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Original article

Leptospira spp. in cats from tropical Mexico

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Summary

The natural exposure of domestic cats to *Leptospira* spp. and some associated risk factors were studied in a large city from tropical Mexico. Two hundred and sixty blood samples were collected from clinically healthy cats in Merida Yucatan, Mexico, and tested for 13 *leptospira* serovars by using the microscopic agglutination test (MAT). Forty-six (17.7%) of the cats were serologically positive to some of the antigens of seven serovars, particularly *australis* (54.3%), *pyrogenes* (15.3%), *gryppotyphosa* (13%) and others. Titters recorded were within 1:100 and 1:200. No clinical signs of the disease were observed in the cats. Cats with no access to outdoors were more prone to become in contact with the agent (P<0.01). There is a wide distribution of *Leptospira* spp. in the population of owned cats in the studied tropical area, particularly the serovar *australis*. These results give important information about the epidemiology of leptospirosis, including the role of cats as a potential reservoir.
Keywords: *Leptospira australis*, domestic cats, tropics, Mexico.

Introduction

Domestic cats have an important role in the epidemiology of leptospirosis since they are susceptible to the infection and may become asymptomatic carriers capable of excreting *leptospira* organisms in their urine up to 3 months (Willoughby and Bennett, 2004). Clinical cases of leptospirosis are rarely seen in cats even when leptospiremia and leptospiuria are present. Cats are considered resistant to natural and experimental infections

of leptospirosis (Larsson et al., 1984; 1985). However, in a study, cats with polyuria-polydipsia were found seropositive in 48% of cases (Luciani, 2004). In cats, transmission through water contact is less likely due to cats' natural aversion to water. However, cats can become infected by feeding on animals harbouring leptospires (Hartmann et al., 2013). The prevalence of *Leptospira* spp. antibodies in cats vary from regions i.e., in Europe is reported 10% in Scotland (Agunloye and Nash, 1996), 20% in Germany (Batza and Weiss, 1987), and 5-20% in the UK (Willouughby and Bennet, 2004). In other latitudes, it is reported 8.1% in Chile (Azócar-Aedo et al., 2014), 30% in Iran (Jamshidi et al., 2009), 4.8% in Massachusett USA (Markovich et al., 2012), and 20% in Quebec Canada (Lapointe et al., 2013). No previous epidemiological studies about the serological status of Leptospirosis in cats are available in Mexico. In Yucatan, located in the south of Mexico, a preliminary study from rural cats showed a seroconversion of 15.2% (Ortega-Pacheco et al., 2017). *Leptospira* serovars, including *canicola*, *grippotyphosa*, and *icterohaemorrhagie* are reported in cats probably by cohabitating with dogs (Craig et al., 2012). Other serovars such as *icterohaemorrhagie*, *pomona* (Hartmann et al., 2013) *sejroe*, and *australis* (Luciani, 2004) have been identified in cats, but other may be found particularly in stray cats or cats having access to the streets which are exposed as a result of their hunting activities.

The aims of this study were: (i) to estimate the exposure of domestic cats to *Leptospira* spp. through a serological survey and (ii) to determine probably associated risk factors of seroconversion in a large city from tropical Mexico.

Materials and Methods

This study was performed in February in the city of Merida Yucatan, Mexico (19º30´ and 21º35´ N latitude, and 87º30´ 90º24´ W longitude). The climate is characterized as tropical sub-humid with a mean annual temperature of 25-28ºC (range of 15-40ºC during the winter and summer respectively) and relative humidity of approximately 80%. Annual rainfall is 400-1500mm.

Two hundred and sixty clinically healthy domestic cats attending a spay/neuter campaign were randomly selected for a serological survey study. The sample size was determined considering an expected seroprevalence of 35% from dogs of the same region (Jiménez-Coello et al., 2008), an error of 5% and a 95% confidence interval. The sample size was calculated using the software Win Episcope 2.0 (Win Episcope, 2000). From each patient information about their gender (male/female), age, and access to the street. Under the consentient of owners, blood samples were collected from the jugular vein

into sterile vacutainer tubes. Samples were centrifuged at 400g for 10 minutes to obtain serum; samples were identified individually and stored at -20°C until processing.

To detect the presence of *Leptospira* spp. antibodies, the microscopic Agglutination Test (MAT) was used. MAT is the gold standard of reference for the diagnosis of leptospirosis and was performed using live antigens as previously reported (Ortega-Pacheco et al., 2017) including serovars *L. australis, L. brastislava, L.* autumnalis, *L.canicola, L. gryppotyphosa, L. icterohaemorrhagie, L. pomona, L. pyrogenes, L. panama, L. hardjo, L. wolffi* and *L. patoc* (Ortega-Pacheco et al., 2017).

The estimated seroprevalence was calculated using a formula for disease occurrence (Thrusfield, 2007). The independent variables gender, age, and access to the street were used to determine their association with serological positivity to *Leptospira* spp. Contingency tables (2X2) were built with these variables, and a Chi^2 analysis was performed to obtain *p*- value, odds ratio (OR), and confidence intervals (CI95%) using the EpiInfo software (Dean et al., 1994).

Results

The overall prevalence was 17.7% (46 out of 260). The most predominant serovar found was *australis* with titters ranging from 1:100 to 1:200. Other serovars found were *bratislava*, *canicola*, *grippotyphosa*, *icterohaemorragie*, *pyrogenes*, and *patoc* but in smaller proportion (Table 1).

Table 1. Frequency of *Leptospira* serovars and titters from 260 domestic cats from Yucatan, Mexico.

Serovar	Positive		Titters		
	n	%	1:100	1:200	1:400
australis	25	54.3	23	2	-
bratislava	2	4.3	2	-	-
autumnalis	0	0.0	-	-	-
canicola	1	2.2	1	-	-
gryppotyphosa	6	13.0	5	1	-
icterohaemorrhagie	1	2.2	1	-	-
pomona	-	0.0	0	-	-
pyrogenes	7	15.3	7	-	-
panama	-	0.0	-	-	-
hardjo	-	0.0	-	-	-
wolfii	-	0.0	-	-	-
patoc	4	8.7	3	-	1
Total	46	100	42	3	1

As seen in table 2, no associations were observed with gender or age, nevertheless not having access to the streets was a factor associated with higher seropositivity ($P > 0.01$).

Table 2. Risk factors, odds ratios (OR), and confidence interval (CI) for *Leptospira* spp. seropositivity in domestic cats from Yucatan, Mexico.

Risk factor	Total	Positive (n) %	OR	CI	*P*-value
Sex					
Male	87	20 (23.0%)			
Female	173	26 (15.0%)	1.6	0.88-3.23	0.11
Age					
> 1	98	20 (20.4%)			
≤ 1	162	26 (16.0%)	1.3	0.7-2.5	0.37
Access to street					
No	21	8 (38.1%)			
Yes	239	38 (15.9%)	3.2	1.2-8.3	0.01

Discussion

Leptospirosis has a worldwide distribution with higher incidences in tropical and subtropical zones where exposure seems to be more feasible. In cats, few epidemiological studies or clinical cases of leptospirosis are available in the literature, probably because clinical cases are rarely diagnosed and the apparently natural resistance of this species to the spirochete. However, once in contact with the agent, cats may develop leptospiremia and leptospiuria (Larsson et al., 1984; 1985) and thus become an important reservoir of the agent and capable of excreting leptospira in their urine up to 3 months (Willoughby and Bennett, 2004). Serosurveys of feline *Leptospira* spp. infection are scarce in the veterinary literature, and this appears to be the first report of a serological survey of leptospiral infection in cats in Mexico. Although the prevalence found is not as high as the 35% reported in dogs from the same region (Jimenez-Coello et al., 2008), seropositivity found may suggest that cats are an important reservoir of Leptospira and the disease could be of more clinical importance than previously recognized in cats; additional studies are required to determine the role of the leptospira found in clinical cases in the domestic cat and their capacity to eliminate the spirochetes.

Worldwide epidemiological studies indicate a common exposure of cats to leptospira. However, serovars circulating in their populations may vary. It is expected to find in cats the same circulating *leptospira* serovars of cohabitating dogs as they share the same environment (Greene et al., 2102). In the studied region, serovar *canicola* in dogs is very

common with prevalences up to 65% of positive cases reaching titters of 1:25, 600 (Jimenez-Coello et al., 2008). However, in the present study, serovar *canicola* was only detected in one case, suggesting that contact with the agent was originated from another source. Since those studies were performed in different years, environmental factors may have been involved, or probably, the different behaviour of the dogs may reduce the risk of contact in cats.

Yucatan has the ideal tropical conditions of high humidity and temperature along the year, alkaline soil, and water pH for the longer survival of leptospiras on the environment; in such conditions, leptospiras may survive up to 180 days (Heath and Johnson, 1994). In a tropical climate like Malaysia, serovar *hardjo* may survive in river water for up to 11 days (Khairani-Bejpo et al., 2004); other serovars can even maintain its virulence for 20 months (Andre-Fontaine et al., 2015). Serovar *australis* found with a high seroprevalence in the present study, may survive in culture-infected soil for 43 days (Smith and Self, 1955); this finding is significant and may contribute to the understanding of the epidemiology of leptospirosis in Mexico. In a study performed in France, *Luciani* (2004) found that 12% of cats with Polyuria-Polydipsia syndrome were found positive to serovar Australis. A more specific study on the health of serovar *australis* seropositive cats and a deep epidemiological investigation that indicates the source of infection is required. In a city of Guatemala, near the Yucatan peninsula 30.0% of inhabitants were seropositive to *Leptospira* spp. where 11.1% were positive to serovar *australis* (García et al., 2013). Rodents are the first recognized carriers of leptospires, including serovar *australis*; cats can acquire leptospirosis from the ingestion of infected mice, as well as the ingestion of contaminated water (Greene et al., 2012). Cats may also become in contact with the spirochetes because of their hunting activities of small infected prey, which seems to be at an early age in the studied population, even when cats are not having access to the streets; indoor rodents may be the source of infection. However, the potential role of invertebrates has been suggested in the transmission of leptospiras (Wojcik-Fatla et al., 2012) including synanthropic cockroaches (Gonzalez-Astudillo et al., 2016) particularly when high environmental humidity is present (Greene *et al*., 2012), which is high during the summertime in the studied region. It is important to determine the enzootic serovars and serogroups to include them in the MAT-antigen testing battery for a better describtion of the patterns of diseases and trends in the exposed animal populations, including humans.

It is concluded that domestic cats in tropical Mexico are exposed to *Leptospira* spp. with the high circulation of serovar *australis*, and are important reservoirs of the spirochete in the domestic population of the studied area. Additional studies are required to determine the origin of infection and the role of *L. australis* in clinical disease in the domestic cat, since the disease could be of more clinical importance than previously recognized.

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