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

Nicolás Céspedes Cárdenas[a,*], Gina Polo Infante[a], Dina Andrea Rangel Pacheco[b], Juan Pablo Diaz Diaz[b], Diana Carolina Mejia Wagner[c], Ricardo Augusto Dias[a], José Soares Ferreira Neto[a], Marcos Amaku[a], Piero Vargas-Pinto[b], Luis Polo[b], Jose Henrique Hildebrand Grisi-Filho[a]

[a] Universidade de São Paulo, 05508 Sao Paulo, Sao Paulo, Brazil
[b] Universidad Nacional de Colombia, Bogotá, Colombia
[c] Colombian Agricultural Institute, Bogotá, Colombia

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

* Corresponding author.
E-mail addresses: ncespedesc@unal.edu.co, ncespedesc@usp.br (N.C. Cárdenas).

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

wastewater 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).

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

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.

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 [?? to better define?] 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

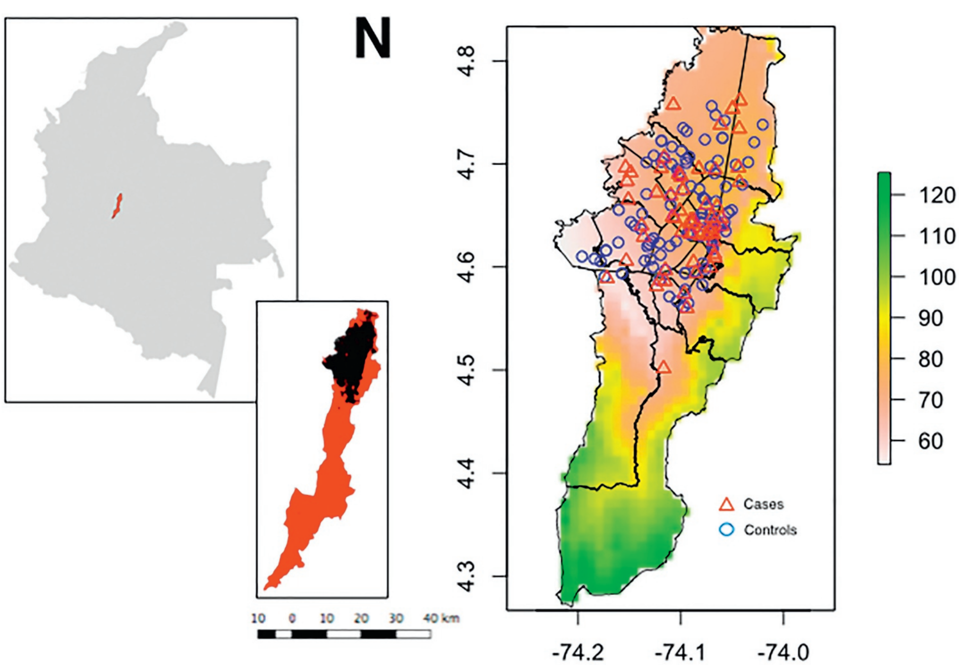

Fig. 1. A. Location of Bogotá in Colombia, the red part represents the urban area of Bogotá. B Distribution of reactive dogs and unreactive dogs in the urban area of Bogotá according to the average annual rainfall expressed as the amount of rain per square meter in one hour (mm).

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, rainfall 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 (Etikan, 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.

Acknowledgements

The authors want to thank the Laboratory and the Clinic of Veterinary Medicine of the National University of Colombia. We also thank the Colombian Agricultural Institute (Instituto Colombiano Agropecuario, ICA).

Declaration of interest

The authors declare that they have no conflicts of interest.

References

Adler, B., & de la Peña Moctezuma, A. (2010). Leptospira and leptospirosis. *Veterinary Microbiology, 140*(3–4), 287–296. https://doi.org/10.1016/j.vetmic.2009.03.012.
Agudelo-Flórez, P., Restrepo-Jaramillo, B. N., & Arboleda-Naranjo, M. (2007). Situación de la leptospirosis en el Urabá antioqueño colombiano: Estudio seroepidemiológico y factores de riesgo en población general urbana. *Cadernos de Saúde Pública, 23*, 2094–2102.
Álvarez, L., Calderón, A., Rodríguez, V., & Arrieta, G. (2011). Seroprevalencia de Leptospirosis canina en una comunidad rural del municipio de Ciénaga de oro, Córdoba (Colombia). *Revista U.D.C.A Actualidad & Divulgación Científica, 14*, 75–81.
André-Fontaine, G. (2006). Canine leptospirosis—Do we have a problem. *Veterinary Microbiology, 117*(1), 19–24. https://doi.org/10.1016/j.vetmic.2006.04.005.
Azócar-Aedo, L., & Monti, G. (2016). Meta-Analyses of Factors Associated with Leptospirosis in Domestic Dogs. *Zoonoses and Public Health, 63*(4), 328–336. https://doi.org/10.1111/zph.12236.
Barwick, R. S., Mohammed, H. O., McDonough, P. L., & White, M. E. (1998). Epidemiologic features of equine Leptospira interrogans of human significance. *Preventive Veterinary Medicine, 36*(2), 153–165. https://doi.org/10.1016/S0167-5877(98)00069-5.
Bharti, A. R., Nally, J. E., Ricaldi, J. N., Matthias, M. A., Diaz, M. M., Lovett, M. A., & Vinetz, J. M. (2003). Leptospirosis: A zoonotic disease of global importance. *The Lancet Infectious Diseases, 3*(12), 757–771. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S1473309903008302.
Carroll, A. G., & Campbell, R. S. (1987). Reproductive and leptospiral studies on beef cattle in central Queensland. *Australian Veterinary Journal, 64*(1), 1–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3593131.
Cole, J. R., Sulzer, C. R., & Pursell, A. R. (1973). Improved Microtechnique for the Leptospiral Microscopic Agglutination Test. *Applied Microbiology, 25*(6), 976–980. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC380950/.
Corcho, D. B., Molina, C. F., Margarita, A., & Santana, B. G. (2007). Leptospirosis humana en la atención primaria de salud: Pautas para su prevención y control. *Revista Cubana de …, 23*(3), 1–8. Retrieved from http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-21252007000300009.
Dechner, A. (2014). A retrospective analysis of the leptospirosis research in Colombia. *The Journal of Infection in Developing Countries, 8*(3), 258–264. Retrieved from http://www.jidc.org/index.php/journal/article/view/24619254.
Etikan, I., Musa, S. A., & Alkassim, R. S. (2016). Comparison of convenience sampling and purposive sampling. *American Journal of Theoretical and Applied Statistics, 5*(1), 1–4.
Hagan, J. E., Moraga, P., Costa, F., Capian, N., Ribeiro, G. S., Wunder, E. A., Jr., & Ko, A. I. (2016). Spatiotemporal Determinants of Urban Leptospirosis Transmission: Four-Year Prospective Cohort Study of Slum Residents in Brazil. *PLoS Neglected Tropical Diseases, 10*(1), e0004275. https://doi.org/10.1371/journal.pntd.0004275.
Hijmans, R. J., Phillips, S., Leathwick, J., Elith, J., & Hijmans, M. R. J. (2017). Package 'dismo'. *Circles, 9*(1).
Greiner, M., & Gardner, I. A. (2000). Application of diagnostic tests in veterinary epidemiologic studies. *Preventive Veterinary Medicine, 45*(1–2), 43–59. https://doi.org/10.1016/S0167-5877(00)00116-1.
Kelly, H., Riddell, M. A., Gidding, H. F., Nolan, T., & Gilbert, G. L. (2002). A random cluster survey and a convenience sample give comparable estimates of immunity to vaccine preventable diseases in children of school age in Victoria, Australia. *Vaccine, 20*(25-26), 3130–3136.
Lee, H. S., Levine, M., Guptill-Yoran, C., Johnson, A. J., von Kamecke, P., & Moore, G. E. (2014). Regional and temporal variations of Leptospira seropositivity in dogs in the United States, 2000-2010. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine, 28*(3), 779–788. https://doi.org/10.1111/jvim.12335.
Levett, P. N. (2004). Leptospirosis: A forgotten zoonosis. *Clinical and Applied Immunology Reviews, 4*(6), 435–448. https://doi.org/10.1016/j.cair.2004.08.001.
Core Team, R. (2017). R: A Language and Environment for Statistical Computing. Retrieved from https://www.r-project.org/.
Raghavan, R., & Brenner, K. (2012). Evaluations of hydrologic risk factors for canine leptospirosis: 94 cases (2002–2009). *Preventive Veterinary …, 107*(1–2), 105–109. https://doi.org/10.1016/j.prevetmed.2012.05.004.
Reiczigel, J., Földi, J., & Ózsvári, L. (2010). Exact confidence limits for prevalence of a disease with an imperfect diagnostic test. *Epidemiology and Infection, 138*(11), 1674–1678. https://doi.org/10.1017/S0950268810000385.
Rogan, W. J., & Gladen, B. (1978). Estimating prevalence from the results of a screening test. *American Journal of Epidemiology, 107*(1), 71–76. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/623091.
Romero, P. M., & Sanchez, V. J. (2009). Seroprevalencia de la leptospirosis canina de tres municipios del departamento del Tolima - Colombia. *Revista MVZ Córdoba, 14*, 1684–1689.
Rubel, D., Seijo, A., Cernigoi, B., Viale, A., & Wisnivesky-Colli, C. (1997). Leptospira interrogans en una población canina del Gran Buenos Aires: Variables asociadas con la seropositividad. *Revista Panamericana de Salud Pública, 2*(2), 102–106. https://doi.

org/10.1590/S1020-49891997000800002.

Sakundarno, M., Bertolatti, D., Maycock, B., Spickett, J., & Dhaliwal, S. (2013). Risk Factors for Leptospirosis Infection in Humans and Implications for Public Health Intervention in Indonesia and the Asia-Pacific Region. *Asia Pacific Journal of Public Health, 26*(1), 15–32. https://doi.org/10.1177/1010539513498768.

Sánchez - García, A. E., Ballut Pestana, J. C., Calderón - Rangel, A., & Rodríguez - Rodríguez, V. C. (2010). Leptospirosis: Enfermedad Endémica en Caninos de Áreas Rurales de Montería (Córdoba). *ORINOQUIA, 14*, 160–167.

Schuller, S., Francey, T., Hartmann, K., Hugonnard, M., Kohn, B., Nally, J. E., & Sykes, J. (2015). European consensus statement on leptospirosis in dogs and cats. *Journal of Small Animal Practice, 56*(3), 159–179. https://doi.org/10.1111/jsap.12328.

Sergeant, E. S. G. (2016). *Epitools epidemiological calculators. Ausvet Pty Ltd. Available at:* http://epitools.ausvet.com.au.

Sonrier, C., Branger, C., Michel, V., Ruvoën-Clouet, N., Ganière, J. P., & André-Fontaine, G. (2000). Evidence of cross-protection within Leptospira interrogans in an experimental model. *Vaccine, 19*(1), 86–94. https://doi.org/10.1016/S0264-410X(00)00129-8.

Sykes, J. E., Hartmann, K., Lunn, K. F., Moore, G. E., Stoddard, R. A., & Goldstein, R. E. (2011). 2010 ACVIM Small Animal Consensus Statement on Leptospirosis: Diagnosis, Epidemiology, Treatment, and Prevention. *Journal of Veterinary Internal Medicine, 25*(1), 1–13. https://doi.org/10.1111/j.1939-1676.2010.0654.x.

Vargas, A., Santos, A. N. A., Cárdenas, E., & Obregón, N. (2011). Análisis de la distribución e interpolación espacial de las lluvias en Bogotá. *Colombia. Dyna, 78*(167), 151–159. Retrieved from http://www.redalyc.org/articulo.oa?id=49622358017.

Vijayachari, P., Sugunan, A. P., & Shriram, A. N. (2008). Leptospirosis: An emerging global public health problem. *Journal of Biosciences, 33*(4), 557–569. https://doi.org/10.1007/s12038-008-0074-z.

Ward, M. P. (2002a). Clustering of reported cases of leptospirosis among dogs in the United States and Canada. *Preventive Veterinary Medicine, 56*(3), 215–226. https://doi.org/10.1016/S0167-5877(02)00160-5.

Ward, M. P. (2002b). Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. *Preventive Veterinary Medicine, 56*(3), 203–213. https://doi.org/10.1016/S0167-5877(02)00183-6.

Wojcik-Fatla, A., Zajac, V., Wasinski, B., Sroka, J., Cisak, E., Sawczyn, A., & Dutkiewicz, J. (2014). Occurrence of Leptospira DNA in water and soil samples collected in eastern Poland. *Annals of Agricultural and Environmental Medicine : AAEM, 21*(4), 730–732. https://doi.org/10.5604/12321966.1129924.