.1
Canicola	6	20.0	9.6-37.5
Copenhageni	4	13.3	5.4-29.8
Icterohaemorrhagiae	2	06.7	2.0-21.4
Grypottyphosa	2	06.7	2.0-21.4
Total	30	100.0	-

<sup>a</sup> CI95%: 95% confidence interval.

Table 2. Univariate analysis for the association between the epidemiological variables and serological results for leptospirosis.

Variables		N	MAT <sup>a</sup>	Reagent; CI95% <sup>b</sup>	OR (CI95%) <sup>c</sup>	p-value <sup>d</sup>
Sex	Male	95	13	13.7; 08.2–22.0	0.9 (0.4–1.9)	0.44 <sup>f</sup>
	Female	110	17	15.4; 09.9–23.4		
Breed	DEF	34	3	8.8; 03.2–23.1	0.5 (0.2–1.8)	0.22 <sup>f</sup>
	UDF	171	27	15.8; 11.1–22.0		
Age (years)	0-4	80	9	11.2; 06.1–20.0	-	0.13 <sup>e</sup>
	4-8	73	9	12.3; 06.7–21.8		
	8-12	52	12	23.1; 13.8–36.2		
Access to the street	Yes	84	8	9.5; 05.0–17.7	0.5 (0.2–1.1)	0.06 <sup>f</sup>
	No	121	22	18.2; 12.3–26.0		
Access to untreated water	Yes	77	8	10.4; 05.4–19.2	0.6 (0.2–1.3)	0.13 <sup>f</sup>
	No	128	22	17.2; 11.6–24.7		
Vaccination	Yes	77	0	00.0; 00.0–00.2	-	0.07 <sup>f</sup>
	No	128	30	15.9; 11.4–21.8		
Presence of rodents	Yes	80	11	13.8; 07.9–23.0	0.9 (0.4–2.0)	0.47 <sup>f</sup>
	No	125	19	15.2; 10.0–22.5		

Legend: DEF, defined breed; UDF, undefined breed; <sup>a</sup> Titer  $\geq$  100; <sup>b</sup> Frequency of positive animals based on the studied variable; <sup>c</sup> OR (CI95%): Odds ratio (95% confidence interval) <sup>d</sup> p-value to  $\alpha = 5\%$ ; <sup>e</sup> Chi-Square test ( $\chi^2$ ); <sup>f</sup> Fisher's exact test.

## DISCUSSION

Leptospirosis is a important disease, mainly in tropical areas, and dogs may develop an important role on the maintenance of the infection. This study reports an infection occurrence of 14.6% in dogs, which quite similar to the observed in other canine populations sampled in Brazil and other countries around the world, ranging from 2.7 to 28.9% (4,8-10,18-23), dependent on the social and economic factors, as well as the climate of each area. For example, Blazius et al. (9) observed 10.5% (62/590) reagent dogs in another part of the Brazilian coast. The tropical and subtropical conditions reported by Blazius et al. (9) and this study suggest that the climate in both areas allowed the maintenance of the bacterium, mainly by the presence of dogs and rodents, which keep the causative agent in the nature.

Dogs play an important role in the transmission of this disease to the humans. They keep the bacterium in their kidneys for long periods, and can eliminate it intermittently through the urine without presenting clinical signs, or even after clinical improvement. This is a serious problem due to their domestic habits and close contact to the owners and other humans (1,19), which allows the dissemination of the infection to the humans and other animal species. Stray dogs, or even those spending most of time out of their houses, are longer exposed to infectious *Leptospira* spp. specimens present in the environment, eliminated by other infected dogs, and constitute the major infection sources to humans (9,18).

Rats, *R. norvegicus*, are the main carriers of *L. interrogans* serovar Icterohaemorrhagiae and, once infected can transmit this and other serovars intermittently during their life, without any clinical sign or death (1,2). This serovar has been frequently observed in tropical regions, i.e. São Paulo City (6). However, the present study observed low frequency for this serovar (2/30; 6.7%), and, as the others, no significant association with the presence of rodents. On the other hand, the answer to the questionnaire can be masked since rodents have nocturnal habits and might have not been seen even inside the house and surrounding areas. No significant association was neither observed to other important related variables to leptospirosis, i.e., access to the street. It is different from the observed by Silva et al. (21), in

Botucatu City, SP, a non-coastal region, who detected significant association of 22.1% positive dogs with the access to the street.

Serovars *Canicola* and *Icterohaemorrhagiae* are considered the most frequent cause of canine leptospirosis in the USA. However, in these days, *Grippotyphosa*, *Pomona* and *Bratislava* are considered very important for dogs, not only in North America but also in South America, Africa, Europe and Asia (7,24). Birnbaum et al. (25) detected 91.2% (31/34) dogs infected with *L. interrogans* serovar *Pomona* or *Grippotyphosa*, presenting renal and hepatic disease in New York, USA. Okewole and Ayoola (23) observed 64.7% (11/38) dogs positive to *Grippotyphosa* in Nigeria. Nielsen et al. (26), Van den Broek et al. (27), Stokes et al. (10) and Okewole and Ayoola (23) observed that, in addition to the others, *Bratislava* is also important in the USA, United Kingdom and Nigeria.

As shown in Table 1, *Pyrogenes* (9/30; 30.3%), *Autumnalis* (7/30; 23.3%) and *Canicola* (6/30; 20%) are the main causative serovars in the studied region of the Brazilian coast. Similar results were obtained by Blazius et al. (9), who detected *Pyrogenes* (26/62; 18%), *Canicola* (20/62; 13.8%), and *Icterohaemorrhagiae* and *Copenhageni* (18/62; 12.5%) in dogs from Itapema, a coastal region from Santa Catarina State, South Region of Brazil.

However, Aguiar et al. (22) detected *Autumnalis* (20/90; 22.2%) as the prevalent serovar in Monte Negro, RO, North Region of Brazil, followed by *Pyrogenes* (11/90; 12.2%), *Canicola* (9/90; 10%), and *Shermani* (7/90; 7.5%). Similar results were observed by Mascoll et al. (4) with *Autumnalis* (34.8%), *Copenhageni* (24%) and *Icterohaemorrhagiae* (10.3%), as well as by Silva et al. (28) with *Castellonis* (28.68%), *Autumnalis* (19.12%), and *Pyrogenes* (17.65%).

In other hand, Yasuda et al. (18) observed *Canicola* as the prevalent one (50.7%). *Canicola* is the most frequent serovar in dogs, and causes important infection in humans. In dogs, it may cause acute disease, fever (40°C), difficulty to move the posterior members, apathy, anorexia, pain to abdominal and posterior member palpation, bloody diarrhea, yellowish vomits, bloody salivation, and necrosis of the tongue edges (3,5,7,9,10,17,19,21).

Serovar *Pyrogenes* is the most common among production and herbivorous animals (cattle, horses, sheep and goats), as well as and other species co-habiting the same places. Therefore, this serovar commonly occurs in rural environments and is considered accidental to dogs (8). Accidental hosts are not important infection reservoirs, and the transmission occurrence is low. The transmission from an accidental host is relatively rare (8). To the epidemiological point of view, accidental hosts work much better as sentinels for the infection in humans and dogs. In this study, the infection by *Pyrogenes* has probably occurred because most of dogs and herbivores (mainly horses) share the same area the live in this region.

No significant association was observed between the epidemiological variables and the serological results ( $p$ -value > 0.05). In other hand, Aguiar et al. (22) observed significant association to the sex, in which males had 2.3 more chances to have positive results to the serology than females (CI95% = 1.3-3.9;  $p$ -value < 0.05). The high occurrence in males was also reported by Modolo et al. (20), with 18.4% infected males, compared to 11% females ( $p$ -value < 0.05).

In the studied region, leptospirosis has a seasonal character since the rainfall index is high throughout the year, which is different from Itapema (9), where rainfall index is higher from December to January with consequent increase of the incidence (2,7). This constitutes an alert for both public health offices and the population to improve the control of such animals. Contaminated water is the main responsible for the dissemination of leptospirosis by the direct contact (8); however, in this study it did not present the expected importance in the transmission to dogs.

Maintenance hosts are generally wild species and, sometimes, pets or production animals. The transmission among these hosts is efficient and the incidence is relatively high.

The contact with maintainance hosts or areas contaminated with their urine may disseminate the infection (8).

The present study highlight the circulating *Leptospira* spp. in the studied Brazilian coastal region, but the studied risk factors did not present the expected importance for the transmission and maintainance of the infection, which contributes to the keep dogs as sources of infection to the humans. Further studies should be conducted in the studied region focusing the detection of antibodies in stray dogs, which are more exposed to the bacterium in coastal and tropical areas, as well as live close to the production and herbivorous animals.

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