

Prevalence and risk factors associated with serovars of *Leptospira* in dogs, related human seropositive

Abstract

To determine the seroprevalence and the main risk factors associated with serovars of *Leptospira* in dogs from Culiacan, Sinaloa, we obtained serum from the blood samples of 106 dogs, related humans seropositive (blood donors of public hospitals). The samples were stored at -40 °C until use and were analyzed by the microbiology laboratory of the Centro Nacional de Sanidad Animal (CENASA) using the leptospirosis microscopic agglutination test (MAT). An epidemiological survey was conducted in order to identify risk factors. Statistical analyzes were done using the chisquare test. The risk (OR: odds ratio) and the confidence intervals were calculated using the logistic regression model. A value of $P < 0.05$ was considered statistically significant. The prevalence of *Leptospira* was 17% (18/106), and we identified eleven serovars and their respective frequency: wolffi (66.6%), bratislava (22.2%), australis (16.6%), canicola (11.1%), grippotyphosa (11.1%), pyrogenes (11.1%), hardjo (5.5%), icterohaemorrhagiae (5.5%), pomona (5.5%), hebdomadis (5.5%), shermani (5.5%). The risk factors associated with the detection of antibodies to this bacteria, based on epidemiological surveys, include the sex of pets female ($P < 0.05$; OR=2.9) and the dogs who stay at home and have access to the street was marginally significant ($P = 0.06$).

Keywords: leptospirosis, *leptospira*, dog, prevalence, risk factors, blood donors

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Introduction

Leptospirosis is the most widespread zoonotic disease in the world, with great economic and health importance. Infection is typically transmitted through direct contact of oral or nasal mucosa, with contaminated urine or water, and dogs are at risk of infection from drinking contaminated water.^{1,2} Dogs play an important role as potential indicators of areas with high endemicity for leptospirosis. Thus, recognizing and preventing canine leptospirosis has implications for human health as well as dogs.³ This disease is caused by a spirochete of pathogenic strains of the genus *Leptospira*, affecting wild and domestic animals, as well as man. Despite being a treatable disease, human leptospirosis is a significant public health problem.

Leptospire are classified into pathogenic, non-pathogenic, and intermediate species based on DNA hybridization. The old phenotypic classification system based on agglutination absorption test identified approximately 250 serovars among the *Leptospira* species and serogroups.⁴ Humans are incidental hosts and get infected by exposure to an environment contaminated with the urine of an animal reservoir. Leptospire are strictly aerobic microorganisms. Morphologically, they are spirochetes that are approximately 0.1µm wide and 6-15µm long, with flexion, translation, and propulsion movements, as well as active undulation. These bacteria are Gram-negative and divide by binary fission. *Leptospira* spp. are spirochetes that can rapidly move through the blood, reaching the kidneys, heart, lungs and liver. These bacteria damage tissues while moving within organs and can cause multiorgan failure in a few weeks.^{5,6}

Rodents are the most frequent reservoirs, with wild carnivores,⁷ no mammal can be excluded as a possible host.⁸ The pathogenic *Leptospira* lives in the proximal renal tubules of the kidneys of carriers,^{9,10} other tissues and organs may serve as a source of infection. From the kidneys, leptospire are excreted into the urine

and can contaminate mud, water, streams and rivers¹¹ for this reason; this disease is occasionally epidemic outbreak, especially in situations of great abundance of rodents and floods.¹² The diagnosis of leptospirosis is performed by various methods, however, plate micro agglutination (MAT) is regarded as the gold standard. The reactions determine the presence of agglutinating antibodies against the tested serovars.¹³ Antibodies generally appear between the sixth and twelfth day of infection and increase rapidly until the second or fourth week, subsequently manifesting a gradual decline of levels.¹⁴

The main source of infection for animals, especially dogs, is the urine of asymptomatic carrier animals (dog to dog), as well as vectors, rodents being a natural reservoir.^{15,16} The serovars icterohaemorrhagiae and pomona produce hemolysins, which are responsible for hemoglobinuria. In the case of the serovar icterohaemorrhagiae, it causes severe jaundice in dogs. Person-to-person transmission is exceptional, but several cases of congenital infection have been described. Furthermore, a case of transmission through breast milk has been described in the United States. In Mexico, serological studies have been carried out in dogs from various states of the country. Moles et al.¹⁷ analyzed sera from a total of 218 dogs from the anti-rabies center of Culhuacán, Mexico City, and found that 28.44% were seropositive to one or more serovars; *Leptospira canicola* was the most prevalent, with 22% seropositivity. Flores & Solana,¹⁸ observed a frequency of seropositivity of 61.7% in all dogs studied. In the same year, Garcia & Ibarra,¹⁹ found 41.5% seropositivity in dogs from Toluca, in the State of Mexico. Luna,²⁰ analyzed a total of 485 sera from dogs in the area of Naucalpan, State of Mexico, and found 48.4% seropositivity. The main source of infection for animals, and dogs in particular, is the urine of asymptomatic carrier animals, and vectors, of which the most important are rodents, due to their capacity to act as natural reservoir for the bacteria. Dogs are considered the most important domestic species in the transmission

of leptospirosis to man.²¹ In the state of Sinaloa, this disease has been diagnosed in humans, ruminants and pigs.²² The real extent of the disease is unknown, as is the presence of *leptospira* serovars in dogs. Thus, it is important to determine the seroprevalence of the disease and to identify risk factors associated with serovars of *Leptospira* Interrogans in dogs of Culiacán, Sinaloa, this information could help find alternatives for controlling the disease. The state of Sinaloa is endemic zone of Leptospirosis, and occupies the first National place in mortality by this disease with 117 deaths in the period 2005-2014 (48% of lethality). As for morbidity, it occupies the third national place with 201 cases in the mentioned period below only the states of Tabasco and Veracruz.²³ Leptospirosis in dog is caused by *L. canicola* and *L. icterohaemorrhagiae*, these two serovars are considered in some areas, the most important in this species.^{24,25} Other serovars are considered accidental transmitted by wildlife,²⁶ it manifests mainly in subclinical form, so that the serological findings are more frequent than the manifestation of the disease, however when it is present, it can cause death, the recovered animals remain in the carrier for months or years.^{27,28} In 1916, the first report published highlighting the importance of canine leptospirosis in public health by observing Weill's disease in two individuals who had been in contact with an icteric dog.^{29,30} The dog has been identified as the most important domestic species in the transmission of leptospirosis to man.^{27,29,31,32}

Infection in dogs by serovar *canicola* is considered the most common, serovar *icterohaemorrhagiae* is less frequent and is related to the rats as carrier and transmitter.³³⁻³⁶ Leptospirosis can be transmitted after a subclinical or clinical infection, as well as in the last stage of acute disease and the chronic phase, since infected dogs excrete leptospire through the urine. The quantity of leptospire is greater in the first weeks post-infection and elimination can last up to four years, thus achieving the transmission of animal to animal and animal to human.^{27,37} The habits of dogs (sniffing, lick, cortege), and the places where they habit favor the transmission of infection and dog-to-dog disease.³⁸⁻⁴⁰

Materials and methods

This study was conducted in the city of Culiacán, Sinaloa, Mexico, located at 24° 48' N and 107° 23' W, 60 meters above sea level; the climate of the region is classified as semi-dry, with a very warm average temperature of 25.5°C, maximum temperatures of 45 °C in the months of July and August, minimum temperatures of 7°C in December and January, and an annual rainfall of 671.14mm. Blood samples were collected from 106 canine in domicile and colonies where seropositive blood donors from public hospitals in Culiacán, Sinaloa were identified, the dogs not vaccinated against leptospirosis. Before taking the blood samples, we applied a questionnaire to the pet owners in order to obtain their address and information related to the conditions and characteristics of the place where the dogs lived; the questions were directly related to the epidemiological variables that are determining factors in the transmission of leptospirosis.

We also asked for their authorization to take the blood samples through an informed consent form; the samples (3ml) were obtained by puncturing the jugular vein, were deposited in a vacutainer tube without anticoagulant and centrifuged for five minutes at 1008g. The blood was free of pollutants, not hemolyzed, and the serum samples thus obtained were frozen at -40°C in an ultra-freezer. Once collected, the samples were taken to the laboratory of the National Animal Health Centre in Tecamac, State of Mexico, where they were processed using

the Microscopic Agglutination Test (MAT), with specific reactions for each serovar; we used a panel that included the following serovars: *ballum*, *canicola*, *hardjo*, *pomona*, *pyogenes*, *icterohaemorrhagiae*, *bratislava*, *wolffi*, *australis*, *grippotyphosa*, *hebdomadis* and *shermani*, 106 samples were taken in vacutainer tubes without anticoagulant; they were centrifuged for five minutes at 1008g to obtain serum, which was then frozen. The cut-off points of the tests considered titers of 1:100 or greater as positive. The questionnaire asked for the name of the owner, address, number of dogs per owner, sex, race, age, vaccinations applied place of habitation, types of floor, presence of rodents, water supply in the house, presence of pools, open water containers, presence of drainage and number of residents per dwelling. We performed a transversal analytical study. An epidemiological survey was applied to the people responsible for the dogs in order to identify risk factors. The homogeneity of proportions was tested using the chi-square statistic test; the risks (OR, odds ratio) and the confidence intervals were estimated using the logistic regression model. A value of $P < 0.05$ was considered statistically significant. All analyzes were performed using the statistical package Stata Intercooled V.13.1.

Results and discussion

We studied 106 serological samples from dogs associated with seropositive blood donors from public hospitals of Culiacán, resulting in 18 canine positive cases. The descriptive analysis of the survey data indicates the number of pets with owner was 96% as well as 201 dogs related to sampled animals and 444 humans. The age data sampled indicate that 53% were taken from animals older than two years, and 47% pets under that age. Regarding the type of dog breed, we found that 50% of the serum samples corresponded to mixed breeds, followed by crosses of small breeds such as poodle and chihuahua (29%); these two groups made up 79% of the samples, the sex of the sampled animals, 55% correspond to males and 45% to females. The place of habitation of the dogs was also considered an important factor related to the epidemiology of the disease; 94.34% of the dogs lived within the home, while 53% of the sampled dogs had contact with the street. We observed that 10.38% of the dogs remained inside the house, 38.68% inhabited both the interior and exterior of the house, and the majority (45.28%) remained only in courtyards. Regarding the type of floor in the place inhabited by the dogs, 36% corresponded to cement and 39% to cement and dirt; the rest of the animals stayed only on dirt (25%). With regard to the water supply in the homes, 88% have piped water, and 12% reported using drums or basins for water storage.

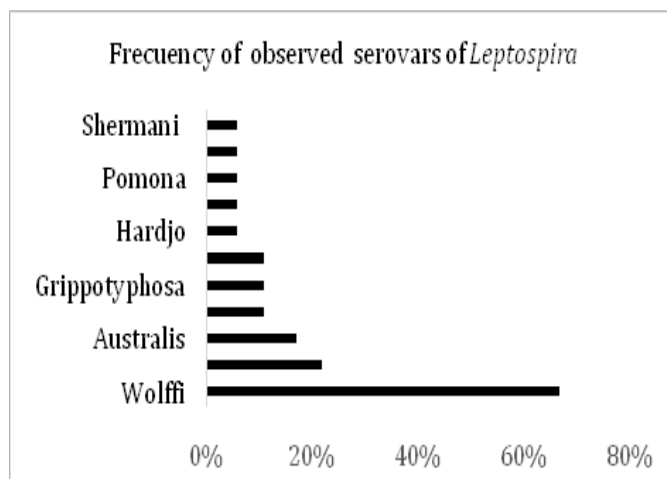
Drainage was present in 90% of the households. Regarding the number of people living in each household, the most frequent value was five residents per household (25.47%), followed by households with four inhabitants (23.58%) as well as other with less people. Rodents were reported in 75% of the households. A serological study of 106 samples from dogs living in the city of Culiacán, Sinaloa, indicated a prevalence of *Leptospira* Interrogans of 17%; the serovars detected were: *wolffi*, *bratislava*, *australis*, *canicola*, *grippotyphosa*, *pyogenes*, *hardjo*, *icterohaemorrhagiae*, *pomona*, *hebdomadis* y *shermani* (Table 1). The observed frequency for serovars, from high to low was: *Wolffi* 66.6%, *Bratislava* 22.2%, *Australis* 16.6%, *Canicola* 11.1%, *Grippotyphosa* 11.1%, *Pyogenes* 11.1%, *Hardjo* 5.5% *Icterohaemorrhagiae* 5.5%, *Pomona* 5.5%, *Hebdomadis* 5.5%, *Shermani* 5.5% (Figure 1).

Table 1 Serovars leptospira interrogans identified in dogs

Sample number	Serovar	Titles
CI-05	Canicola	0.319444
	Pomona	0.111111
	Bratislava	0.111111
	Grippotyphosa	0.319444
	Pyrogenes	0.597222
C4-04	Grippotyphosa	0.180556
	Icterohaemorrhagiae	0.111111
	Bratislava	0.180556
	Canicola	0.180556
	Pyrogenes	0.319444
C5-01	Wolffi	0.180556
	Wolffi	0.180556
C5-02	Wolffi	0.180556
C5-03	Wolffi	0.180556
C5-04	Wolffi	0.180556
C5-05	Wolffi	0.319444
	Australis	0.111111
C5-06	Wolffi	0.111111
C5-07	Wolffi	0.111111
	Shermani	0.111111
C5-08	Wolffi	0.180556
	Australis	0.111111
C5-10	Wolffi	0.111111
C5-15	Australis	0.111111
	Shermani	0.111111
	Wolffi	0.111111
C5-17	Wolffi	0.111111
	bratislava	0.180556
	Hebdomadis	0.111111
	Bratislava	0.111111
C5-18	Australis	0.111111
	Wolffi	0.180556
	Wolffi	0.180556
C5-19	Wolffi	0.180556
C6-01		
C8-03		
C8-13	Wolffi	0.111111
	Wolffi	0.111111
	Bratislava	0.111111

The epidemiological variables corresponding to neighborhoods (socioeconomic stratum), number of pets per household, dogs with or without owner age, breed, stay of pets type of floors water supply,

drainage, water stored in drums and basins and presence of rodents showed no significant differences ($P < 0.05$). There was a significant difference in the sex of pets female ($P < 0.05$; $OR = 2.9$) and the dogs who stay at home and have access to the street was marginally significant ($P = 0.06$). Serological positive samples were found in colonies with similar characteristics, of housing construction, access routes and similar socioeconomic status, unpaved streets with garbage presence in yards and street, with accumulation of "pots", with poor basic sanitation and poor management of stored water all these characteristics are risk factors for the disease.¹⁶

**Figure 1** Frequency of observed serovars of *Leptospira*.

The eleven identified serovars include those mentioned as very pathogenic to humans of their characteristics of hemolysins production mainly by serovars Icterohaemorrhagiae and Pomona, which are responsible for hemoglobinuria and vascular damage.⁴¹ Positive animals no presents clinical signs characteristic of the disease and their owners not mention sighth them sick. We also observed the presence of multiple infections in a single host(up to six serovars).The vaccines conventionally applied in veterinary clinics in Culiacan usually include four serovars: canicola, grippotyphosa, icterohaemorrhagiae and pomona but the serovars, bratislava, pyrogenes, shermani, ballum, australis, hardjo y hebdomadis which were found in the study and are pathogenic to humans, are not included, creating the potential risk that pet owners could get infected by these serovars.⁴² The observed prevalence (17%) is higher than that reported by Hernández et al.⁴³ In Culiacan in apparently healthy dogs from a general population, without association with seropositive humans. However, it is close to that reported for Mexico by several authors in other studies were conducted with animals suspected of leptospirosis or at high risk, including stray dogs, animals in veterinary clinics and rabies centers, and dogs in close coexistence with domestic animals such as cattle, pigs, goats and sheep.⁴⁴ The serovars vary from region to region,⁴⁵ observed a predominance of the serovars autumnalis, icterohaemorrhagiae and canicola in healthy dogs of the state of Washington, in Mexico in the state of Veracruz the serovars detect tarassovi, canicola pyrogenes y autumnalis with a seroprevalence of Sinaloa have detected 20 serovars between humans and animals, Studies in Mexico refer up to five serovars in dogs detected in the different regions studied⁴⁶ unlike the eleven serovars found in this study .The sex of pets was also statistically significant ($P < 0.05$; $OR = 2.9$),with females having a higher prevalence compared to males;⁴⁰ Ward et al.⁴¹ reported male

dogs used for work and shepherding are in greater risk of contracting the disease (Table 2). The dogs who stay at home and have access to the street was marginally significant ($P=0.06$), (Table 3) to have contact with other animals, urine or contaminated water, can be inferred there is a greater risk of infected with bacteria.^{15,16,43,46}

Table 2 Sex pets

Sex	Negative	Positive	Total
Female	36	12	48
Male	52	6	58
Total	88	18	106
%	83.02	16.98	100

pearson chi-square, 4.001; GL, 1; P value, 0.045

chi-square likelihood rate, 4.019; GL, 1; P value, 0.045

Table 3 Dogs have access to the street

Sex	Negative	Positive	Total
Home/street	43	13	56
home	45	5	50
Total	88	18	106
%	83.02	16.98	100

pearson chi-square, 3.272; GL, 1; P value, 0.070

chi-square likelihood rate, 3.389; GL, 1; P value, 0.066

Conclusion

In the city of Culiacan Sinaloa, there are seropositive dogs to antileptospira interrogans antibodies related to seropositive humans associated to risk factors in the streets that favor the contact with the bacteria while maintaining the possibility of contagion to other animals and humans. The prevalence observed was higher in dogs associated with human cases, than in the general population, in the same area studied, it is probable that these animals have an important role in the transmission to humans.

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Conflict of interest

The authors declare that there is no conflict of interest.

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