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

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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
wastewater and garbage (Bharti et al., 2003; Levett, 2004; Sakundarno, Bertolotti, 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).

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 Hardjo prajitno; 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 MB4; Wolfli (WOL) strain 3705; Copenhageni (COP) strain M20; Ballum (BAL) strain Ballum; Tarasovi (TAR) strain Perepelcin; 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, & Özsávári, 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.

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; 6.9%) 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 serogroups. There is a lack of consensus regarding the correlation between active infection and serological titers (Adler et al., 2010). Results regarding the seroprevalence of leptospirosis from surveys on dog populations are difficult to compare because of the different MAT cutoff titers and serovars included. Results with cutoff titers ranging from ≥ 50 to ≥ 800 have been reported (Schuller et al., 2015). In this study, we considered MAT titers ≥ 200 and took Bogotá to be an endemic zone for leptospirosis (Agudelo-Flórez, Restrepo-Jaramillo, & Arboleda-Naranjo, 2007; Sánchez - García, Ballut Pestana, Calderón - Rangel, & Rodríguez - Rodríguez, 2010).

In Colombia, the reported prevalences have varied, e.g. Tunja 67.2%, Cali 41.1%, Tolima 20.2% and Cienaga de Oro, Córdoba 47.14% (Álvarez, Calderón, Rodríguez, & Arrieta, 2011; Dechner, 2014; Romero, & Sanchez, 2009). These studies reported MAT cutoff values > 100, which may have increased the percentage of positive animals. In the present study, we found that the most common serovars in the urban area of Bogotá were Autumnalis and Canicola. This suggests that the dynamics of these serovars varies across the country, which may be related to the great diversity of the country's ecosystems. Specifically, regarding the Autumnalis serovar, other studies did not include it in the panel for MAT and its presence was attributed to possible cross-reactions between Canicola and Autumnalis serovars (Sonrier et al., 2000). Hebdomadis, Cynoptery, Wolffi and Ballum serovars were found for the first time in dogs in Bogotá, Colombia, in the present study. Further studies are needed to determine the presence of these serovars and their impact on public health. Therefore, it is proposed to include more serovars in making the diagnosis of leptospirosis in dogs in Bogotá.

We found a statistical association between occurrences of Leptospirosis in dogs in Bogotá and both the presence of water bodies near the home (p < 0.05) and the observation of dogs hunting rodents (p < 0.05). A similar result was reported by Rubel, Seijo, Cernigoi, Viale, & Wisnivesky-Colli, 1997, who correlated areas with wastewater

Table 2
MAT results according to survey questions asked.

| Categories                                      | Response       | MAT+ | MAT- | %   | p value | OR      | 95% CI  |
|------------------------------------------------|----------------|------|------|-----|---------|---------|---------|
| Have you observed your pet hunting small prey? | Yes            | 20   | 26   | 26.90 | 0.28    | 1.46 (0.72-2.93) |
|                                                | No             | 43   | 82   | 73.10 |         |         |         |
| Have you noted the presence of rodents in your home or surrounding areas? | Yes            | 26   | 44   | 40.94 | 0.94    | 1.02 (0.53-1.92) |
|                                                | No             | 37   | 64   | 59.06 |         |         |         |
| Are there any water bodies near your home?     | Yes            | 35   | 42   | 45.03 | 0.03    | 1.95 (1.04-3.70) |
|                                                | No             | 28   | 66   | 54.97 |         |         |         |
| Has your pet been in contact with garbage?    | Yes            | 34   | 50   | 49.12 | 0.33    | 1.35 (0.72-2.74) |
|                                                | No             | 29   | 58   | 50.88 |         |         |         |
| Have you observed your pet hunting rodents?    | Yes            | 12   | 9    | 12.28 | 0.04    | 2.58 (1.02-6.54) |
|                                                | No             | 51   | 99   | 87.72 |         |         |         |
| What are the water sources for your pet?       | Water in the environment | 61   | 100  | 94.15 | 0.32    | 2.44 (0.5-11.86) |
|                                                | Potable water  | 2    | 8    | 5.85  |         |         |         |
| Does your pet have one or more clinical signs of leptospirosis? | Yes            | 33   | 49   | 47.95 | 0.38    | 1.32 (0.70-2.48) |
|                                                | No             | 30   | 59   | 57.89 |         |         |         |
| Did you know that this is leptospirosis?       | Yes            | 23   | 39   | 36.26 | 0.95    | 1.01 (0.52-1.94) |
|                                                | No             | 40   | 69   | 63.74 |         |         |         |
| How many times a day does your pet go outside? | One or more times | 45   | 79   | 72.51 | 0.8     | 0.91 (0.45-1.86) |
|                                                | Less than once | 18   | 29   | 27.49 |         |         |         |

MAT + : positive in microscopic agglutination test; MAT-: negative in microscopic agglutination test; OR: odds ratio; 95% CI: 95% confidence interval.

Fig. 1. A. Location of Bogotá in Colombia, the red part represents the urban area of Bogotá. B Distribution of reactive dogs and unreactive dogs in the urban area of Bogotá according to the average annual rainfall expressed as the amount of rain per square meter in one hour (mm).
and rodents with occurrences of *Leptospira*. It is probable that some factors are dependent on the owners’ perceptions, such as observing dogs in contact with garbage, clinical signs associated with leptospirosis and the number of times dogs go outside in the day. These were not identified as risk factors because the owners were not spending enough time with the dogs to provide complete information about their dogs’ behavior. Questionnaires that aim to determine the risk factors for canine leptospirosis may not be suitable for use in preventive programs because of the limited degree of observation that owners may have in relation to their own dogs. The variable relating to observation of rodents within the house or in the surroundings may be affected by the nocturnal behavior of these animals, which makes it difficult to observe them.

Since the presence of water bodies near homes was identified as a risk factor, it is likely that these water bodies consist of residual rainwater, which is a very common type of water body in Bogotá. Bogotá is characterized by having a bimodal regime of rainfall characterized by amounts ranging from 69 to 142 mm and has an average temperature of 13°C, with a range from 7°C to 18°C (Vargas, Santos, Cárdenas, & Obregón, 2011). However, there is high spatial-temporal variability of precipitation within the city (Vargas et al., 2011), which may be an important factor for understanding the spatial patterns of diseases within this capital city. Exposure to rainwater was reported to be a risk factor for leptospirosis among dogs in northern California (Ward, 2002a). Carroll & Campbell (1987), found that leptospirosis in cattle due to the serovar Hardjo was more prevalent following the rainy season on farms where soils had high water-holding capacity. In a study on 2551 horses, an association between the water index in the soil and the exposure risk to five serovars was found (Barwick, Mohammed, McDonough, & White, 1998). In countries with high seasonal variability, rainwater can indeed be correlated with leptospirosis (Ward, 2002b). However, there are places in the tropics where there is high variation of precipitation. In Bogotá, we found that cases of leptospirosis occurred in places where the average annual rainfall was higher.

This study provides the first epidemiological approach to leptospirosis in dogs in Bogotá, Colombia. It will support the planning of interventions aimed at preventing future cases. The dogs were examined at the Veterinary Hospital of the National University of Colombia, and this sample was built up according to convenience. It was thus inferior to probability sampling regarding its representativeness of the rest of the city. Hence, although the results obtained from this study relate to the city of Bogotá, they cannot be extrapolated to the whole city. On the other hand, some recent papers have stated that convenience sampling is useful for providing population immunity data, especially when the population is very large or when researchers have limited resources and workforces (Etkin, Musa, & Alkassim, 2016; Kelly, Riddell, Gidding, Nolan, & Gilbert, 2002).

5. Conclusions

We found high occurrence of antibodies against *Leptospira*, which demonstrated that this pathogen was circulating in the urban area of Bogotá, Colombia. Autumnalis was the most common serovar found in this city. Serovars not commonly reported in dogs, such as Hebdomadis, Cynoptery, Ballum and Wolffi, were also found. Living near water bodies and observation of dogs hunting rodents were identified as risk factors for occurrences of leptospirosis in Bogotá. Annual precipitation was higher at the geographical locations of reactive dogs than at those of unreactive dogs. 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 preventing leptospirosis.

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Declaration of interest

The authors declare that they have no conflicts of interest.

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