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Leptospira serovars and of contagion risks in humans and dogs from Culiacan City, in Sinaloa, Mexico

Serovariedades de *Leptospira* y riesgos de contagio en humanos y perros de la ciudad de Culiacán, Sinaloa, México

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ABSTRACT

This work aimed to identify the *Leptospira* interrogans serovar and the risk factors in humans and dogs in shared areas, 247 samples of human serum, were analyzed by the Micro Agglutination technique (MAT), prior informed consent from public hospital blood banks. To obtain information from the participants regarding the presence of dogs a survey was used. A total of 106 dog sera samples were collected inside and outside the homes of seropositive humans and analyzed by MAT. The statistical analysis consisted of a Pearson's *Chi* square test of homogeneity. The OR estimation was made using a simple logistic regression model using the Stata Intercooled version 13.1 program. Five serovars were detected in humans; from the highest to the lowest frequency, these were: Canicola, Icterohaemorrhagiae, Pyrogenes, Autumnalis, and Pomona. Eleven serovars were identified in dogs: Wolffi, Bratislava, Australis, Canicola, Grippotyphosa, Pyrogenes, Hardjo, Icterohaemorrhagiae, Pomona, Hebdomadis, and Shermani. All the serovar present in dogs were also observed in humans, the serovariety Autumnalis is not included in the dog search panel. The main risk factor for humans was job occupation ($p < 0.05$); in dogs, it was sex, with females being at greater risk ($p<0.05$, OR = 2.9) of infection. A total of 12 serovars were identified among humans and dogs.

Keywords: leptospirosis, Leptospira interrogans, prevalence, risk factors, humans, canines.

RESUMEN

La leptospirosis es la zoonosis más difundida en el mundo, en México es un padecimiento de notificación obligatoria, se relaciona al perro como el más importante en la transmisión al hombre. Para Identificar las serovariedades y factores de contagio en humanos y perros, se analizaron por la técnica de aglutinación microscópica (MAT) 247 muestras de suero humano de bancos de sangre. Mediante una encuesta epidemilógica, se obtuvieron datos relacionados al trabajo. Se recolectaron 106 muestras de sueros de perros, en domicilios de los humanos seropositivos analizándose por MAT. Se utilizó la prueba de *Ji* cuadrada de *Pearson*; las estimaciones de OR fueron realizadas utilizando un modelo de regresión logística simple, mediante el programa Stata Intercooled versión 13.1. En los humanos se detectaron anticuerpos antileptospira para cinco serovariedades, Canicola, Icterohaemorragiae, Pyrogenes, Autumnalis, Pomona. En los perros se identificaron anticuerpos contra once serovariedades; Wolffi, Bratislava, Australis, Canicola, Grippotyphosa, Pyrogenes, Hardjo, Icterohaemorragiae, Pomona, Hebdomadis y Shermani. Todas las serovariedades probadas en los caninos se observaron en humanos. Los factores de riesgo asociados para los seres humanos, fue su ocupación laboral $p<0.05$. Para los perros las hembras tuvieron mayor riesgo ($p<0.05$; OR= 2.9). En humanos y caninos hubo anticuerpos antileptospira, en total 12 serovariedades.

Palabras clave: leptospirosis, leptospira, prevalencia, factores de riesgo, humanos, caninos.

INTRODUCTION

Annually, more than one million cases of leptospirosis are estimated in humans, in the world with 59,000 deaths (Costa *et al.,* 2015), however, the exact number is unknown since epidemiological diagnostic and registration systems are difficult to apply (Berlios *et al.,* 2010; Jobbins *et al.,* 2014). The African continent has the highest incidence of endemic species of leptospira and the incidence rate in humans reached 95.5/100,000 inhabitants. In Asia, a seroprevalence of leptospirosis was observed in a Malaysian hospital in 8.4% of febrile patients (Noor *et al*., 2013), likewise in Korea, up to 12.4% of these patients were reported seropositive to *Leptospira* spp. (Kim, 2013), in India in a study carried out in febrile patients from 15 private hospitals and clinics, 4% of positive patients were registered by the microscopic agglutination test (MAT) (Basker *et al*., 2014). In 1916, the first report was recorded that highlighted the importance of canine leptospirosis in public health, by observing the disease in two people who had been in contact with a jaundiced dog (Jansen *et al.,* 2005). The human being is susceptible to a large number of serovars and the incubation period of the disease generally lasts from one to two weeks (García *et al.,* 2013). Although there are cases with incubation, as short as two days and others of more than three weeks (Bofill *et al.,* 1988), the signs and symptoms are nonspecific so they can be easily confused with other infectious processes of a bacterial or virus, such as dengue, influenza, malaria and brucellosis (Dircio *et al*., 2012; NOM-029-SSA2-1999; Adler and de la Peña 2011; Haake y Levett, 2015).

The main source of contagion for animals and in particular dogs is the urine of asymptomatic infected animals, due to their status as carriers, rodents are the most important natural reservoirs (Songer and Thiermann 1988). Not all serovars occur in the same geographic areas, but they affect humans in the same way (Torres *et al*., 2016). In dogs, infection by the serovar Canicola is considered the most common, the serovar Icterohaemorragiae is less frequent and is related to the rodents *Rattus rattus* and *Mus musculus* as carriers and transmitters (Torres, 2017; Socolovschi *et al.,* 2011; Calderon *et al*., 2014). The age, breed, and gender of the dogs represent risk factors for leptospirosis, and environmental characteristics, such as increased rainfall and ambient temperature, have been shown to be related to an increased incidence of the disease (Alton *et al.,* 2009). Contact with the street is an important risk factor for the canine population, adult males roam the streets more than females and puppies, so they have more contact with other animals. In addition, dogs have the behavior of marking territory, which causes them to release and spread the bacteria, which contributes to the transmission cycle (Raghavana *et al*., 2012). Research related to the etiology of leptospirosis in some species of domestic animals, especially dogs, indicates that these represent a risk of direct infection for humans, given their close relationship (Hernández *et al.,* 2017), the presence of dogs and infected rodents in urban ecosystems that are

largely under human control, appear less prone to natural seasonal variations by maintaining constant populations of these animals throughout the year (Himsworth *et al.,* 2014). Dogs play a very important role in the permanence of the bacteria in the environment (Calderón *et al.,* 2014; Rodríguez *et al.,* 2014), since, as pets, they are an important source for the transmission of leptospirosis for humans (Allwood *et al.,* 2014; Jiménez *et al.,* 2009), mainly the serovars Icterohaemorrhagiae and Canicola (Gualtieri *et al.,* 2012; Stokes *et al.,* 2007). Unlike human infection, the risk factors for leptospirosis in animals are not fully understood and further study is required (Kikuti *et al.,* 2012). To prevent leptospirosis in dogs, bacterins are used with the serovars Canicola, Grippotyphosa, Icterohaemorrhagiae and Pomona (da Silva *et al.,* 2010; Shekatkar *et al.,* 2010; Tian *et al.,* 2011; Barmettler *et al.,* 2011). The application of this biological is the responsibility of the owners, since it is not done in a massive way.

In Mexico, the first works on leptospirosis were carried out in 1920 in Yucatán state, by Noguchi and Kleiger, who for the first time isolated the spirochete from patients diagnosed with yellow fever, in 1954 Dr. Gerardo Varela carried out the first seroepidemiological surveys in Veracruz and Tampico later in other states of the Mexican Republic, in 1961 he observed cases in 19 entities, mainly Campeche, Tabasco, Colima and the Federal District (Varela *et al*., 1972). Studies of seroprevalences of leptospirosis have been carried out in humans and dogs in some regions of the country, in Chiapas the Center for Ecological Research of the Southeast mentions a seropositivity of 14.5% in humans, in domiciled dogs (with owner) 23% and in stray dogs 55% seropositive (Zavala *et al.,* 1984). The National Epidemiological Reference Institute (InDRE) conducted studies on the man-dog pairing of various regions of Mexico and found titers of 1: 160 or higher in 46% of the owners and in 62% of the animals (Zuñiga and Caro, 2012). As of the year 2000, the national registry of leptospirosis cases began in Mexico. When analyzing the data, it is observed that the state of Sinaloa has historically remained within the first places of human leptospirosis at the national level, during the period 2005-2016, 297 cases of leptospirosis were reported with 124 deaths (mortality of 41.7%), In addition, for 17 years the state has occupied the first national place in mortality from this disease (DGIS, 2017).

According to the results of human samples sent to the State Laboratory of Public Health of the State of Sinaloa and samples of dogs from Sinaloa, examined in the Animal Health laboratory in Tecámac Edo from Mexico, antileptospira antibodies have been identified in humans and dogs. The risk factors in the areas where both species coexist can determine the presence of the disease, therefore, the objective of this research is to identify the presence of antibodies to Leptospira serovars in humans and dogs, as well as contagion factors associated with the disease.

MATERIAL AND METHODS

The study was carried out in the city of Culiacán, Sinaloa, Mexico, a locality located at 24º 48 'North latitude and 107º 23' West longitude, at an average altitude above sea level of 60 meters; the region's climate is classified as semi-dry, very warm BS1 (h ') with an average annual temperature of 25.5 ºC with maximums of 45 ºC in July and August and minimums of 7 ºC in December and January. It presents an annual rainfall of 671.14 mm, with maximum rainfall in the months of July, August and September (INEGI, 2017). The sample size was determined taking into account a prevalence of antileptospira reactivity of 18% in the Mexican population (Benavides *et al.,* 2006; De Igartua *et al.,* 2005; Gavaldón, *et al.,* 1995) and it was calculated from according to the formula for estimating the proportions for finite samples (Wayne, 2006).

$$n = \frac{N * Z * p * (1 - p)}{d * (N - 1) + Z * p * (1 - p)}$$

Where
n = sample size
N = susceptible population, annual average blood donors (6,000)
Z = standard normal distribution value (1.96)
d = reliability coefficient (0.05)
p = proportion (0.18)
n = 219
Expected proportion of losses (R) 10%
Adjusted sample n = 243

Random sampling was conducted over a six-month period (June-December) in blood banks of two public hospitals. The samples were obtained from 247 blood donors from the city of Culiacán, without distinction of gender or age, accepted in accordance with the Official Mexican Standard NOM-253-SSA1-2012. Exclusion criteria, none, except for the same participant (self-elimination). Once the samples were obtained, they were centrifuged for 10 min at 1008 *Xg*, the serum was kept deep-frozen at -40 ºC until use. The study of the serological samples was carried out in the State Laboratory of Public Health of the State of Veracruz using the MAT test. In accordance with the Guidelines for the Epidemiological Surveillance of *Leptospirosis*, by means of Microscopic Agglutination of the Institute for Epidemiological Diagnosis and Reference (InDRE). The detection of agglutinating antibodies is observed from 4 dilutions (1:80, 1:160, 1:320, 1:640) however, this is not indicative of the disease, it only shows that the person was infected at some point with the bacteria. In accordance with the aforementioned guidelines, for

the confirmatory diagnosis, in humans, it was performed with a second sample obtained 15 days after the first.

Blood samples from donors were obtained with prior authorization by means of informed consent in accordance with the provisions of the Hospitals Ethics and Research Committee.

In follow-up to the cases of blood donors, with positive results, their homes were visited to proceed with the sampling of dogs, both inside the house and in the surroundings of it; 116 serological samples were obtained from dogs, not immunized with the bacterin against *Leptospira* spp. This was done with the prior informed consent of the owners or managers of the animals. Before taking the blood samples, a questionnaire was applied to those responsible for the pets to obtain information related to the conditions and characteristics of the place where they lived. Blood samples (3 ml) were obtained by puncture of the jugular vein, they were deposited in a vacuum tube without anticoagulant, they were centrifuged for ten minutes at 1008 *Xg* and serum samples were obtained, free of contaminants, not hemolyzed and they were kept at -40 °C in a deep freezer. Once collected, all the samples were transferred to the laboratory of the National Animal Health Center in Tecamac, State of Mexico, where they were processed using the MAT test, which detects the presence of agglutinating antibodies for each of the serovars tested (Gautam *et al*., 2010), a panel was used that included the following serovars: Ballum, Canicola, Hardjo, Pomona, Pyogenes, Icterohaemorrhagiae, Bratislava, Wolffi, Australis, Grippotyphosa, Hebdomadis and Shermani. The cut-off points of the tests considered titers of 1:100 or more as positive (OMS, 2008; NOM-253-SSA1-2012; Lau *et al.,* 2010).

Statistical analysis. To show whether there was a statistically significant difference between the different Leptospira serovars identified, the results were analyzed by Pearson's *Chi*-square test for homogeneity of proportions considering a statistically significant value of $P < 0.05$. The absolute estimates of odds ratio (OR) were performed using a simple logistic regression model. All analyzes were performed using the Stata intercooled version 13.1 statistical package.

RESULTS AND DISCUSSION

247 samples from blood donors from public hospitals were analyzed, resulting in 8 seropositive (3.23%). A trend of the cases was observed towards the northeast of the city of Culiacán, where three cases were registered in contiguous colonies, all located near improvised corrals in front of the main entrance of the municipal garbage dump. In these colonies, its inhabitants routinely coexist with pigs, sheep and goats, most of which are fed organic waste and which come to drink water from the same ponds as dogs, scavengers and rodents. In addition, all seropositive samples come from sectors of a low socioeconomic level, in these homes it is common to find accumulation of garbage, streets without pavement, plastic containers, poor management of stored water and water stagnations, these factors and the presence of reservoirs increase the risk of contagion of leptospirosis in humans and dogs (Lau *et al.,* 2010; Hernández, 2019). The serovars that reacted to the MAT test, from highest to lowest frequency of seropositives were: Canicola 4 (50%), Icterohaemorragiae 3 (37.5%), Pyrogenes 2 (25%), Autumnalis 2 (25%), Pomona 2 (25 %). The highest titers (1: 360) were observed for the serovars Canicola, Icterohaemorragiae and Pyrogenes (Table 1). These three serovars are considered the most pathogenic in humans. In an investigation carried out in dogs from the same city, Hernández *et al*. (2017) report the highest prevalences in the serovars, Canicola and Icterohaemorragia. For there to be anti-leptospira antibodies in humans, they had to have suffered an infection (OMS, 2008) since vaccines are not used in our country to prevent the disease.

Table 1. *Leptospira* serovars and titers identified in blood donors

Colony seropositive cases	Serovariety	Title
El Mirador	Canicola Icterohaemorrhagiae	1:80 1:80
Adolfo López Mateos	Canicola Icterohaemorrhagiae	1:80 1:160
Rafael Buelna	Canicola Pyrogenes	1:320 1:320
Pemex	Canicola Pyrogenes	1;80 1:80
Buenavista	Pomona	1:80
Fracc. Nueva Galicia	Pomona	1:160
Rosario Uzárraga	Autumnalis	1:80
Fracc. San Fernando	Icterohaemorrhagiae Autumnalis	1:320 1:80

The general information obtained from the surveys carried out with blood donors indicates the origin of the samples, 138 colonies and subdivisions of the city of Culiacán, which were divided into three strata, popular 83%, medium 15% and high 2%. Regarding their occupational group, the following was observed: 5% carry out activities within the area of agriculture and livestock, 63% carry out some type of trade and 32% are professionals, the number of residents per household on average was 4.3 people, 53% of the participants stated that they owned a dog. The characteristics of the dogs in the

study were as follows: 53% were mongrels and 47% of some breed, in addition, the majority were younger than 13 months (94%) and 70% had an anti-rabies vaccine, 64% correspond to males, of the total only 10% stay inside the house, 32% live in the yard and 58% enter and leave the house. 59% of the surveys reveal the presence of rodents in houses or streets, 94% of the people surveyed refer to the presence of other dogs in the street; where the dogs live indicates that 49% live in patios with cement floors, 30% on dirt, and 21% on both types of flooring. It was observed that 99% of homes have piped water, in 65% of homes there are drums or basins to store water, and 95% have drainage. The number of pets found in the homes indicates that the owners have an average of 1.4 dogs per home.

No statistically significant differences were observed for the presentation of the infection, in the variables related to: socioeconomic stratum, number of inhabitants in the home, whether they own a dog or not, age of the pets, race, sex, whether they live at home. In the patios in both spaces, there were also no significant differences related to the permanence in the street and the house, the presence of rodents, the presence of other dogs in the street, in the types of floors in which the pets live (cement, earth, both), if the homes have piped water, drums, sinks and drainage. A statistically significant difference (p=0.02) was observed for people who have a job as a job, in the specific case of seropositive blood donors they corresponded to the jobs of construction employees, warehouse workers, department store workers, electrician, merchant, locksmith and a public servant (Table 2). There is research carried out to determine the prevalence of leptospirosis in risk groups such as slaughterhouse workers, markets, garbage collectors, agricultural laborers, workers and others (Rahman *et al*., 2018; Alinaitwe *et al*., 2019; Azafar *et al*., 2018), but not in groups apparently unrelated to the disease, as is the case in this research.

Table 2. Labor occupation

Occupation	Negatives	Positives	Total
Agriculture and Livestock	79	0	79
Jobs	148	8	156
Professionals	12	0	12
Total	239	8	247
%	100.	100	100

Pearson's Chi-square = 4.823, GL = 2. Likelihood ratio Jj-square = 7.508, GL = 2. OR = 8.2.

The serological samples of the dogs (n = 106) were analyzed at the National Animal Health Center in Tecámac Estado de México (CENASA) using the MAT test, resulting in 18 seropositives located in five of the eight colonies sampled (62.5%), identifying Antibody titers for eleven serovars, from highest to lowest frequency were: Wolffi, Bratislava, Australis, Canicola, Grippotyphosa, Pyrogenes, Hardjo, Icterohaemorragiae, Pomona, Hebdomadis and Shermani. The antibodies tested in dogs include all those

identified in blood donors, the Autumnalis serovar, not included in the CENASA panel. In this study, the observed prevalence of 17% (18/106), in the samples of dogs living with seropositive humans, was higher than that reported by Hernández *et al*. (2017) in the same city in dogs of the general population, since they reported a prevalence of 9% (15/165). According to the surveys applied to the owners, the sampled dogs are related to 201 other dogs that could be at risk, as well as 444 humans. The age of the animals sampled indicates that 53% were older than two years and 47% were pets younger than that age. Regarding the type of dog breed, we found that 50% of the serum samples corresponded to mongrel dogs, followed by crosses of small breeds such as Poodles and Chihuahuas (29%); these two groups constituted 79% of the samples. The place of residence of the dogs was also considered an important factor related to the epidemiology of the disease, 94.34% of the dogs lived inside the home, while 53% of the dogs in the sample had contact with the street. In relation to the sex of the animals sampled, 55% correspond to males and 45% to females, this variable was statistically significant (P <0.05; OR = 2.9), females have a higher frequency compared to males (Table 3).

Results of studies carried out in Canada and the United States of North America, obtained by Ward et al., (2002, 2004), refer the opposite, observing a higher incidence in male dogs, (Ward *et al.,* 2002; Ward *et al*., 2004), however, Hernandez *et al.*, (2017) report a higher frequency in females in the City of Culiacán Sinaloa.

Table 3. Sex of the dogs

Sex	Negatives	Positives	Total
Females	36.00	12.00	48
Males	52.00	6.00	58
Total	88.00	18.00	106
%	83.02	16.98	100

Pearson's Chi-square = 4.001; GL = 1; P value = 0.045. Chi-square of the likelihood ratio = 4.019; GL = 1; P value = 0.045. OR = 2.9

The data of the serological tests, analyzed for the dogs that remain in the houses and have access to the street, were marginally significant (P=0.06) (Table 4). This may be due to contact with other animals, urine or contaminated water, for which there is an increased risk of infection with *Leptospira* (Alton *et al.,* 2009; Kikuti *et al.,* 2012).

The serovars observed in the eight positive cases in humans and canines in their corresponding areas are described below:
In the “San Fernando” fractionation, the blood donor presented antibodies to the serovars Icterohaemorrhagiae (1:320) and Autumnalis (1:80), in contrast the dog reacted to the serovars Grippotyphosa (1:200), Canicola (1:200), Bratislava (1:200), Hardjo (1:100), Pyrogenes (1:400), Icterohaemorrhagiae (1:100), in this dog antibodies were identified for six serovars, all these leptospires are the most frequent and the most

pathogens, grouped within the «interrogans complex» (Céspedes, 2005; Sun *et al.,* 2020), for humans the antibody titer of the serovar Icterohaemorrhagiae is higher than that observed in dogs, the serovar Autumnalis is not included in the test panel for canines.

In another case, colony "El Mirador" the blood donor gave seropositivity to two serovars, Icterohaemorrhagiae (1:80), Canicola (1:80), for the dog antibodies to the Wolffi serovar (1: 100) were observed.

Table 4. Dogs with access to the street

Dogs	Negative	Positive	Total
Home and street	43.00	13.00	56
Home	45.00	5.00	50
Total	88.00	18.00	106
%	83.02	16.98	100

Pearson's chi-square = 3,272 GL = 1; P value = 0.070. Chi-square of the likelihood ratio = 3.389; GL = 1; P value = 0.066

In the "Adolfo López Mateos" colony, human antileptospira antibodies were detected for the serovars Icterohaemorrhagiae (1: 160) and Canicola (1:80), no seropositive dogs were found. In these first three cases in seropositive humans, antibodies to the serovar Icterohaemorrhagiae were identified, considered one of the most pathogenic due to its characteristics of production of hemolysins and toxins that can trigger a severe phase of the disease known as hemorrhagic syndrome, jaundice fever or Weil's disease, it is the form of presentation with the worst prognosis and extreme severity (Carranza *et al.,* 2020).

Antibodies to the Canicola (1: 320) and Pyrogenes (1: 320) serovars were detected in the seropositive sample from the blood donor from the "Rafael Buelna" colony, seropositivity for antibodies to the Canicola serovars (1: 100), Bratislava (1: 400), Grippotyphosa (1: 100), Pyrogenes (1: 800), Pomona (1: 100), the titration for the Canicola serovar in humans is higher in relation to the canine Antibodies to the Pyrogenes serovar were observed in both samples, the observation of several serovars is notorious mainly in dogs, as well as being asymptomatic.

In the positive case of the “Pemex” colony, the serovars of antibodies identified in humans were Canicola (1:80) and Pyrogenes (1:80), for canines antibodies to the Wolffi serovars ranges of (1: 200 at 1: 400), Hebdomadis (1: 100), Bratislava (1: 100), Australis (1: 100), Shermani (1: 100). The antibodies observed do not coincide between humans and dogs, however, for all these serovars that are pathogenic for humans, determining proteins have been identified as virulence factors using molecular biology techniques (Martínez *et al.,* 2018).

In the colony "Rosario Uzárraga" antibodies to the Autumnalis serovar (1:80) were observed in humans, for the dog the serovars that reacted to the test were Wolffi (1: 100), Bratislava (1: 100). In the Buenavista and Nueva Galicia colonies in humans, anti-leptospira antibodies were detected for the Pomona serovars with titers of 1:80 and 1: 160, without finding seropositive dogs (Table 5).

Table 5. Serovars observed in blood donors and dogs

Colony positive blood donors	Serovarieties	Title	Positive dogs	Serovarieties	Title
El Mirador	Canicola	1:80	1	Wolffi	1:100
	Icterohaemorrhagiae	1:80			
Adolfo López	Canicola	1:80	0	not observed	
Mateos	Icterohaemorrhagiae	1:160			
Rafael Buelna	Canicola	1:320	1	Canicola	1:400
	Pyrogenes	1:320		Grippotyphosa	1:400
				Pomona	1:100
				Bratislava	1:100
				Pyrogenes	1:800
Pemex	Canicola	1:80	13	Wolffi	1:200
	Pyrogenes	1:80		Hebdomadis	1:100
				Bratislava	1:100
				Australis	1:100
				Shermani	1:100
Buenavista	Pomona	1:80	0	not observed	
Nueva Galicia	Pomona	1:160	0	not observed	
Rosario Uzárraga	Autumnalis	1:80	2	Wolffi	1:100
				Bratislava	1:100
San Fernando	Icterohaemorrhagiae	1:320	1	Canicola	1:200
	Autumnalis	1:80		Grippotyphosa	1:200
				Hardjo	1:100
				Bratislava	1:200
				Pyrogenes	1:400
				Icterohaemorrhagiae	1:100

CONCLUSION

Antibodies against *Leptospira* of 12 serovars were identified in living with humans and dogs in the same shared areas. The samples tested in the dogs include the serovars observed in humans and the contagion factors for the canines included sex (female), the dogs that remain at home with access to the street, were marginally significant. In humans, occupational occupation (trades) was a significant risk factor.

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