

## Seroprevalence and factors associated with *Leptospira* infection in an urban district of Cali, Colombia

Seroprevalencia y factores asociados a la infección por *Leptospira* en un distrito urbano de Cali, Colombia

Soroprevalência da infecção por *Leptospira* e fatores associados em um distrito urbano de Cali, Colômbia

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### Abstract

Few studies have addressed *Leptospira* seroprevalence and risk factors in urban populations in Colombia. This study aimed to determine seroprevalence and factors associated with *Leptospira* infection in inhabitants of an urban district of Cali, Colombia. We collected sociodemographic and environmental data, as well as blood samples, from 353 subjects selected through a multistage cluster sampling design. We performed microagglutination test for the eight main *Leptospira* serogroups circulating in the region, considering a cut-off titer of  $\geq 1:100$ . Most participants were female (226, 64.8%), with mean age 41.4 years, and 89 (32.6%) lived in low-low socioeconomic stratum (SES-1). Overall seroprevalence was 12.2% (95%CI: 10.3%-14.4%). Factors associated with *Leptospira* infection were SES 1, older age, single marital status, ethnic groups (Afro-Colombian and white/mestizo), school students, absence of toilet, barefoot walking, travel outside Cali in the previous month, and absence of skin and mucous-membrane lesions in the previous month. Our study suggests domestic and peridomiliary transmission of *Leptospira* likely related to activities of daily living and inadequate environmental conditions. SES-1 is a major factor associated with *Leptospira* infection (adjusted OR = 4.08; 95%CI: 2.54-6.53;  $p < 0.001$ ), suggesting that social and environmental conditions are key elements for endemicity of *Leptospira* infection in the study area. Epidemiological surveillance, improvement of environmental and sanitary conditions in various SES-1 areas, and community educational campaigns are recommended.

*Leptospirosis*; *Leptospira*; Communicable Diseases

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## Introduction

Leptospirosis is a zoonotic disease of public health importance worldwide, especially in developing and tropical countries. It is caused by pathogenic bacterial species of the genus *Leptospira*, and although it was initially considered an occupational disease associated with rural environments, it is now a reemerging disease in both urban and rural contexts<sup>1,2,3,4</sup>. Given that leptospirosis affects both people's health and subsistence and that lack of health and research resources exist, it is regarded as a neglected infectious disease that perpetuates poverty<sup>5</sup>.

The epidemiology of leptospirosis is complex given the wide number of determinants for human infection in different areas<sup>6</sup>. Risk factors for leptospirosis are usually linked to *Leptospira* survival and persistence in water and moist soil contaminated with animal reservoirs' urine<sup>7</sup>. Occupational exposure, high seasonal rainfall, flooding, poor sanitation, inadequate solid waste accumulation, barefoot walking, contact with domestic and wild animals, rat infestations, and even water sports are among the most important determinants in the transmission dynamics of leptospirosis<sup>2,5,6,8</sup>.

In Colombia, several regions have socioeconomic and climatic conditions that favor *Leptospira* transmission; however, few local studies have addressed both *Leptospira* seroprevalence and risk factors in urban populations in the country<sup>9,10</sup>. Statistics from the Colombian National Public Health Surveillance System (SIVIGILA) showed that 135 (16%) of 853 confirmed cases of leptospirosis during 2014 occurred in the Valle del Cauca Department, ranking it second after Antioquia Department (data from SIVIGILA, available at <http://www.ins.gov.co>, accessed on 10/Jul/2015). In 2006, one study determined by microagglutination test (MAT) a 23.3% *Leptospira* seroprevalence in 259 inhabitants from three urban neighborhoods of Cali, Valle del Cauca<sup>9</sup>. Another study of 150 patients with clinical suspicion of leptospirosis between 2003 and 2006 in the Valle del Cauca reported 20.6% confirmed cases (titer  $\geq$  1:800) and determined eight different serogroups circulating in the region as the main ones<sup>11</sup>.

Of the 22 urban districts of Cali, Districts 18 and 20, historically considered "hot spots", accounted for 25.2% and 10.4% of leptospirosis cases in 2012, respectively (data from the Epidemiological Bulletin of the Vector-Borne Diseases and Zoonoses Group, Secretaría de Salud Pública Municipal, Cali, available at <http://www.cali.gov.co>, accessed on 10/Jul/2015) but *Leptospira* seroprevalence and the local risk factors for infection have not been assessed through epidemiological studies. As local studies are needed to understand the transmission dynamics of leptospirosis in specific populations<sup>6</sup>, we performed a seroepidemiological study aimed at determining seroprevalence and factors associated with *Leptospira* infection in the urban District 18 of Cali, Colombia.

## Materials and methods

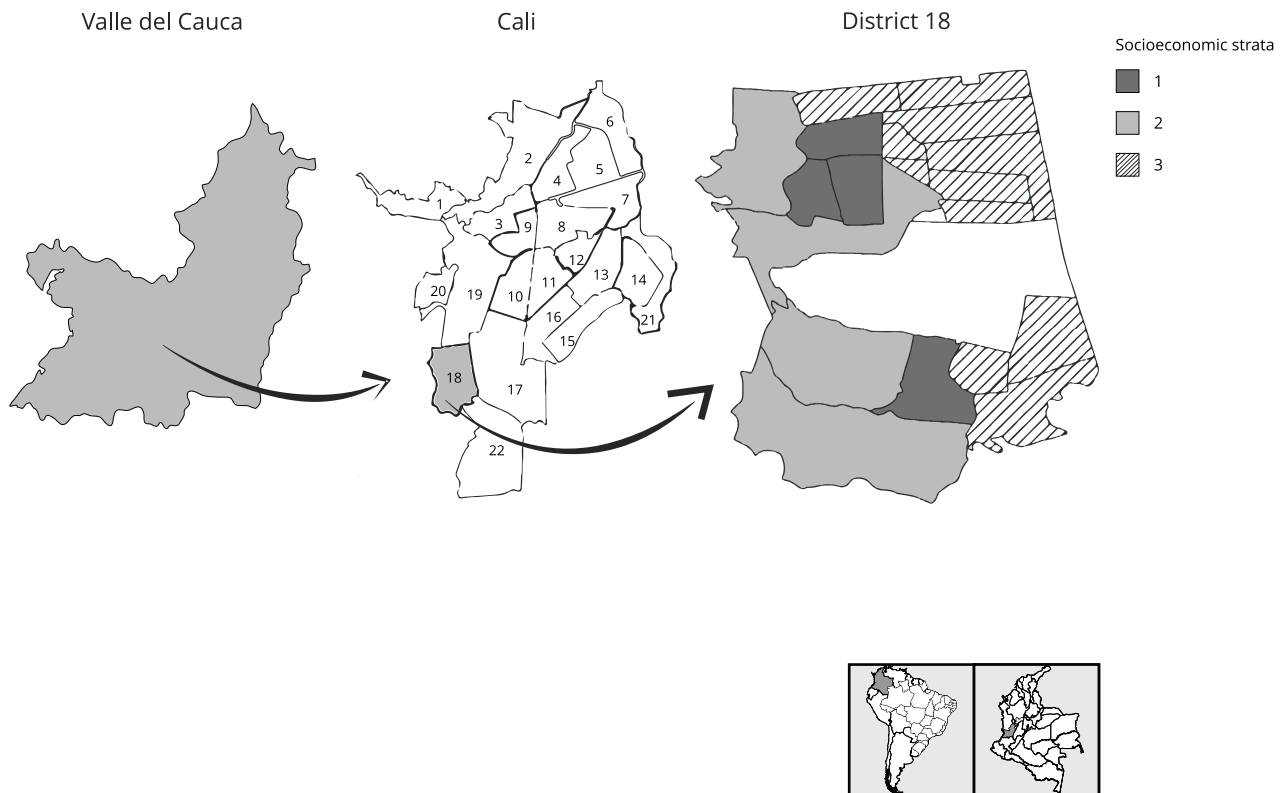
### Study area

Cali is the third largest city in Colombia, with 2.3 million inhabitants, and is located in the southwestern region of the country (Figure 1) as the capital of the Valle del Cauca Department. Cali lies at 1,070m above sea level and has an average temperature of 24.7°C (76.5°F) and an average annual rainfall of 1,019mm. District 18, one of the 22 urban divisions or communes of the city, has 542.9ha, or 4.5% of the entire urban area, and consists of 20 neighborhoods and sectors which are distributed in approximately 595 blocks. One of these sectors is non-residential and comprises the military brigade in Cali.

The socioeconomic stratification system in Colombia divides all cities' residential neighborhoods and sectors into six socioeconomic strata, ranging from 1 (SES-1; the lowest income areas) to 6 (SES-6; the highest income areas). This government classification system is based on physical characteristics of the households (e.g., type of construction, building materials of the façade and main door, presence of front yard and garage), and the surrounding environment (e.g., presence of sidewalks, type of streets and roads, presence of environmental contamination sources), and is used to bill for public utilities and facilitate the distribution of subsidy programs (data from the Colombian National Administrative Department of Statistics (DANE), available at <http://www.dane.gov>).

**Figure 1**

District 18 (Cali, Valle del Cauca, Colombia) with its socioeconomic stratification.



co/index.php/estratificacion-socioeconomica/generalidades, accessed on 05/Oct/2015). Of the 19 residential neighborhoods and sectors of District 18, 11 belong to SES-3 (medium-low), 4 to SES-2 (low), and 4 to SES-1 (low-low) (Figure 1). Most SES-1 and several SES-2 areas of District 18 are urban slums and have environmental conditions that favor *Leptospira* transmission. District 18 has been reported as the urban city administrative division with the majority of suspected human cases of leptospirosis, especially in the neighborhoods Meléndez and Los Chorros which have historically been considered notification “hot spots” (data from the Epidemiological Bulletin of the Vector-Borne Diseases and Zoonoses Group, Secretaría de Salud Pública Municipal, Cali, available at <http://www.cali.gov.co>, accessed on 10/Jul/2015).

### **Study design and population**

A cross-sectional study was conducted from November 2013 to March 2014 in residents of District 18 of Cali, Colombia. People of either sex, 5 years and older, and living in houses other than residential units and apartments were eligible to enter the study. Written informed consent was obtained from all participants before entering the study. Children 8 to 17 years of age were asked to read and sign an informed assent form, besides the consent form signed by parents or guardians. The study was approved by the Institutional Review Board, Universidad del Valle.

### **Sample size and sampling**

We calculated a sample size of 277 persons for complex sampling based on an estimated population of 124,105 in District 18 in 2013 (data from document *Cali in Numbers, 2013*, prepared by the Administrative Department of Municipal Planning, Alcaldía de Santiago de Cali, Cali, available at [http://planeacion.cali.gov.co/Publicaciones/Cali\\_en\\_Cifras/Caliencifras2013.pdf](http://planeacion.cali.gov.co/Publicaciones/Cali_en_Cifras/Caliencifras2013.pdf), accessed on 10/Jul/2015), 23% *Leptospira* seroprevalence<sup>9</sup>, 95% confidence interval (95%CI), and 7% precision. Sample size was increased to 360 persons to account for non-response. Multistage cluster sampling was conducted using a list of all the blocks in District 18, from which 30 blocks (primary sampling unit) were randomly selected through a systematic sampling method. Twelve houses (second sampling unit) were selected in each selected block using computer-generated random numbers. All selected houses were visited by one of six trained field technicians, supervised by one of two supervisors. In each house, the number of homes (home defined as the group of people living in the same house and eating meals together) was recorded, and one home (third sampling unit) was randomly selected using colored cardboards. The field technicians then asked the date of birth of all individuals in the selected home that met inclusion criteria in order to complete a table on a written form and determine the closest date of birth to the date of the visit; this subject (fourth sampling unit) was selected to enter the study. If the eligible person was absent, a second visit was performed. When a person declined to participate, the supervisors carried out random replacement of a non-response house with another house on the same block until a participant was included. During fieldwork, two more blocks were added to the sampling for a total of 32 blocks, because some blocks showed very low response rates.

### **Procedures**

Sociodemographic and environmental data were obtained from participants through in-home interviewer-administered written questionnaires. A 5mL blood sample was drawn by antecubital venipuncture into a non-anticoagulant tube. All blood samples were allowed to clot for 30 minutes at room temperature and were then transported in a box cooler with ice packs to the Leptospirosis Laboratory of the Department of Microbiology, Universidad del Valle.

In the laboratory, the clotted blood samples were centrifuged at 3,500 x g for 10 minutes at 25°C to separate the sera, which were then stored at -20°C until performing the serological tests. MAT was done to determine the presence of anti-*Leptospira* antibodies in the study participants. MAT was performed following standard procedures<sup>12</sup> using available serogroups provided by the WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute in Amsterdam, the Netherlands. We tested a panel with 8 serovars representing the following eight serogroups of pathogenic *Leptospira* previously found to be circulating in the Valle del Cauca<sup>11</sup>: Australis (serovar Bratislava), Autumnalis (serovar Autumnalis), Bataviae (serovar Bataviae), Canicola (serovar Canicola), Cynopteri (serovar Cynopteri), Icterohaemorrhagiae (serovar Copenhageni), Mini (serovar Mini), and Shermani (serovar Shermani). *L. biflexa* serogroup Semarang (serovar Patoc) was the non-pathogenic reference strain for controls. Serial serum dilutions were performed with phosphate-buffered saline (PBS, pH 7.2) from 1:20 to 1:1,280. Tests were interpreted as positive when agglutination at  $\geq 1:100$  dilution of at least 50% of leptospires for any serovar was observed through a dark-field microscope with the 10x objective; this cut-off was chosen based on previous serosurveys performed in Colombia<sup>10,13,14,15,16,17</sup>. Since the methodology used in our laboratory started MAT dilutions at 1:20, serum titers at 1:80 were reassessed under traditional dilutions to determine whether they reached 1:100. In positive sera, the presumptive infecting serovar was considered as the pathogenic serovar with the highest agglutination titer. Coagglutinations were regarded as the cases in which a serum reacted to two or more serovars at the same titer<sup>14,15</sup>.

### **Statistical analysis**

Data were double-entered into EpiInfo 6.04 (Centers for Disease Control and Prevention, Atlanta, USA). The final database was exported to Stata 11 (StataCorp LP, College Station, USA) where *svy*

commands were used to derive statistics. All calculations were thus adjusted for sampling design effect and weighted for sampling probabilities. Relative frequencies and percentages were calculated for qualitative variables, while measures of central tendency and dispersion were used for quantitative variables. Chi-square ( $\chi^2$ ) test was used to compare categorical variables between *Leptospira*-seropositive and seronegative participants, and Student's t test was used to analyze age. We also used *svy* commands to obtain estimates for the odds ratios (OR) with their 95% confidence interval (95%CI). P-values < 0.05 were considered statistically significant. We identified independent factors associated with *Leptospira* seroprevalence in a multivariate logistic regression model using a backward stepwise approach. Sociodemographic and environmental variables with p-value < 0.2 in univariate analysis were placed all at once into the full model and removed sequentially if corresponding p-value of the adjusted Wald test exceeded 0.05. Factors that were significantly and independently associated with the outcome were retained in the final model.

## Results

A total of 353 participants were included in the study. Two serum samples were unsuitable for serological testing and were not included in seroprevalence calculation. Most participants were female (226, 64.8%), with a mean age of 41.4 years (Table 1). Participants lived in 14 neighborhoods and sectors of District 18 (Alfárez Real, Alto Nápoles, Buenos Aires, Caldas, Colinas del Sur, El Jordán, Los Farallones, Horizontes, Lourdes, Meléndez, Nápoles, Prados del Sur, Sector Alto Jordán, and Sector Meléndez); there were no participants randomly selected from Alto Meléndez-Polvorines, Francisco Eladio Ramírez, Los Chorros, Mario Correa Rengifo, or Sector Alto de los Chorros. While 89 (32.6%) participants belonged to SES-1, 87 (27.2%) belonged to SES-2 and 177 (40.2%) to SES-3. Most participants (92.9%) had health insurance. Although 97.1% reported having any schooling, only 18.5% reported greater than a high school diploma. The most frequent occupation was housewife (39.6%), followed by student (17.1%).

Regarding environmental data, we found that almost everyone (99.7%) used water from the public aqueduct system for bathing. The majority of participants (347, 98.8%) had access to a toilet. A total of 339 (96.1%) of all participants reported no house flooding in the previous month, and 182 (53.9%) reported walking barefoot. Of all the participants, 57 (17.8%) had contact with water sources other than the aqueduct in the previous month, such as swimming pools (46.3%) or rivers, lakes, and ponds (53.7%). Dog ownership was reported by 162 participants (43.9%). Observation of rodents in the house was reported by 205 participants (64.6%). Only 14 participants (3.6%) reported swimming. Some 12% of participants reported skin and mucous-membrane lesions in the previous month.

MAT was positive in 44 of 351 participants (seroprevalence 12.2%, 95%CI: 10.3%-14.4%). The highest *Leptospira* seroprevalence rates were 26.5% in Los Farallones, 20.3% in Alto Nápoles, 18.3% in Prados del Sur, and 16.9% in Sector Alto Jordán. *Leptospira* seroprevalence was 0% in Alfárez Real and Buenos Aires. Individual seroprevalence rates for SES-1, SES-2, and SES-3 were 19%, 11.7% and 7.1%, respectively. Among all participants, the lowest seroprevalence was observed in the 15-to-44-year group (10.4%), and the highest in the  $\geq 65$ -year group (18.9%). Seroprevalence in children (5-to-14 years old) was 16.4%. We found seroreactivity for 7 of the 8 serogroups tested; there was no seroreactivity to serogroup Autumnalis. Twenty-seven (61.4%) of the seropositive participants reacted to serogroup Australis (Table 2). The rest reacted to any of the other six serogroups. There were only two coagglutinations (4.5%), both caused by serogroups Canicola and Icterohaemorrhagiae, one at 1:160 and the other at 1:1,280. The participant with the 1:1,280 coagglutination and two others with 1:640 titers for serogroup Australis had the highest titers among all participants with positive MAT, but all were asymptomatic.

All sociodemographic and environmental variables were compared to the seroprevalence to find significant associations (Table 1). Single marital status (OR = 0.47; 95%CI: 0.25-0.85; p = 0.02) was a statistically significant factor associated with lower prevalence of *Leptospira* infection. Ethnic groups whites/mestizos (OR = 2.53; 95%CI: 1.27-5.04; p = 0.01) and Afro-Colombians (OR = 2.84; 95%CI: 1.29-6.25; p = 0.01) were statistically significant factors associated with higher prevalence of

**Table**

Sociodemographic and environmental characteristics of residents included in the study, and univariate analysis of factors associated with *Leptospira* infection. District 18, Cali, Colombia.

Variable	Frequency (N = 353) n (%)	MAT result [n (%)]		OR	95%CI	p-value
		Positive (n = 44)	Negative (n = 307)			
<b>Sociodemographic variables</b>						
Age, years (mean, range)	41.4 (7-91)	44.4 (7-87)	41 (7-91)	1.01	1.00-1.02	0.130
Sex						
Male	127 (35.2)	13 (30.2)	114 (36.1)	1.00		
Female	226 (64.8)	31 (69.8)	193 (63.9)	1.31	0.86-1.98	0.200
Marital status						
Single	123 (36.3)	14 (29.2)	108 (37.1)	0.47	0.25-0.85	0.020
Married or civil union	183 (52.5)	23 (53.2)	159 (52.5)	0.60	0.34-1.05	0.070
Other	47 (11.2)	7 (17.6)	40 (10.4)	1.00		
Ethnic group						
Indigenous	26 (6.0)	3 (2.6)	23 (6.5)	1.00		
White or mestizo	290 (80.4)	35 (82.0)	253 (80.0)	2.53	1.27-5.04	0.010
Afro-Colombian	37 (13.6)	6 (15.4)	31 (13.5)	2.84	1.29-6.25	0.010
Socioeconomic stratum						
3	177 (40.2)	18 (23.6)	159 (42.8)	1.00		
2	87 (27.2)	8 (25.8)	78 (27.2)	1.72	1.02-2.91	0.040
1	89 (32.6)	18 (50.6)	70 (30.0)	3.05	2.01-4.64	< 0.001
Health system affiliation						
Uninsured	22 (7.1)	2 (2.0)	20 (7.9)	1.00		
Insured	331 (92.9)	42 (98.0)	287 (92.1)	4.16	1.89-9.18	0.001
Education	342 (97.1)	42 (96.5)	298 (97.1)	0.81	0.30-2.23	0.670
Occupation						
Housewife	132 (39.6)	15 (36.8)	117 (40.2)	1.14	0.71-1.85	0.570
Student	57 (17.1)	10 (24.7)	46 (15.8)	1.96	1.11-3.46	0.020
Health professional	6 (0.8)	1 (3.2)	5 (0.5)	8.11	2.48-26.58	0.001
Farmer or rancher	6 (0.9)	0 (0.0)	6 (1.0)			
Plumber	5 (1.6)	0 (0.0)	5 (1.8)			
Garbage collector	1 (0.3)	0 (0.0)	1 (0.4)			
Unemployed or retired	40 (10.6)	6 (11.5)	34 (10.5)	1.36	0.73-2.55	0.320
Other	106 (29.1)	12 (23.8)	93 (29.8)	1.00		
<b>Environmental variables</b>						
Toilet location						
Indoors	315 (89.8)	38 (87.6)	275 (90.0)	1.00		
Outdoors	32 (9.0)	4 (9.1)	28 (9.1)	1.04	0.55-1.94	0.910
Absence of a toilet	6 (1.2)	2 (3.3)	4 (0.9)	3.75	1.33-10.60	0.010
History of house flooding *	14 (4.0)	2 (3.3)	11 (3.7)	0.91	0.38-2.22	0.840
Barefoot walking	182 (53.9)	26 (64.6)	155 (52.4)	1.66	1.13-2.43	0.010
Contact with water sources other than aqueduct *	57 (17.8)	5 (9.2)	51 (18.7)	0.44	0.25-0.78	0.006
Dog ownership	162 (43.9)	19 (30.0)	142 (45.9)	0.51	0.35-0.74	0.001
Observation of rodents in the house	205 (64.6)	28 (67.3)	176 (64.2)	1.15	0.79-1.68	0.460
Swimming	14 (3.6)	1 (1.0)	13 (4.0)	0.23	0.05-1.23	0.080
Travel outside Cali *	70 (20.5)	10 (27.8)	60 (19.7)	1.58	1.00-2.49	0.050
Skin and mucous-membrane lesions *	39 (12.0)	1 (1.5)	38 (13.6)	0.10	0.03-0.28	< 0.001

95%CI: 95% confidence interval; MAT: microagglutination test; OR: odds ratio.

\* In the previous month.

**Table 2**Distribution of *Leptospira* microscopic agglutination titers in sera of 351 inhabitants of District 18, Cali, Colombia.

	Number of subjects with agglutination titer					Total (%)
	1:100	1:160	1:320	1:640	1:1,280	
Serogroup (serovar)						
Australis (Bratislava)	15	9	1	2		27 (61.4)
Autumnalis (Autumnalis)						0 (0.0)
Bataviae (Bataviae)		3				3 (6.8)
Canicola (Canicola)	3					3 (6.8)
Cynopteri (Cynopteri)		1				1 (2.3)
Icterohaemorrhagiae (Copenhageni)		1	1			2 (4.5)
Mini (Mini)	1	1	1			3 (6.8)
Shermani (Shermani)		2	1			3 (6.8)
Coagglutinations (serogroups)						
Canicola-Icterohaemorrhagiae		1			1	2 (4.5)
<b>Total (%)</b>	19 (43.2)	18 (40.9)	4 (9.1)	2 (4.5)	1 (2.3)	44 (100.0)

*Leptospira* infection. SES-1 (OR = 3.05; 95%CI: 2.01-4.64;  $p < 0.001$ ) and SES-2 (OR = 1.72; 95%CI: 1.02-2.91;  $p = 0.04$ ) were significantly associated with higher odds of *Leptospira* infection, compared to SES-3. Education was not associated with *Leptospira* infection. There were increased odds of *Leptospira* infection for school students (OR = 1.96; 95%CI: 1.11-3.46;  $p = 0.02$ ) and health professionals (OR = 8.11; 95%CI: 2.48-26.58;  $p = 0.001$ ). Other factors associated with higher prevalence of infection were the absence of a toilet (OR = 3.75; 95%CI: 1.33-10.60;  $p = 0.01$ ) and barefoot walking (OR = 1.66; 95%CI: 1.13-2.43;  $p = 0.01$ ). History of house flooding in the previous month, observation of rodents in the house, and swimming were not associated with *Leptospira* infection. We initially found that contact with water sources other than the aqueduct in the previous month (OR = 0.44; 95%CI: 0.25-0.78;  $p = 0.006$ ) and dog ownership (OR = 0.51; 95%CI: 0.35-0.74;  $p = 0.001$ ) were associated with lower prevalence of *Leptospira* infection, but these variables were dropped from further analysis in the multivariate model due to lack of significance. The presence of skin and mucous-membrane lesions in the previous month was also inversely associated with *Leptospira* infection (OR = 0.10; 95%CI: 0.03-0.28;  $p < 0.001$ ).

In the final multivariate model, factors independently associated with higher *Leptospira* seroprevalence included older age, female sex, ethnic group (whites/mestizos and Afro-Colombians), SES-1, occupation (school student, health professional), absence of a toilet, barefoot walking, travel outside Cali in the previous month, and absence of skin and mucous-membrane lesions in the previous month (Table 3). Single marital status and being unemployed or retired were independent factors associated with lower seroprevalence of *Leptospira* infection.

## Discussion

This seroepidemiological survey aimed to determine *Leptospira* seroprevalence and associated factors in the general population of District 18 of Cali. We found an overall seroprevalence of 12.2% (95%CI: 10.3%-14.4%) among 351 participants from 14 neighborhoods and sectors of District 18. The highest seroprevalence (19%) was reported for people living in SES-1, suggesting an endemic leptospirosis pattern in that zone. The older age group showed the highest *Leptospira* seroprevalence (18.9%), as described in previous studies, likely due to longer lifetime exposure<sup>9,18,19,20</sup>.

*Leptospira* seroprevalence in the Colombian general population has been estimated between 6% and 67.9%<sup>9,10,13,14,21,22</sup>. Occupational risk groups such as farmers, ranchers, garbage collectors,

**Table 3**Multivariate logistic regression results for factors associated with *Leptospira* infection.

Variable	aOR	95%CI	p-value
Age	1.03	1.01-1.05	0.001
Female sex	2.16	1.12-4.16	0.020
Marital status			
Married	0.96	0.51-1.82	0.900
Single	0.28	0.12-0.69	0.007
Ethnic group			
Afro-Colombian	3.16	1.13-8.85	0.030
White or mestizo	3.07	1.15-8.20	0.030
Socioeconomic stratum			
1	4.08	2.54-6.53	< 0.001
2	1.64	0.94-2.88	0.080
Uninsured	2.94	1.01-8.59	0.050
Occupation			
Student	7.07	3.41-14.64	< 0.001
Housewife	1.22	0.54-2.74	0.620
Health professional	23.02	5.17-102.48	< 0.001
Unemployed or retired	0.40	0.22-0.74	0.005
Toilet location			
Absence of a toilet	4.48	1.32-15.23	0.020
Outdoors	1.95	0.95-4.01	0.070
Barefoot walking	2.22	1.44-3.43	0.001
Travel outside Cali in the previous month	2.38	1.49-3.80	0.001
Skin and mucous-membrane lesions in the previous month	0.06	0.02-0.17	< 0.001

95%CI: 95% confidence interval; aOR: adjusted odds ratio.

veterinarians, zoo workers, and slaughterhouse workers, have been screened for agglutinating antibodies against *Leptospira*, and seroprevalence rates in these specific populations range from 9.8% to 75.8%<sup>15,17,23,24,25,26,27,28</sup>.

The only other seroepidemiological study in the general population in Cali, by Ferro et al.<sup>9</sup>, reported 23.3% seroprevalence in three urban neighborhoods of other districts of Cali; two neighborhoods were SES-2 and one was SES-1. However, this seroprevalence was probably overestimated, since the MAT cut-off was lower ( $\geq 1:50$ ) than in our study and also because the authors included the non-pathogenic serogroup Semarang in the seroepidemiological analysis.

We found that serogroup Australis was the most frequent (61.4%), with dilutions from 1:100 to 1:640. In Colombia, Australis has been reported as the most common serogroup (24.9%) according to a recent national surveillance study of leptospirosis in 2007-2011<sup>4</sup>. Ferro et al.<sup>9</sup> and Astudillo Hernández et al.<sup>11</sup> also found serogroup Australis circulating in the Valle del Cauca, but serogroups Grippityphosa and Icterohaemorrhagiae accounted for the greater seroreactivity in the first of these studies, while Icterohaemorrhagiae and Mini were the most common serogroups in the second. Although cross-reactions between serogroups have been shown to occur<sup>29</sup>, identification of serogroups by MAT is useful as some hypotheses could be derived for understanding the epidemiology of leptospirosis, namely the likely source of infection and the potential reservoir and its location<sup>8,12</sup>. Prevalence of particular serovars greatly depends on the exposure to specific domestic and wild mammals, and the environmental conditions for *Leptospira* persistence in contaminated water and moist soil<sup>2,8</sup>. Reservoirs of serogroup Australis are pigs, horses, hedgehogs, dogs, cattle, sheep, goats, and donkeys<sup>16,30,31,32,33,34,35</sup>; rodents do not seem to be important reservoirs for this



serogroup. Therefore, findings for Australis and other serogroups reported in this study could be related to the presence of several reservoir animals not assessed in the study.

The Canicola-Icterohaemorrhagiae coagglutinations are likely explained because of the presence of several common leptospiral antigens or history of contact with different serovars<sup>8</sup>. Of all participants, three subjects (6.8%) were found with high *Leptospira* agglutination titers, i.e., two with 1:640 and one with 1:1,280, but all were asymptomatic. The fact that infection is asymptomatic even at such high *Leptospira* titers is probably the consequence of the endemicity. It has been recognized in many tropical countries that higher cut-off titers, even as high as  $\geq 1:1,600$ , are necessary for defining confirmed leptospirosis in single titer results, instead of the U.S. Centers for Disease Control and Prevention (CDC) titer of  $\geq 1:800$ <sup>8</sup>.

The vast majority of the studied population had access to public running water, and house flooding history in the previous month was only recorded in 4% of participants. These findings are likely explained by the study sampling and large improvement of the public services in recent years in some areas of District 18, but there are several SES-1 areas without access to public services or with flooding areas. Many studies on leptospirosis patients have widely documented that the disease is particularly prevalent in communities with inadequate sanitation<sup>3,16,20,36,37,38,39</sup>.

There were statistically significant and plausible associations with the presence of leptospiral antibodies. After multivariate adjustment, older age was associated with higher *Leptospira* seroprevalence (aOR = 1.03 per one-year increase; 95%CI: 1.01-1.05;  $p = 0.001$ ), and women showed higher *Leptospira* seroprevalence than men (aOR = 2.16; 95%CI: 1.12-4.16;  $p = 0.02$ ). As female sex was found to be directly associated with *Leptospira* infection, thus perhaps domestic factors play a major role in disease transmission more than rural occupational factors which are usually attributable to men. However, of note, we could have selected more females than males given the design of our study. Unlike our study, predominance of seroprevalence in men over women has been reported in several seroepidemiological studies in Colombia and elsewhere<sup>7,9,10,18,19,21,40,41</sup>, and most of the laboratory-confirmed clinical cases of leptospirosis have been reported to occur in men<sup>4,11,42,43,44,45,46,47</sup>. Single marital status was associated with lower odds of *Leptospira* seropositivity (aOR = 0.28; 95%CI: 0.12-0.69;  $p = 0.007$ ). One possible explanation for this finding could be that single persons are more prone to engage in occupational or leisure-time activities or even have social drivers that are riskier for *Leptospira* infection. When compared to indigenous people, both Afro-Colombians and white-mestizos were associated with higher prevalence of infection. In a study from Urabá, Antioquia, the same ethnic groups were compared, but no significant difference was found<sup>10</sup>. In contrast, black race was reported as an independent risk factor for infection in a *Leptospira* survey performed in slum residents from Salvador, Brazil<sup>39</sup>. Ethnicity could be an indicator of health inequalities. Participants from SES-1 had significantly higher *Leptospira* seroprevalence (aOR = 4.08; 95%CI: 2.54-6.53;  $p < 0.001$ ). The Colombian socioeconomic stratification system reflects the living conditions of the inhabitants. Several SES-1 areas of District 18 present open sewers, temporary accumulation of garbage piles, unpaved streets, and rainwater puddles; all of which are environmental conditions that increase proliferation of rodents and wandering of stray animals, and could ultimately explain a higher risk for *Leptospira* infection. Although the socioeconomic stratification systems (if available or existing) could vary among countries, leptospirosis-endemic urban areas are characterized by environmental conditions as the aforementioned<sup>3,9,10,16,20,22,36,38,39</sup>. Being uninsured was directly associated with *Leptospira* infection, but it was marginally associated after multivariate analysis (aOR = 2.94; 95%CI: 1.01-8.59;  $p = 0.05$ ), therefore confounding is possible. Students (aOR = 7.07; 95%CI: 3.41-14.64;  $p < 0.001$ ) and health professionals (aOR = 23.02; 95%CI: 5.17-102.48;  $p < 0.001$ ) were also directly associated with *Leptospira* infection. However, the precision of the health profession as a factor associated with infection cannot be properly established because of the wide confidence interval. Student status as a risk factor might indicate *Leptospira* household transmission rather than other occupational transmissions, although housewives were not associated with *Leptospira* exposure. As in our study, students and housewives were the main occupations in previous *Leptospira* seroepidemiological studies performed in the urban population in Colombia; however, student status had not been reported as an occupational risk for *Leptospira* infection<sup>9,10</sup>. Interestingly, students and housewives have also been reported as the main occupational groups among the confirmed cases of leptospirosis (1,519 cases, 33%) in Colombia in 2007-2011<sup>4</sup>. Romero-Vivas et al.<sup>16</sup> reported that most of the confirmed

leptospirosis patients in their study were housewives, unemployed men, and  $\leq 17$ -year-old students, suggesting household transmission.

Regarding environmental variables, factors associated with higher odds of *Leptospira* infection were absence of a toilet (aOR = 4.48; 95%CI: 1.32-15.23;  $p = 0.02$ ), barefoot walking (aOR = 2.22; 95%CI: 1.44-3.43;  $p = 0.001$ ), and travel outside Cali in the previous month (aOR = 2.38; 95%CI: 1.49-3.80;  $p = 0.001$ ). Walking barefoot indoors or outdoors was not significantly associated with the presence of leptospiral antibodies in the study by Ferro et al.<sup>9</sup>, but it has been reported as a risk factor elsewhere<sup>20,40,41</sup>. Interestingly, one study from Rural Durango, Mexico found that *Leptospira* exposure was positively associated with history of domestic travel in the general population<sup>19</sup>. Although no information exists regarding *Leptospira* infection and history of travel in Colombia, our finding also suggests exposure to unidentified sources other than the domicile. Contact with water sources other than the aqueduct in the previous month and dog ownership were associated with lower risk of *Leptospira* infection in the univariate analysis, but were removed from the final logistic regression model because of lack of statistical significance. Presence of skin and mucous-membrane lesions in the previous month was associated with lower prevalence of *Leptospira* infection, even after multivariate adjustment (aOR = 0.06; 95%CI: 0.02-0.17;  $p < 0.001$ ). One possible explanation could be that persons with skin or mucous-membrane lesions were more careful and avoided walking barefoot or other daily exposures. However, measurement errors regarding risk exposures and residual confounding are a possibility. Conversely, skin cuts and abrasions during flooding have been reported elsewhere as a risk factor<sup>40</sup>.

Our study found no increased prevalence associated with dog ownership or observation of rodents, which have been reported as risk factors for urban leptospirosis in several studies elsewhere<sup>2,7,8,18,39</sup>. In Cali, Ferro et al.<sup>9</sup> found that contact with domestic animals, namely pigs and rabbits, was the only statistically significant risk factor for the presence of leptospiral antibodies; dogs and rodents were not associated with *Leptospira* seroprevalence. Only one study has estimated *Leptospira* seroprevalence in urban-dwelling animals in Cali, reporting MAT agglutination in 41.1% of 197 dogs from 12 districts of Cali with serovars Icterohaemorrhagiae, Hardjo, Grippotyphosa, and Canicola<sup>34</sup>. Since we recorded animal ownership rather than animal observation or exposure, dogs should not be ruled out as possible urban reservoirs in District 18. As serogroup Australis accounted for the majority of positive MAT results, further epidemiological surveys and *Leptospira* analysis should be considered in mammals including pigs, equines, dogs, and cows.

Some limitations of this study need to be considered. Although the multistage design used for this study helped obtain confident data for the majority of District 18, some "hot-spots" (e.g., Los Chorros) and other very deprived areas (Alto Meléndez-Polvorines and Sector Alto de los Chorros), which are SES-1, were not randomly included and therefore our findings are not representative of the entire district. Further, eight *Leptospira* serovars representing eight different serogroups were tested based on the findings by Astudillo Hernández et al.<sup>11</sup> in clinically suspected cases from the Valle del Cauca; however, others serogroups, such as Grippotyphosa, Panama, Pomona, Sejroe, Hebdomadis, Ballum, and Pyrogenes have been documented in our city<sup>9</sup> and should be used in MAT panels for further local studies.

We encourage continued epidemiological surveillance as an essential measure for tracking leptospirosis cases and ultimate planning of effective interventions for prevention and control of this zoonosis in District 18<sup>9,45</sup>. Environmental and sanitary conditions of several areas of District 18, especially SES-1, should be improved, and public health promotion and sanitation policies are needed. Measures for leptospirosis control and prevention implemented in Cali include leptospirosis educational sessions for health care personnel, organized by the Secretaría de Salud Pública Departamental of the Valle del Cauca, capture of stray dogs, and anti-rat campaigns, but unfortunately the first were suspended in 2013. Educational campaigns for residents are critical for increasing awareness of leptospirosis and reducing human exposure to common *Leptospira* environmental sources. Practitioners in the health network setting should also be familiar with leptospirosis. Control of rodents and stray dogs should be continued even though this study did not show evidence of these animals as risk factors for *Leptospira* infection; rats and dogs are the main urban reservoirs for *Leptospira* worldwide, and their feeding and shelters are favored by deprived environmental conditions<sup>2,7</sup>.

## Conclusions

We confirmed the circulation of *Leptospira* spp. in urban District 18 of Cali, reporting 12.2% overall seroprevalence for 14 of its 19 neighborhoods and sectors. This finding indicates that a substantial proportion of residents of District 18 have been exposed to *Leptospira* spp. Lower socioeconomic stratum (SES-1) was found to be directly associated with *Leptospira* infection, suggesting that social and environmental conditions are key elements for endemicity of *Leptospira* infection in the area. Other important factors directly associated with *Leptospira* infection were student status and absence of a toilet. Altogether, our results suggest domestic and peridomestic transmission of *Leptospira*, likely related to activities of daily living and inadequate environmental conditions. We recommend epidemiological surveillance of leptospirosis cases, improvement of environmental and sanitary conditions of several SES-1 areas, and community educational campaigns.

## Contributors

K. Escandón-Vargas and M. Astudillo-Hernández conceived and designed the study. K. Escandón-Vargas drafted the manuscript and coordinated the recruitment activities. M. Astudillo-Hernández conducted the laboratory tests. K. Escandón-Vargas and L. Osorio performed the statistical analysis and data interpretation. All authors reviewed and approved the manuscript.

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## Resumen

Pocos estudios se han centrado en la seroprevalencia por *Leptospira* y sus factores de riesgo en poblaciones urbanas de Colombia. Este estudio tuvo como meta determinar la seroprevalencia y los factores asociados a la infección por *Leptospira* en una población residente de un distrito urbano de Cali, Colombia. Recogimos datos sociodemográficos y ambientales, así como muestras de sangre, de 353 sujetos seleccionados a través de un diseño de muestreo por conglomerados en etapas múltiples. Se realizó un test de microaglutinación para los ocho serogrupos principales de *Leptospira* circulando en la región, considerando una zona corte de  $\geq 1:100$ . La mayoría de los participantes fueron mujeres (226, un 64,8%), con un promedio de edad de 41,4 años, con 89 (32,6%) que vivían en condiciones socioeconómicas bajas o muy bajas (SES-1). La seroprevalencia global fue de un 12,2% (IC95%: 10,3%-14,4%). Los factores asociados con la infección por *Leptospira* fueron SES-1, edad más avanzada, estado civil soltera, grupo étnico (afrocolombiano y blanco/mestizo), estudiantes escolares, ausencia de baño, caminar descalzo, viajar fuera de Cali durante el mes previo al estudio, y ausencia de lesiones en la piel y membranas mucosas durante el mes previo al mismo. Nuestro estudio sugiere que la transmisión doméstica y peridomiliar de la *Leptospira* está vinculada a actividades de la vida diaria y a condiciones ambientales inadecuadas. SES-1 es un factor importante, asociado a la infección por *Leptospira* (OR ajustada = 4,08; IC95%: 2,54-6,53;  $p < 0,001$ ), sugiriendo que las condiciones sociales y ambientales son elementos clave para la endemicidad de la infección por *Leptospira* en el área de estudio. Se recomienda vigilancia epidemiológica, mejora de las condiciones ambientales y sanitarias en varias áreas SES-1, así como campañas educativas dirigidas a la comunidad.

*Leptospirosis; Leptospira; Enfermedades Transmisibles*

## Resumo

Poucos estudos trataram da soroprevalência para *Leptospira* e fatores de risco em populações urbanas da Colômbia. Este estudo teve como objetivo determinar a soroprevalência e fatores associados com a infecção por *Leptospira* nos habitantes de um distrito urbano de Cali, Colômbia. Os autores coletaram dados sociodemográficos e ambientais, além de amostras de sangue, de 353 indivíduos selecionados através de uma amostragem de aglomerados em múltiplas fases. Foi realizado o teste de microaglutinação para os oito principais grupos sorológicos da *Leptospira* circulantes na região, considerando um título de  $\geq 1:100$ . A maioria dos participantes era do sexo feminino (226, 64,8%), com média de idade de 41,4 anos; 89 (32,6%) pertenciam ao estrato socioeconômico mais baixo (SES-1). A soroprevalência global era 12,2% (IC95%: 10,3%-14,4%). Os fatores associados à infecção por *Leptospira* eram SES-1, idade, estado civil solteiro, grupo étnico (afro-colombiano e branco/mestiço), estudantes, ausência de privada no domicílio, andar descalço, viagem fora de Cali no mês anterior e ausência de lesões mucocutâneas no mês anterior. O estudo sugere a transmissão domiciliar e peridomiliar da *Leptospira*, provavelmente associada a atividades cotidianas e condições ambientais adversas. SES-1 é um fator importante associado à infecção com *Leptospira* (OR ajustado = 4,08; IC95%: 2,54-6,53;  $p < 0,001$ ), indicando que as condições sociais e ambientais são elementos importantes na endemicidade da leptospirose na área do estudo. Recomendamos a vigilância epidemiológica, melhora das condições ambientais e sanitárias em diversas áreas SES-1 e campanhas educativas comunitárias.

*Leptospirose; Leptospira; Doenças Transmissíveis*

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