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Frequency of canine leptospirosis in dog shelters in Veracruz, Mexico

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A cross-sectional study was conducted to determine the frequency of canine leptospirosis in 92 dogs from two shelters in the city of Veracruz, Mexico. The microaglutination test (MAT) was used for detecting antibodies against 12 serovars of *Leptospira interrogans* in dog serum. Of the 92 dogs, eight were positive, resulting in an overall frequency of leptospirosis of 8.6% ($CI_{95\%}$ 3.8 to 16.41). With reference to shelter, frequency was 8.3% (4/48; $CI_{95\%}$ 2.3 to 19.9) in shelter 1 and 9.0% (4/44; $CI_{95\%}$ 2.5 to 21.6) in shelter 2. The most frequent serovar of *L. interrogans* was Canicola. Frequency by sex of the dogs was 8.8% (4/45; $CI_{95\%}$ 2.4 to 21.2) for females and 8.5% (4/47; $CI_{95\%}$ 2.3 to 20.3) for males. In conclusion, leptospirosis is present in dogs housed in shelters in the city of Veracruz, Mexico.

Key words: Leptospirosis, dog, epidemiology, serovar.

INTRODUCTION

Leptospirosis is a zoonotic infectious disease of world-wide distribution that is endemic in tropical and temperate climates, with higher prevalence in tropical countries (Levett, 2001; Adler and de la Peña, 2010; Desvars et al., 2011). Leptospirosis affects humans, domestic and wild animals (Båverud et al., 2009), and the reservoirs are wild or domestic animals such as rodents, cattle or dogs (Kikuti et al., 2012). In urban areas, rodents (mostly rats) are the main carriers of the disease (Oliveira et al., 2012), whereas the dog is considered a dead-end host (Prescott, 2008; Reis et al., 2008). Nonetheless, due to their close contact, dogs pose a risk of infection for humans (Greene, 2006; Adler and de la Peña, 2010).

Leptospirosis is caused by several pathogenic serovars within the species *Leptospira interrogans* (Båverud et al., 2009; Adler and de la Peña, 2010). Rodents, particularly rats (*Rattus norvergicus*), are considered natural reservoirs of serovar Icterohaemorrhagiae, and domestic dogs are natural reservoirs of serovar Canicola (André-Fontaine, 2006). However, canine leptospirosis can be caused by other serovars such as Grippotyphosa, Pomona, Bratislava (Birnbaum et al., 1998; Ward et al., 2004; Greene, 2006; Ghneim et al., 2007), Australis, Autumnalis, Ballum, Bataviae and Hardjo (Ward, 2002), which have, among others, mice, pigs and cattle as reservoirs (Bolin, 1996; Birnbaum et al., 1998; Adin and Cowgill, 2000). In dogs, infection usually results from direct contact with urine from an infected animal, or indirectly from contaminated water or moist soil, where the bacteria can survive for several months (Adler and de la Peña, 2010; Raghavan et al., 2011). Acute leptospirosis is characterized by hepatic and mainly renal failure, and common manifestations include anorexia, vomiting, lethargy, jaundice, diarrhoea and bloody urine, and if not treated quickly it can lead to death (André-Fontaine, 2006; Minke et al., 2009). Although serovars Canicola and Icterohaemorrhagiae are usually responsible for acute leptospirosis, serovars Australis, Pyrogenes, Autumnalis and Grippotyphosa have also been found in this form of the disease (Adamus et al., 1997; André-Fontaine, 2006). Chronic

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Table 1. Frequency of canine leptospirosis in two shelters from Veracruz, Mexico.

Shelter	Total	Positive	Frequency	$CI_{95\%}$
1	48	4	9.0	2.5-21.6
2	44	4	8.3	2.3-19.9
Total	92	8	8.7	3.8-16.41

X_2= 0.06, P= 0.80.

Table 2. Frequency of canine leptospirosis with regard to sex in two shelters from Veracruz, Mexico.

Sex	Total	Positive	Frequency	$CI_{95\%}$
Female	45	4	8.8%	2.5-21.2
Male	47	4	8.5%	2.3-20.3
Total	92	8	8.7%	3.8-16.41

X_2= 0.09, P = 0.76.

leptospirosis is more difficult to diagnose because vaccinated animals can show signs of the disease (Adamus et al., 1997). A vaccinated dog may get infected when exposed to a highly infectious environment or a highly virulent strain (Andre-Fontaine et al., 2003; Klaasen et al., 2003), which could be the case of a dog shelter. Subclinical forms are more common and usually occur in chronically infected animals, which can be carriers for years or for life (OIE, 2004). Clinically recovered dogs can turn into asymptomatic renal carriers and become an important source of infection for humans (CDC, 1972; Trevejo et al., 1998). Seroprevalence of canine leptospirosis has been reported from 1.9% in shelter dogs to 35% in serological surveys(Meeyametal.,2006;Davisetal.,2008; Zwijnenberg et al., 2008). There are few studies on canine leptospirosis in Mexico, and seroprevalence reported ranged from 4.9 to 35% (Ortega-Pacheco et al., 2008; Jimenez-Coello et al., 2008; 2010). In Mexico, vaccination of pet dogs against leptospirosis is practiced by responsible owners, but in the case of shelters, most of the dogs that are taken in at these places are stray, and others have been neglected by their owners, so it is unlikely that these dogs have been vaccinated against leptospirosis. If these dogs are carriers of the disease, they may be a source of infection for their care givers or adopters. Therefore, the aim of this study was to determine the frequency of anti-*Leptospira* spp. antibodies in dogs from two shelters in the city of Veracruz, Mexico.

MATERIALS AND METHODS

A cross-sectional study was conducted in two dog shelters from the city of Veracruz, Mexico (19°12' North latitude and 96°07' West longitude). Climate is tropical and mean annual rainfall is 1500 mm.

These shelters are the only ones in the city and take in stray or unwanted dogs. A total of 92 adult dogs (shelter 1: n = 48; shelter 2: n = 44) were included in the study. The vaccination status of these dogs was unknown because most of them had been picked up as strays, and vaccination is not usually practiced in the shelters due to economic restraints.

Blood samples collection

One blood sample was collected via cephalic or jugular venipuncture from each of the 92 dogs to detect antibodies against *Leptospira interrogans*. Samples were kept in refrigeration and transported to the laboratory, where they were centrifuged at 3000 rpm for 10 min to separate the serum, which was kept frozen at -20°C until analyzed.

Serodiagnosis of leptospirosis

Diagnosis was performed using the gold-standard serological microscopic agglutination test (MAT) using live antigens for antibody detection (Faine, 2000). Twelve pathogenic serovars were used: *L. interrogans* serovars Canicola Hound Utrech IV, Hardjo LT 1085, Pomona Johnson, Icterohaemorrhagiae, Pyrogenes Salinem, Bratislava Jez, Autumnalis Akiyami A, Ballum Mus 127, Grippotyphosa Moskva V, Tarassovi Perepicilin, Lai Lai and Muenchen C90. Samples were considered as positive when titers were equal to or greater than 100 (Faine, 2000; WHO, 2003).

Data analysis

The frequency of seropositive animals was calculated as a percentage of the total number of samples tested. Seropositive animals were examined in relation to sex and shelter. The chi-square test was used to compare both shelters. The STATA® software was used to evaluate the frequency and to calculate the confidence intervals.

RESULTS AND DISCUSSION

Positive results for anti-*Leptospira* spp. antibodies were observed in eight of the 92 samples, for an overall frequency of 8.7% ($CI_{95\%}$: 3.8-16.41%). With regard to shelter, frequency was 8.3% (shelter 2) and 9.0% (shelter 1; Table 1). Overall frequency of canine leptospirosis reported in previous studies is 4.9% in Chiapas, Mexico (Jimenez-Coello et al*.,* 2010), 4.4% in stray dogs in Trinidad and Tobago (Adesiyun et al*.,* 2006), 7.1% in Brazil (Oliveira et al., 2012), 34% (Ortega-Pacheco et al., 2008) and 35% (Jimenez-Coello et al., 2008) both in Yucatan, Mexico, and 14 to 55% in other countries (O'Keefe et al., 2002; Adesiyun et al., 2006; Houwers et al., 2011).

According to the sex of the dogs, no difference was found in the present study (Table 2). In Australia, Zwijnenberg et al. (2008) indicate that the female dogs are risk factor associated with the seropositive status, and Miller et al. (2007) mentioned that young and male dogs are more commonly affected by leptospirosis.

The Canicola serovar was the most prevalent, followed by serovars Icterohaemorrhagiae, Autumnalis, Ballum and Grippotyphosa. The agglutinating antibody titers ranged from 1:50 to 1:1600 (Table 3).

Table 3. Frequency of serovars of *Leptospira* spp. in two dog shelters from Veracruz, Mexico.

L. interrogans Serovar	1:50	1:100	1:200	1:400	1:800	1:1600	+	Frequency	$CI_{95\%}$
Canicola	9	1	3	1	2	1	8	8.7%	3.8-16.41
Hardjo	-	-	-	-	-	-	-	-	-
Icterohaemorrhagiae	1	-	-	-	-	-	-	-	-
Pomona	-	-	-	-	-	-	-	-	-
Pyrogenes	-	-	-	-	-	-	-	-	-
Bratislava	-	-	-	-	-	-	-	-	-
Autumnalis	2	-	-	-	-	-	-	-	-
Ballum	1	-	-	-	-	-	-	-	-
Grippotyphosa	2	-	-	-	-	-	-	-	-
Tarassovi	-	-	-	-	-	-	-	-	-
Lai lai	-	-	-	-	-	-	-	-	-
Muenchen	-	-	-	-	-	-	-	-	-

+ = Positive.

In the present study, the serovar Canicola was the most frequent. In Yucatan, Mexico, the serovars present are Canicola and Icterohaemorrhagiae (Jimenez-Coello et al*.,* 2008). In Sao Paulo, Brazil, the seroprevalence in dogs is 20.08%, and the most prevalent serovars are Canicola (6.7%), Copenhageni (5.0%), Icterohaemorrhagiae (2.9%), Autumnalis (2.9%), Pyrogenes (2.8%), Pomona (2.0%), Hardjo (2.0%), Australis (1.8%), Bratislava (1.6%), Cynopteri (1.4%), Grippotyphosa (1.3%) and Djasiman (1.0%) (Kikuti et al., 2012). Although an animal was considered as positive to leptospirosis when the antibodies titers were equal to or greater than 100, the presence of dogs with an antibody response at titers 1:50 for different serovars might indicate the exposure to the bacteria via contact with other animals that act as reservoirs for these serovars, such as rats and mice (serovars Icterohaemorrhagiae and Grippotyphosa).

In conclusion, leptospirosis was present in the canine population from the two shelters surveyed. Thus, if these dogs or those that tested negative were carriers of the disease, they might spread it to the rest of the population, causing a serious health problem, or they might also transmit it to their human care givers or adopters. Thus, it is important to determine the presence of leptospirosis in dogs from shelters to take preventive sanitary measures to avoid its transmission to the rest of the pack or to the people that are in contact with them.

REFERENCES

Adamus C, Buggin-Daubie M, Izembart A, Sonrier Pierre C, Guigand L, Masson MT, Andre-Fontaine G, Wyers M (1997). Chronic hepatitis associated with field strain Leptospira infection in a vaccinated colony of laboratory Beagle dogs. Comp. Pathol. 117: 311-328.

Adesiyun A, Hull-Jackson C, Mootoo N, Halsall S, Bennett R, Clarke N, Whittington C, Seepersadsingh N (2006). Sero-epidemiology of canine leptospirosis in Trinidad: serovars, implications for vaccination and public health. J. Vet. Med. B: Infect. Dis. Vet. Public Health. 53 : 91-99.

Adin CA, Cowgill LD (2000). Treatment and outcome of dogs with leptospirosis: 36 cases (1990–1998). J. Am. Vet. Med. Assoc. 216 : 371-375.

Adler B, De la Peña MA (2010). Leptospira and leptospirosis. Vet. Microbiol. 140: 287-296.

André-Fontaine G (2006). Canine leptospirosis: Do we have a problem?. Vet. Microbiol. 117 : 19-24.

Andre-Fontaine G, Branger C, Gray AW, Klaasen E (2003). Comparison of the efficacy of three commercial bacterins used to prevent canine leptospirosis. Vet. Rec. 153 : 165-169.

Båverud V, Gunnarsson A, Olsson Engvall E, Franzén P, Egenvall A (2009). *Leptospira* seroprevalence and associations between seropositivity, clinical disease and host factors in horses. Acta Vet. Scand. 51 : 15.

Birnbaum N, Barr SC, Center SA, Schermerhorn T, Randolph JF, Simpson KW (1998). Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. J. Small Anim. Pract. 39 : 231-236.

Bolin CA (1996). Diagnosis of leptospirosis: a reemerging disease of companion animals. Sem. Vet. Med. Surg. (Small Anim.) 11:166-171.

CDC. Center for Disease Control (1972). Leptospirosis surveillance. Annual Summary, p 3.

Davis MA, Evermann JF, Petersen CR, VancerSchalie J, Besser TE, Huckabee J, Daniels JB, Hancock DD, Leslie M, Baer R (2008). Serological survey for antibodies to *Leptospira* in dogs and raccoons in Washington State. Zoonoses Public Health. 55 : 436-442.

Desvars A, Cardinale E, Michault A (2011). Animal leptospirosis in small tropical areas. Epidemiol. Infect. 139 : 167-188.

Faine S (2000). Leptospira and Leptospirosis. Boca Raton, CRC Press, USA.

Ghneim GS, Viers JH, Chomel BB, Kass PH, Descollonges DA, Johnson ML (2007). Use of a case–control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. Vet. Res. 38: 37-50.

Greene CE (2006). Laboratory diagnosis of canine leptospirosis and babesiosis. In: 24th Annual ACVIM Forum , 31 May - 3 June 2006, Louisville, KY, USA, pp 490-491.

Houwers D, Goris M, Abdoel T, Kas J, Knobbe S, van Dongen AM, Westerduin F, Klein W, Hartskeerl R (2011). Agglutinating antibodies against pathogenic Leptospira in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections. Vet. Microbiol. 148 : 449-451.

Jimenez-Coello M, Ortega-Pacheco A, Guzman-Marin E, Guirs-Andrade DM, Martinez-Figueroa L, Acosta-Viana KY (2010). Stray dogs as reservoirs of the zoonotic agents Leptospira interrogans, Trypanosoma cruzi, and Aspergillus spp. In an urban area of Chiapas in Southern Mexico. Vector Borne Zoonotic Dis. 10: 135-141.

Jimenez-Coello M, Vado-Solís L, Cárdenas-Marrufo MF, Rodríguez-Buenfil JC, Ortega-Pacheco A (2008). Serological survey of canine leptospirosis in the tropics of Yucatan using two different test. Acta Trop. 106: 22-26.

Kikuti M, Langoni H, Nobrega DN, Corrêa APFL, Ullmann LS (2012). Occurrence and risk factors associated with canine Leptospirosis. J. Venom. Anim. Toxins incl. Trop. Dis. 18: 124-127.

Klaasen HLBM, Molkenboer MJCH, Vrijenhoek MP, Kaashoek MJ (2003). Duration of immunity in dogs vaccinated against leptospirosis with a bivalent inactivated vaccine. Vet. Microbiol. 95 : 121-132.

Levett PN (2001). Leptospirosis. Clin. Microbiol. Rev. 14: 296-326.

Meeyam T, Tablerk P, Petchanok B, Pichpol D, Padungtod P (2006). Seroprevalence and risk factors associated with leptospirosis in dogs. Southeast Asian J. Trop. Med. Public Health. 37: 148-53.

Miller RI, Ross SP, Sullivan ND, Perkins NR (2007). Clinical and epidemiological features of canine leptospirosis in North Queensland. Aust. Vet. J. 85: 13-19.

Minke JM, Bey R, Tronel JP, Latour S, Colombet G, Yvorel J, Cariou C, Guiot AL, Cozette V, Guigal PM (2009). Onset and duration of protective immunity against clinical disease and renal carriage in dogs provided by a bi-valent inactivated leptospirosis vaccine. Vet. Microbiol. 137: 137-145.

OIE. Office International des epizooties (2004). Leptospirosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals Volume 1. 5th ed. OIE Biological Standards Commission and adopted by the International Committee of the OIE. Paris, France, pp. 316-327.

O'Keefe JS, Jenner JA, Sandifer NC, Antony A, Williamson NB (2002). A serosurvey for antibodies to Leptospira in dogs in the lower North Island of New Zealand. N. Z. Vet. J. 50: 23-25.

Oliveira Lavinsky M, Said RA, Strenzel GM, Langoni H (2012). Seroprevalence of anti-Leptospira spp. antibodies in dogs in Bahia, Brazil. Prev. Vet. Med. 106: 79-84.

Ortega-Pacheco A, Collin-Flores RF, Gutierrez-Blanco E, Jimenez-Coello M (2008). Frequency and type of renal lesiones in dog naturally infected with Leptospira species. Ann. N. Y. Acad. Sci. 1149: 270-274.

Prescott J (2008). Canine leptospirosis in Canada: a veterinarian's perspective. Can. Med- Assoc. J. 178: 397-398.

Raghavan R, Brenner K, Higgins J, Van der Merwe D, Harkin KR (2011). Evaluations of land cover risk factors for canine leptospirosis: 94 cases (2002–2009). Prev. Vet. Med. 101: 241-249.

Reis RB, Ribeiro GS, Felzemburgh RDM, Santana FS, Mohr S, Melendez AXTO, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI (2008). Impact of environment and social gradient on *Leptospira* infection in urban slums. PLoS Negl. Trop. Dis. 2 : e288.

Trevejo RT, Rigau-Pérez JG, Ashford DA, McClure EM, Jarquín-González C, Amador JJ, de los Reyes JO, Gonzalez A, Zaki SR, Shieh WJ, McLean RG, Nasci RS, Weyant RS, Bolin CA, Bragg SL, Perkins BA, Spiegel RA (1998). Epidemic leptospirosis associated with pulmonary hemorrhage-Nicaragua, 1995. J. Infect. Dis. 178: 1457-1463.

Ward MP (2002). Clustering of reported cases of leptospirosis among dogs in the United States and Canada. Prev. Vet. Med. 56 : 215-226.

Ward MP, Guptill LF, Wu CC (2004). Evaluation of environmental risk factors for leptospirosis in dogs: 36 cases (1997–2002). J. Amer. Vet. Medical Assoc. 225: 72-77.

WHO. World Health Organization (2003). Human leptospirosis: guidance for diagnosis, surveillance and control. WHO Library Cataloguing-in-Publication Data, pp. 63-69.

Zwijnenberg RJG, Smythe LD, Symonds ML, Dohnt MF, Toribio JA (2008). Cross-sectional study of canine leptospirosis in animal shelter populations in mainland Australia. Aust. Vet. J. 86: 317-323.