Veterinary and Animal Science 6 (2018) 64–68

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Seroprevalence of *Leptospira* spp infection and its risk factors among domestic dogs in Bogotá, Colombia

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ARTICLE INFO

Keywords:
Leptospirosis
Seroprevalence
Precipitation
Epidemiology, Dogs

ABSTRACT

Different analytical tools were used to determine the seroprevalence of and risk factors associated with *Leptospira* spp infection in 192 domestic dogs (*Canis familiaris*) in Bogotá, Colombia. Using the microscopic agglutination test (MAT), a battery of 16 *Leptospira* serovars were tested. The seroprevalence of *Leptospira* spp was calculated as 36.46% (95% CI 0.30-0.43). A questionnaire was applied to the dogs' owners at the time of sampling and the variables "Water sources near home" and "Dog hunting rodents" were identified as risk factors for leptospirosis occurrence in the urban area of Bogotá. Geographical coordinates relating to the dogs' households were obtained in order to map out the spatial distribution of reactive and unreactive dogs. Additionally, we found that the mean annual precipitation was higher at geographical locations with reactive animals than at those with unreactive dogs ($p < 0.05$). Preventing exposure of dogs to rodents and waste-water bodies that could be contaminated with *Leptospira* might effectively reduce occurrences of leptospirosis. Moreover, promoting preventive programs and vaccination of dogs against leptospirosis in areas of higher precipitation and prior to rainy months could be an effective strategy for leptospirosis prevention.

1. Introduction

Leptospirosis is one of the most widespread zoonotic diseases worldwide (Vijayachari, Sugunan, & Shriram, 2008). In dogs, it can be acute and may produce signs such as jaundice, kidney damage, liver damage and vasculitis (André-Fontaine, 2006; Schuller et al., 2015; Sykes et al., 2011). The microscopic agglutination test (MAT), with 92% sensitivity and 60%−100% specificity, is the gold standard method for diagnosing leptospirosis (Sykes et al., 2011). However, interpreting MAT is not trivial since it depends on the antibody titer established as the threshold, the host's immune status, the serovars involved in the infection and some cross-reactivity among different serogroups (Adler *et al.*, 2010). Moreover, the presence of antibodies may be affected if antibiotic treatment was started before samples were taken (Schuller et al., 2015). Seroconversion occurs at between five and seven days post-infection, but MAT is usually positive at between seven and fourteen days after the onset of symptoms (Sykes et al., 2011). Vaccine-induced antibody titers may be greater than 600. with persistence for up to six months. Moreover, low titers may be explained by the high degree of cross-reactions that occur between different serogroups or because the samples were taken during the early stage of convalescence. In this stage, paired serological tests with an interval of eight to fifteen days are suggested (Sykes et al., 2011).

Leptospirosis transmission usually results from direct or indirect exposure to urine or other body fluids from leptospiruric animals. Indirect exposure usually occurs through contact with contaminated water and soil (Wojcik-Fatla et al., 2014). This transmission route is crucial, especially for prolonging the survival of leptospires in warm and humid conditions. Thus, rainfall contributes notably towards transmission of *Leptospira* (Lee et al., 2014; Raghavan *et al.*, 2012). Among humans, the factors that are commonly reported to play a role in getting the disease include poor socioeconomic conditions, inhabiting urban and peri-urban areas, flooding events, contact with wild and peridomestic animals, presence of rivers and contact with waste-water and garbage (Bharti et al., 2003; Levett, 2004; Sakundarno, Bertolatti, Maycock, Spickett, & Dhaliwal, 2013; Ward, 2002a). However, some of these factors have not yet been elucidated as presenting risks among dogs (Corcho, Molina, Margarita, & Santana, 2007).

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https://doi.org/10.1016/j.vas.2018.08.002
Received 19 May 2017; Received in revised form 16 April 2018; Accepted 8 August 2018
Available online 11 August 2018

Risk factor analyses need to be conducted to understand the transmission dynamics of leptospirosis in urban areas and to plan preventive strategies (Azócar-Aedo *et al.*, 2016; Hagan *et al.*, 2016). The aims of the present study were to determine the seroprevalence of *Leptospira* spp among dogs in Bogotá, Colombia, and to identify risk factors for occurrences of leptospirosis.

2. Materials and methods

2.1. Case selection

This study was conducted in Bogotá, at the Veterinary Hospital of the National University of Colombia in 2013. Through convenience sampling, blood samples were collected from 192 domestic dogs living in the urban area of Bogotá, which had not been vaccinated in the last six months before sampling and were apparently healthy at the time of sampling. No specific tests were performed to determine concomitant diseases. The serum samples collected were stored at −70°C.

2.2. Serological testing and seroprevalence calculation

The microscopic agglutination test (MAT) was performed at the National Laboratory of Veterinary Diagnostics of the Colombian Agricultural Institute (ICA) using a battery of 16 antigens for *Leptospira interrogans* and *Leptospira kirschneri* serovars: Hardjo prajitno (HJOPRAJ) strain Hardjoprajitno; Hardjo bovis (HJOBOV) strain Hardjobovis; Pomona (POM) strain Pomona; Canicola (CAN) strain Hond Utrecht IV; Icterohaemorrhagiae (ITC) strain RGA; Grippotyphosa (GPT) strain Moskva V; Bratislava (BRA) strain Gez Bratislava; Hebdomadis (HEB) strain Hebdomadis; Serjoe (SJO) strain M84; Wolffi (WOL) strain 3705; Copenhageni (COP) strain M20; Ballum (BAL) strain Ballum; Tarasovi (TAR) strain Perepelicin; Autumnalis (AUT) strain Akiyami A; Panama (PAN) strain CZ214K; and Cynoptery (CYN) strain 3522C. All of these serovars were obtained from the Biomedical Research Sector of the Royal Tropical Institute (KIT), Amsterdam, Netherlands. Twofold dilutions of serum (from 1:100 to 1:1600) were tested using the MAT, and the titer was recorded as the reciprocal of the highest dilution of serum that agglutinated ≥ 50% of the leptospires. Dark-field microscopy was used to read the tests. Samples with a titer greater than or equal to 1:200 were considered positive (Cole, Sulzer, & Pursell, 1973).

True seroprevalence was estimated as described by Rogan and Gladen (1978). Confidence limit calculations assumed sensitivity of 92% and specificity of 80%, and then the normal approximation method was used as described by Greiner and Gardner (2000). Wilson confidence limits were calculated as described by Reiczigel, Földi, & Ózsvári, 2010. These calculations were performed using EpiTools epidemiological calculators (Seargent, 2016).

2.3. Risk factor identification

To assess potential risk factors associated with occurrences of leptospirosis, a questionnaire was applied to 171 owners of the 192 sampled dogs. Some of the factors evaluated related to what the owners had seen, such as observing their dogs hunting small animals, presence of rodents within homes or surrounding areas, observing dogs in contact with garbage, presence of clinical signs associated with leptospirosis in dogs and the number of times that the dogs were going outside in a day. Other factors such as the kind of water sources, existence of water bodies near homes and dog owners' knowledge about leptospirosis were also considered (Table 1). Univariate statistical analysis was performed using the chi-square test through the R software (R Core Team, 2017).

Since it has been found that precipitation contributes remarkably towards transmission of *Leptospira* (Lee et al., 2014; Raghavan *et al.*, 2012), we obtained the geographical coordinates of the dogs' households and mapped out the spatial distribution of reactive and unreactive dogs. To obtain detailed annual average precipitation data for each dog's household, we used the monthly WorldClim precipitation dataset for 2013 with a spatial resolution of 10 minutes from the BioClim dataset (www.worldclim.org/bioclim), using the R dismo package (Hijmans, Phillips, Leathwick, Elith, & Hijmans, 2017). Using the Mann Whitney U test, we compared whether the mean values for precipitation differed between the geographical locations of reactive and unreactive dogs.

Table 1
Distribution of positive serum according to serovars.

SEROVAR	100	200 [b]	400 [c]	Total	% [d]
HJOPRAJ		1	2	3	1.09
HJOBOV	3		2	5	1.82
POM	14	15	3	32	11.68
CAN	40	16	7	63	22.99
ICT	13			13	4.74
GPT	2		1	3	1.09
BRA	21	6	2	29	10.58
HEB	6	3	2	11	4.01
SJO			1	1	0.36
WOL			2	2	0.73
COP	3	3	2	8	2.92
BAL	8	2	4	14	5.11
TAR	8	1	2	11	4.01
AUT	43	19	11	73	26.64
PAN	1		2	3	1.09
CYN	3			3	1.09
Total	165	66	43	274	100.00

[a] MAT-positive serovars with a titer of 100;
[b] MAT-positive serovars with a titer of 200;
[c] MAT-positive serovars with a titer of 400;
[d] percentage of positive findings according to serovar.

3. Results

The seroprevalence of *Leptospira* was 36.46% (95% CI: 0.30-0.43), and the most common serovars were: Autumnalis (73 cases; 15.18%), Canicola (63 cases; 12.04%), Pomona (32 cases; 8.9%) and Bratislava (29 cases; 4.19%). Serovar-specific seroprevalences are shown in Table 2. Co-agglutinations occurred in 71 cases (51%): two co-agglutinations were presented on 41 occasions; three on 21 occasions; four on five occasions; five on two occasions; six on seven occasions; and eight once. The most frequent co-agglutinations were Canicola and Autumnalis serovars (37 times), followed by Canicola and Icterohaemorrhagiae (seven times) and Pomona and Bratislava (six times). The distribution of positive serum according to serovars is shown in Table 1.

The questionnaire was applied to 89.1% of the owners of the sampled dogs (Table 2). In the chi-square test, presence of water bodies near homes ($p < 0.05$) and observation of dogs hunting rodents ($p < 0.05$) were identified as risk factors for occurrences of canine leptospirosis in the urban area of Bogotá. Annual average precipitation was also identified as a risk factor, since it was higher at the geographic locations of reactive dogs ($p < 0.05$). Fig. 1 shows the spatial distribution of reactive and unreactive dogs in the urban area and the annual average precipitation in Bogotá.

4. Discussion

Leptospirosis has been described as the most frequent zoonosis (Bharti et al., 2003) and several serological surveys on dogs have been performed worldwide (Schuller et al., 2015). MAT is the standard test for making the serodiagnosis of leptospirosis (Cole et al., 1973). However, interpretation of MAT results is influenced by the antibody titer established as the threshold, the host's immune status, the serovars involved in the infection and some cross-reactivity among different