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Detection of reactive canines to *Leptospira* in Campeche City, Mexico

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ABSTRACT

Leptospira reactivity in stray and household dogs in Campeche as well as associated risk factors to the seropositivity in household dogs have been herein determined. The survey included 323 dogs, 142 of which were stray dogs and 181 household dogs. Nine *Leptospira interrogans* serovars were tested by the microagglutination test. Reactivity was 21.3 % (69/323), 17.2 % corresponded to household dogs and 26.7 % to stray dogs. *Leptospira* Canicola (29 %), *Leptospira* Hardjo (22.58 %), and *Leptospira* Icterohaemorrhagiae (16.12 %) were the most common serovars reacting against the serum of household animals, while *Leptospira* Canicola (15.78 %), *Leptospira* Icterohaemorrhagiae (13.15 %), and *Leptospira* Pomona (7.89 %) were those reacting in stray dogs. Results showed that all dogs have been in contact with different *Leptospira* serovars and outdoor exposure is the main infection risk factor.

Key words: seroreactivity, *Leptospira*, dogs, urban area

RESUMEN

Detección de caninos reaccionantes a *Leptospira* en la ciudad de Campeche, México. Se determinó la reactividad frente a distintos serovares de *Leptospira* de 142 perros callejeros y 181 perros domésticos de la ciudad de Campeche, así como los factores de riesgo asociados a la serorreactividad de los perros domésticos. Se utilizaron 9 serovares de *Leptospira interrogans* en la prueba de microaglutinación. La reactividad global a *Leptospira* fue del 21,3 % (69/323), alcanzó el 17,2 % en perros domiciliados y el 26,7 % en perros callejeros. Las serovariedades que reaccionaron a los sueros de caninos domésticos fueron *Leptospira* Canicola (29 %), *Leptospira* Hardjo (22,58 %) y *Leptospira* Icterohaemorrhagiae (16,12 %); las que reaccionaron a los sueros de perros callejeros fueron *Leptospira* Canicola (15,78 %), *Leptospira* Icterohaemorrhagiae (13,15 %) y *Leptospira* Pomona (7,89 %). Los resultados indican que todos los perros evaluados han estado en contacto con diferentes serovares de *Leptospira* y que la calle es el principal factor de riesgo para la infección.

Palabras clave: serorreactividad, *Leptospira*, perros, área urbana

Leptospirosis is a worldwide zoonosis that is frequently found in tropical areas where spread conditions are favorable; as a result it is considered a public health concern. Leptospirosis is caused by *Leptospira interrogans* affecting man and some wild and domestic animals, which include cows, pigs, horses, sheep, dogs, cats as well as rodents such as mice and rats; the latter being considered the main *Leptospira* reservoirs (14).

Transmission occurs by direct contact with contaminated urine or indirectly through soil and water which has been contaminated by the urine of the above mentioned animals. Even though rodents are considered the main reservoir, dogs could have epidemiological relevance due to their close association with man in non occupational environments.

Recent studies carried out in countries like Colombia have shown a prevalence of 20 % in household dogs (8) and in Aragua, Venezuela, a

prevalence of 100 % in stray dogs (4). On the other hand, in Mexico, a prevalence of 23 % was reported in household dogs in Jalisco (9), of 38.51 % in stray dogs in the north of Mexico City (6), whereas in the Southeast of Mexico, that is, Chiapas, a seropositivity of 23 % in household dogs, 55 % in stray dogs (15) and 30.5 % in reservoirs of livestock in the State of Yucatan (1). The objective of this study was to determine *Leptospira* reactivity in stray and household dogs in the City of Campeche, as well as the serovars and risk factors associated with seroreactivity in household dogs.

The State of Campeche is located at 20°51'N, 17°49'S, 89°9'E and 92°28'W on the Yucatan Peninsula, Mexico. Campeche, its capital city, lies along the coast line. It has annual temperature and precipitation averages of 28 ºC and 300 mm, respectively. The studied population included stray dogs captured by the Vector-Borne and Zoonotic Disease Department staff of the Health Ministry and evaluated by a veterinarian.

The sample size was based on a proportion of 30 % close to the average prevalence in the States of Chiapas and Yucatan. These states are located in the tropical regions of southeast Mexico, as Campeche is, favoring the growth and development of *Leptospira*. On the other hand, based on their epidemiological experience, experts from the Department of Health suggested the usage of 30 % prevalence to make an efficient use of resources for this study since there exists no difference between sampling at 30 % or 50 %.

Based on information from the Department of Vector-Borne and Zoonotic Diseases, the total canine population in the city is estimated at 57,000 canines. (Ing. Carlos Chi Tun, personal communication). The required sample size (n = 323) was determined by the proportion method, through the formula $n = Z^2$. p.q $/d^2$, where $z^2 = 1.96$ for 95 % confidence, p = 30 % prevalence, q = 1-p = 0.7 is the expected variability of the phenomenon to be studied, and d = 5 % is the precision grade.

Blood samples were taken from those household dogs whose owners had granted veterinarian permission, and had answered the relevant questionnaire. Canines vaccinated against *Leptospira* were excluded. A sample of 5 ml of venous blood was taken from all dogs. The samples were analyzed by the Biomed Research Center at the University of Campeche. The blood samples were centrifuged at 800 *x* g for 10 minutes to obtain serum, which was stored at -20 °C until processing.

A total of 9 *Leptospira interrogans* serovars: *L.* Pomona*, L.* Hardjo*, L.* Canicola*, L.* Tarassovi, *L.* Sejroe*, L.* Pyrogenes*, L.* Bataviae, *L.* Icterohaemorrhagiae and *L.* Grippotyphosa, were used as antigens for the microscopic agglutination test as these are the serovars most commonly studied in canines in Mexico. The cultures were donated by the Veterinary School at UNAM. Serum was positive when agglutination equal to or greater than 50 % of *Leptospira* was observed in a 1:100 diluted serum, using an antigen control for each serovar (11, 14).

Descriptive statistics were used to analyze data using frequency distributions. X^2 (chi square test) was used to determine the association between seropositivity and each of the qualitative variables compiled in the survey at a significant level of $p < 0.05$ and confidence intervals.

A total of 323 canine serums were studied, 181 of which (56 %) were from household dogs and 142 (44 %) were from stray dogs. The overall seroprevalence of leptospirosis was 21.3 % (69/323) positive at titres of 100, 200 and 400 for one or more serovars, 17.2 % (31/181) of which were household dogs and 26.7 % (38/142) were stray dogs. The highest titre was 400 which corresponded to an *L*. Canicola serovar, found in a stray dog serum.

The most commonly detected antibodies in household dogs were against *L.* Canicola 29 % (9/31), followed by *L.* Hardjo 22.58 % (7/31), *L.* Icterohaemorrhagiae 16.12 % (5/31), *L.* Pomona 9.67 % (3/31), *L.* Bataviae 9.67 % (3/31) and *L.* Grippothyphosa 3.22 % (1/31). Coagglutinations were observed in 9.67 % of positive serums, 66.66 % of which showed a titre of 100 and 33.33 % showed a titre of 200.

Regarding the stray dogs, the most frequent antibodies found were against *L.* Canicola 15.78 % (6/38), followed by *L.* Icterohaemorrhagiae 13.15 % (5/38), *L.* Pomona 7.89 % (3/38), *L.* Bataviae 5.26 % (2/38), *L.* Grippothyphosa 1.38 % (1/38) and *L.* Hardjo 2.63 % (1/38). Coagglutination was shown in 52.63 % of positive serums. The most frequent titre was 100 with 87 %, followed by 10.52 % at 200 and 2.63 % at 400. Serum agglutination was not observed against *L.* Tarassovi, *L.* Pyrogenes, and *L.* Sejroe serovars.

After evaluating the survey data obtained, where 7 variables were included as risk factors, it was found, according to the bi-factorial analysis, that the factors significantly associated ($p < 0.05$) with seropositivity were exposure of household dogs to the street, the location of ditches near the homes as well as coexistence with other dogs (The complete list of risk factors analyzed is shown in Table 1).

The study confirmed the presence of *Leptospira* reactivity in the canine population and determined a seroreactivity of 21.3 % (69/323) against diverse serovars. This reactivity was higher in stray dogs (26.7 %) than in household dogs (17.2 %). Similar studies in Aragua, Venezuela found 100 % (30/30) seroprevalence,

Table 1. Risk factors associated to *Leptospira* reactivity in a study performed on 181 household dogs

Factor	Exposition Factor	Serology against leptospirosis		RMP[3]	IC 95 %[4]	*p*[5]	X^{2}[6]
		Positive[1] (n = 31)	Negative[2] (n = 150)				
Street	Yes	25	37	12.7	4.84 - 33.32	0.0001	35.74
	No	6	113	1			
Ditch	Yes	15	34	3.2	1.43 - 7.13	0.0017	8.61
	No	16	116	1			
Permanent[7] surface drainage	Yes	1	4	1.28	0.13 - 11.07	0.431	0.03
	No	30	146	1			
Sporadic drainage	Yes	4	16	1.24	4.0 - 0.38	0.3	0.131
	No	27	134	1			
Other canines	Yes	28	91	6.05	1.76 - 20.78	0.0008	10.033
	No	3	59	1			
Other species	Yes	19	117	0.4	0.19 - 1.0	0.025	3.84
	No	12	33	1			
Rodent hunters	Yes	12	123	0.13	0.06 - 0.32	0.0001	25.4
	No	19	27	1			

[1] Positive: serum agglutination at a titre ≥ 100; [2] Negative: absence of agglutination or presence at a titre < 100; [3] RPM: Prevalence Momio Ratio; [4] IC95 %: confidence interval of 95 %; [5] *P*: level of significance $p < 0.05$; [6] X^{2}: Chi square; [7] permanent surface drainage: drainage of rain water or other to a natural drainage system or sewage system.

being *L.* Canicola*, L.* Icterohaemorrhagiae and *L.* Hardjo the most frequent serovars (4). In Cali, Colombia, 41.1 % seroprevalence was found and the most frequent serovars were *L.* Icterohaemorrhagiae*, L.* Hardjo*, L. G*ryppotyphosa and *L.* Canicola (7). In Maringa, Brazil a prevalence of 12.2 % was found in 335 canines, being *L.* Pyrogenes*, L.* Canicola*, L.* Hardjo, *L.* Pomona and *L.* Grippotyphosa the serovars found (3). In Itapema, Brazil a seroprevalence of 10.5 % was found in 590 canines and *L.* Pyrogenes, *L.* Canicola and *L.* Icterohaemorrhagiae serovars were detected (2). In Mexico City, the seroprevalence was 38.51 % (52/135) and the serovars detected were *L.* Canicola*, L*. Icterohaemorrhagiae, *L.* Hardjo and *L.* Pomona (6). The seroreactivity in stray dogs in this study differed from that in the study mentioned before; however, there is a concordance with the serovars in those studies with the exception of *L.* Pyrogenes. It was observed that seroreactivity varies from one region to another, where the climate can be an important factor that affects *Leptospira* prevalence (2).

In Manizales, Colombia, dogs observed by veterinaries, showed a seropositivity of 20.5 % (41/200), where the main serovars represented were *L.* Icterohaemorrhagiae*, L.* Grippotyphosa*, L.* Canicola and *L.* Pomona (10); in Valdivia, Chile there was a prevalence of 14.8 % (59/400) and the most

frequently found serovars were *L.* Ballum*, L.* Canicola and *L*. Icterohaemorrhagiae (11). In Michigan, USA, 1241 household dogs had 24.5 % reactivity to *L.* Grippotyphosa*, L.* Bratislava*, L.* Canicola*, L.* Icterohaemorrhagiae and *L.* Pomona (12). In another study carried out in Yucatan, *L.* Canicola*, L.* Icterohaemorrhagiae*, L.* Grippothyphosa and *L.* Pyrogenes serovars were detected (1). The serovars in our study are similar to those found in the above mentioned studies and seroprevalence is similar to that mentioned in those countries. This study had differences regarding *L.* Pyrogenes reactivity.

The concordance for serovars registered in all the studies mentioned could be due to the similarity between domestic and wild reservoirs such as dogs, rats, pigs, cows, raccoons and skunks that kept the *Leptospira* life cycle by infecting other animals. Nevertheless, the prevalence variation could be due to the ecological differences that exist among regions (2), as well as the methodology differences such as sample size and type of sampling methods.

Regarding the association of seropositivity with the variables studied, it was shown that the contact of household dogs with outdoor surroundings, nearby ditches and other dogs or species, is favorable for infection and spread of bacteria.

The most important risk factor for the canine population is outdoor exposure, as shown in the seropositivity found in stray as well as in household dogs with this kind of exposure. (13). Rats in ditches and drainages, as well as dogs in the surroundings, make both reservoirs important factors for transmission via environmental pollution due to their direct contact.

Regarding the seroreactivity found, the serums reacted preferentially to *L.* Icteohaemorrhagiae and *L.* Canicola transmitted by rats and dogs, respectively. The presence of these serovars in household dogs could have been due to the presence and coexistence of rats, mice and other backyard animals. On the other hand, in the case of stray dogs, some were captured wandering in garbage dump sites, where they could have eaten rats or been in contact with water contaminated by rodent urine. Hygiene and rodent control methods are paramount to reduce the infection risk by dogs. In addition, canines undergoing serological testing were found to have positive serology against *L.* Pomona whose main reservoirs are pigs as well as *L.* Hardjo and *L.* Grippothyphosa, found mainly in cattle. The presence of these serovars could be the result of several factors, one being the contact with water polluted by the urine of cattle and pigs or by their infected blood and tissues discarded in markets and slaughter houses. Another factor could be the contact with other animals as was reported in North America with serovars *L.* Grippothyphosa and *L.* Pomona that dogs acquire from raccoons and skunks found in the urban area (5). In a study done in Jalisco, Mexico, the most common serovars isolated in rats were *L.* Icterohaemorrhagiae, *L.* Grippothyphosa, *L.* Tarasovi and *L.* Hardjo (9). In another study, *L.* Bataviae was hosted by sewer rats. Considering the vagrant behavior of stray dogs, the environmental contamination risk is higher compared to household dogs. These results are a reminder that every pathogenic *Leptospira* has a specific or main host, and that, given the right circumstances, could spread infection to other animals.

Stray and household dogs in Campeche City showed serological reactivity to more than one serovar, which indicates the importance of the fact that canine populations are prone to be reservoirs and carriers of pathogenic *Leptospira.* More importantly though, is the potential public health risk due to their coexistence with humans.

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