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Canine Leptospirosis Serology in Southern Mexico City

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Abstract

Background: Leptospirosis is an important zooanthroponotic disease spread worldwide which infection is recognized as a re-emergent disease. Leptospirosis is a systemic disease of humans and domestic animals, mainly dogs, cattle and swine, characterized by fever, renal and hepatic insufficiency, pulmonary manifestations and reproductive failure. **Objective:** To study the seroprevalence of anti-*Leptospira* antibodies in an urban canine population. **Materials and Methods:** The study was performed in March 2014 in stray dogs that had been captured in the districts of Coyoacan, Iztapalapa, Iztacalco and Benito Juarez in Mexico City; 117 dogs were analyzed, from each of which 5 ml of blood were obtained. Serology was performed using a MAT test (Microscopic Agglutination Test), finding that the antigens corresponded to 13 serotypes of *Leptospirainterrogans*. Three age groups were formed: Group 1) younger than a year (n = 28), Group 2) 1 to 6 years (n = 75), and Group 3) older than 6 years (n = 14). **Results:** Of the analyzed sera, 28.2% were seropositive to one or more *Leptospira* serotypes, 74% of the positive seracoagglutinated with two or more serotypes. The most frequent serotypes were: *Canicola*, *Icterohaemorrhagiae* and *Portland-vere*. Serotypes *Canicola*, *Pyrogenes*, and *Bratislava* resulted statistically significant ($p < 0.001$). The presence of anti-*Leptospira* antibodies in the blood of animals of 1 to 6 years resulted with a $p < 0.001$. The sera from Iztapalapa reacted to nine serotypes. **Conclusion**: The 28.2% of seropositivity indicates *Leptospira* transmission in the canine population that was studied could exhibit a potential public health risk.

Keywords

Leptospira, Leptospirosis, Canicola, Icterohaemorrhagiae, Dogs

1. Introduction

Leptospirosis is a globally distributed bacterial zoonotic disease and a serious health hazard around the world caused by pathogenic spirochaetes of the *Leptospira* genus that affects many wild and domestic animal species, such as rats, dogs, pigs, bovines, which are reservoirs, disseminating agents and infection sources for humans [1]-[5].

Infected dogs and rats are the main source of infection for humans [6]. Serologic tests conducted on canine population in different urban environments report a 24.9% seroprevalence in Michigan, USA. [7], 29.4% in Milan, Italy [8], 10.5% in Itapema, Brazil [9], 41.1% in Cali, Colombia [10], 14.6% in Trinidad and Tobago [11], and 22% in Guelph, Canada [12]. The most common serotypes in urban environments are: *Canicola*, *Icterohaemorrhagiae, Grippotyphosa*, and *Pomona* [6] [10] [12] [13]. Previous studies performed in Mexico City report serologic frequencies of 22% to 76.6%, with *Pomona, Canicola* and *Icterohaemorragiae* as the most common serovars (Rivera *et al*., 1999; Luna *et al*., 2004). Infected animals eliminate the spirochaetes through urine for months or years, and occasionally through vaginal secretions, thus contaminating soils and water [13] [14] [15].

Leptospirosis can be found in dogs of any age, predominantly in males, probably due to their sniffing habits and licking female's genitalia. The bacterium enters the body through the nasal and oral mucosa, conjunctiva or through open skin wounds. *Leptospira* is capable of producing severe renal and hepatic disease in the infected host; it can produce a rupture of erythrocytes and obstructive hepatitis, which gives mucous membranes the characteristic yellow color. The clinical outcome varies and depends on the animal's age, immunologic state and on the serovar's virulence. Diagnosing canine leptospirosis is not easy given that leptospiras can affect multiple organ systems, resulting in an extensive variety of clinical presentations, which range from minor to severe and frequently become chronic and without a characteristic symptomatology. The diagnosis is only confirmed once the bacterium is identified or isolated in clinical tests and/or shows high levels of anti-*Leptospira* antibodies, indicative of an active infection against one or more *Leptospira* serotypes. A Microscopic Agglutination Test (MAT) is the "gold standard" in detecting specific anti-Leptospira antibodies [16] [17] [18].

This study's objective was to determine the prevalence of anti-Leptospira antibodies to 13 serotypes in dogs captured in four zones of Mexico City, contained within the corresponding Canine Control Centers and analyze variables associated with the studied animals.

2. Material & Methods

2.1. Study Universe

A descriptive study was performed in February and March 2014, to determine the pre-

valence of different *Leptospira* serotypes on Mexico City's stray dog population. Blood samples were collected from dogs confined to four Canine Control Centers (CCC) in the districts of Coyoacan, Iztapalapa, Iztacalco and Benito Juarez, without discriminating for age, breed or sex. The subjects were divided into three age groups to analyze the results: Group 1, less than a year old, Group 2, 1 to 6 years, and Group 3, more than 6 years. The animals' age was calculated through their dental development according to the criteria provided in the odontogram CANINE [19].

2.2. Blood Sample Acquisition

Five milliliters of blood were taken from each animal by venipuncture and collected in sterile tubes without anticlotting agents. The serum was separated by centrifuging at 500 g for 10 min, and stored at 4˚C until analyzed.

2.3. Immunology Test

The sera were analyzed using a MAT test; the internationally referenced technique for the detection of anti-*Leptospira* specific antibodies, to which the diagnostic sensitivity and accurateness of the other techniques are compared and evaluated [14] [16] [18]. The test was standardized with the different antigens. Sera were considered positive when presenting agglutination at a dilution titer of 1:100 or higher.

2.4. Antigens

The antigens used corresponded to the following serotypes and strains: *Icterohaemorrhagiae* (RGA), *Grippotyphosa* (Moskva V), *Canicola* (Hond Utrecht IV), *Bratislava* (Jez Bratislava), *Pomona* (Pomona), *Tarassovi* (Perepelicin), *Wolffi* (3707), *Hardjo* (Hardjoprajitno LT 1085), *Pyrogenes* (Salinem), *Icterohaemorrhagiae* (Palo Alto), *Portlandvere* (Sinaloa ACR), *Hardjo* (Hardjoprajitno H-89). The last three were isolated in Mexico.

2.5. Statistical Analysis

Data were analyzed using SPSS and JMP 5.1 software. The association between the different variables and sero positivity was carried out using a chi-squared test.

3. Results

The 117 samples were collected from canines confined to the CCC of Coyoacan, 38; Iztapalapa, 34; Iztacalco, 31; and B. Juarez, 14.in Mexico City. The average age was 45 months (2.69 SE) with a range from 0.25 to 120 months; 61 (52%) males and 56 (48%) females. When distributed according to age groups, 28 were younger than a year (Group 1), 75 between 1 and 6 years (Group 2), and 14 were older than 6 years (Group 3).

Of the sera, 28.2% (33/117) were positive; 74% of the positive sera coagglutinated with two or more serotypes. Table 1 shows the results of the dilution titers obtained for the *Leptospira* antigens using the MAT technique for the 117 serums analyzed. The first

Table 1. Dilution titers obtained using MAT for 13 *Leptospira* serotypes in the 117 canine sera obtained in Mexico City.

	Dilution Titers							
Serotypes and Strains	100	200	400	800	1600	3200	Total	%
Icterohaemorrhagiae (RGA)	5	3	1	-	-	-	9	12.1
Icterohaemorragiae (Palo Alto)[a]	7	8	-	-	-	-	15	20.3
Pyrogenes (Salinem)	1	4	-	-	-	1	6	8.1
Griptotyphosa (Moska V)	-	1	-	-	-	-	1	1.4
Canicola (Hond Utrech IV)	5	10	2	1	-	2	20	27.0
Pomona (Pomona)	-	-	-	-	-	-	0	0
Hardjo (Hardjo-bovis LT1085)	1	-	-	-	-	-	1	1.4
Hardjo (Hardjoprajitno)	-	-	-	-	-	-	0	0
Hardio (hardioprajino H-89)[b]	1	-	-	-	-	-	1	1.4
Wolffi (3705)	2	-	-	-	-	-	2	2.7
Tarassovi (Perepelicin)	-	-	-	-	-	-	0	0
Bratislava (Jesbratislava)	6	-	1	-	1	-	8	10.8
Portland-vere (Sinaloa-ACR)[c]	10	-	1	-	-	-	11	14.8
Total	38	26	5	1	1	3	74	100.00
(%)	(32.4)	(22.2)	(4.2)	(0.8)	(0.8)	(2.5)	(63.2)	

[a]Serotype Icterohaemorrhagiae (Palo Alto strain) isolated in Mexico; [b]Serotype Harjo Hardjoprajitno (H-89 strain) isolated in Mexico; [c]Serotype Portland-vere (Sinaloa-ACR strain) isolated in Mexico.

column shows the *Leptospira* serotypes and strains used in the study. The serotypes with the highest percentage of seropositivity were: *Canicola*, 27%; *Icterohaemorrhagiae* (Palo Alto), 20.3%; *Portland-vere* (Sinaloa ACR), 14.8%; *Icterohaemorrhagie* (RGA), 12.1%; and *Bratislava*, 10.8%. There were also five sera with very high dilution titers, suggesting active infection. One reacted with the *Canicola* serotype at a 1:800 titer, another with *Bratislava*, 1:1600; and three sera at a 1:3200 titer, one reacted with *Pyrogenes* and two with *Canicola.* These three serotypes: *Canicola*, *Pyrogenes,* and *Bratislava* were found to be statistically significant ($p < 0.001$).

Table 2 shows the sera that were seropositive to the different serotypes and *Leptospira* strains by study area. The samples obtained in the districts of Coyoacan, Iztapalapa and Iztacalco resulted seropositive to 10 serotypes and 66 coagglutinations. In Iztapalapa, 9 serotypes and 27 coagglutinations were detected, with *Canicola* as the prevalent serotype as in Coyoacan and Iztacalco. The serotypes that were identified in the four study areas were: *Pyrogenes*, *Canicola Portland-vere* and *Icterohaemorrhagiae* (Palo Alto), whilst *Pomona* and *Hardjo* (Hardjoprajitno) resulted negative. The most frequent coagglutinations pattern in the same animal was *Canicola-Icterohaemorrhagiae* (Palo Alto) (n = 13) followed by *Canicola-Portland-vere* (Sinaloa ACR) (n = 10), and *Portland-vere* (Sinaloa ACR)-*Icterohaemorrhagiae* (Palo Alto) (n = 10).

Table 3 shows the distribution of the positive sera to the different serotypes according to age group and study area. The presence of anti-*Leptospira* antigens in Group 2's (between 1 and 6 years old) sera was statistically significant (p = 0.0001). The largest percentage, 75.8%, of seropositive dogs was found in this age group (25/33).

Table 2. Sero positivity to *Leptospira* serotypes from the 117 canine sera obtained in Mexico City according the study area.

Serotypes and strains	StudyArea				Total reactions	
	Coyoacán (n = 38) positives	Iztapalapa (n = 34) positives	Iztacalco (n = 31) positives	B. Juárez (n = 14) positives	Total (n = 117) positives	%
Icterohaemorrhagiae (RGA)	2	4	3	0	**9**	**12.1**
Icterohaemorrhagiae (Palo Alto)[a]	5	5	2	3	**15**	**20.3**
Pyrogenes (Salinem)	3	1	1	1	6	8.1
Griptotyphosa (Moska V)	0	0	1	0	1	1.4
Canicola (HondUtrech IV)	7	6	5	2	**20**	**27**
Pomona (Pomona)	0	0	0	0	0	0.0
Hardjo (Hardjo-bovis LT1085)	0	1	0	0	1	1.4
Hardjo (Hardjoprajitno)	0	0	0	0	0	0
Hardio (hardioprajino H-89)[b]	0	1	0	0	1	1.4
Wolffi (3705)	0	2	0	0	2	2.7
Tarassovi (Perepelicin)	0	0	0	0	0	0
Bratislava (Jes Bratislava)	3	3	2	0	**8**	**10.8**
Portland-vere (Sinaloa-ACR)[c]	4	4	1	2	**11**	**14.8**
Total	25	27	14	8	74	100.0
(%)	(65.7%)	(79.4%)	(45.1)	(57.1)	(63.2%)	

[a]Serotype Icterohaemorrhagiae (Palo Alto strain) isolated in Mexico; [b]Serotype Harjo Hardjoprajitno (H-89 strain) isolated in Mexico; [c]Serotype Portland-vere (Sinaloa-ACR strain) isolated in Mexico.

Table 3. Distribution of *L. interrogans* seropositive dogs by age group and study area. In Mexico City.

StudyGroups*	Studyáreas					
	Coyoacán	Iztapalapa	Iztacalco	B. Juárez	Total	%
Group 1	1	2	1	0	4	12.1
Group 2	9	8	6	2	25	75.8
Group 3	0	2	1	1	4	12.1
Total	10	12	8	3	33	100
(%)	(30.3)	(36.3)	(24.2)	(9)	(100)	

*Group 1) ≤ 1 year; Group 2) 1 to 6 years; Group 3) > 6 years.

4. Discussion

Little attention has been shown towards leptospirosis, a zoonotic disease, in the metropolitan area of Mexico City, mostly due to the fact that the disease is considered to present itself predominantly in rural areas. However, the 28.2% of seropositivity that was demonstrated by this study is indicative of leptospirosis beginning to circulate among the stray dog population of the metropolis, presenting a significant public health risk. A risk that is intensified in the district of Iztapalapa, where seropositivity was present for 9 of the 13 serotypes included in this study.

The percentage obtained by this study is consistent with the 22% reported by Moles [20] in a study carried out on dogs in Iztapalapa, nevertheless, this percentage is lower than the 76.6% reported by Cisneros [21] for canines in the same district. This disparity in frequencies could be related to the canine population type researched and/or the time of year when the studies were carried out. Leptospira is considered to be a seasonal zoonosis with higher incidence during the rainy season, a factor that should be considered when presenting a clinical case [15] [22] [23] report an average seropositivity level of 30.5% in a longitudinal study performed in three seasons in which the following seropositivity data were obtained: 41.4% in spring, 33.9% in summer, and 25.2% in autumn, with *Canicola* and *Icterohaemorrhagiae* as the most frequently found serotypes [22]. The present study was performed in winter; hence, it was considered that the obtained percentage would be related to the climatological characteristics of Mexico City during this season.

The most frequent serotypes in this study were *Canicola* and *Icterohaemorrhagiae* with some sera presenting dilution titers of 1:600 and 1:3200, respectively. Both are mentioned in the literature as the dominant serotypes in urban environments [3] [7] [10] [12] [17]. However, our results indicate that the *Portland-vere*, a serotype included in the *Canicola* serotype, is becoming an important serotype in the canine population of Mexico City.

According to Adesiyuncriteria [11], for determining leptospirosis infection, 5 dogs with dilution titers between 1:800 and 1:3200 at the moment of the study coursed with acute leptospirosis, and leptospipuria could contaminate their surroundings, facilitating the disease's transmission to other mammals, such as rats, dogs and even humans. The same authors mention that low titers of 1:100 and 1:200 are considered to be indicative of immunological anamnesis due to a previous infection of the animal. The importance of performing seroepidemiological studies in open population, is that the evaluation of the serology among several individuals, includes a low cost strategy and exhibit the experience of the contact between the host studied and parasite antigens.

Llamas *et al*., 1991 [24] comment that the most frequent coagglutination is found between *Canicola* and *Icterohaemorrhagiae* serotypes. The *Canicola-Icterohaemorrhagiae* (Palo Alto) coagglutination was also the most common pattern found in this study, along with *Canicola-Portland-vere* (Sinaloa ACR).

It is worth mentioning that the highest number of seropositive animals to the different *Leptospira* serotypes was found in the district of Iztapalapa, where the highest

agglutination titers corresponded to *Canicola*. This serotype is especially prevalent in urban areas where regular contact between stray dogs occurs. This result is perhaps related to the fact that this area holds the largest food supply market in the metropolitan area of Mexico City, where the continuous arrival of trucks filled with all sorts of food from the country's interior could foster the transport of leptospirosis infected rodents with the *Hardjo* (Hardjobovis), *Hardjo* (Hardjoprajitno) and *Wolffi* serotypes which all gave negative results in the sera obtained from the districts of Coyoacan, Iztacalco and B. Juarez. In addition to this, the immense amount of trash and food waste that is produced daily leads to the spread of mice and rats, as well as a high concentration of canine population. In which asymptomatic dogs have a relevant epidemiological role, as they are the main disseminators and contaminants of the soil and water with their urine. As known, the major source of human infection is the direct exposure to the infected animals' urine or contact with water and/or soil contaminated with urine; thus, municipal cleaning personnel, longshoremen, dairy farmers, butchers, refrigeration and sewage workers, among others, are the populations most likely to become infected in this district. On the other hand, the authors emphasize the epidemiological importance of knowing each of the different sero groups of the different *Leptospira* serovarieties, as well as local isolations [12] [25] [26] [27]. In this investigation, the most frequent sero groups were *Canicola*, which includes the *Portland-vere* (Sinaloa ACR) serotype, and the *Icterohaemorrhagiae,* which includes the Palo Alto and RGA strains. The importance of reporting the local isolations consists in the fact that they need to be considered when designing the regional strategies for leptospirosis vaccination.

The animals' age is an important factor in the dissemination and transmission of leptospirosis, as young animals are the most vulnerable to acquiring the disease. Therefore, it is important to highlight the amount of seropositive animals in Group 2, where the study's most statistically significant result was found ($p < 0.001$). The animal's reproductive age and the *Leptospira* seropositivity are possibly related to sniffing habits and the licking of external genitalia by infected males and females in heat [14] [21]. However, older dogs are more likely to have been exposed to leptospirosis at some point and, therefore, are likely to present antibodies for different serotypes due to immunological anamnesis [11].

The percentage of serological positivity to *Leptospira* antigens obtained in this study indicates that *Leptospira* is being transmitted among the canine population analyzed with a consequential risk to public health, as Rubel [28], reported in 1997, and pointed out that stray dogs are the number one factor for the transmission of human leptospirosis, and that the frequent contamination on the streets and infected animals' urine puddles constitute a microenvironment that is favorable for the survival of some *Leptospira* serotypes.

In Mexico, leptospirosis prevention and control requires cooperative action from the public, private, and social sectors, by means of advocating health, medical attention, basic sanitation, and training medical and epidemiologic personnel [16]. It is recommended to take advantage of the financial and human resources being employed for the

current campaign for anti-rabies vaccination, to design and apply vaccines against canine leptospirosis keeping the serotypes identified in our population in mind [12]. It is necessary to develop informational programs about this disease. Veterinarian clinics and hospitals, as well as animal shelters and pet stores, among others, are the spaces where an anti-leptospirosis vaccine should be mandatorily applied and where veterinarians play an important role in educating the community about this zoonosis [29]

The epidemiological studies allow the improvement of prevention measures and control programs in order to favour the eradication of zoonotic diseases in human and animal populations.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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