

Leptospira infection in people in the city of Durango, Mexico: a cross sectional study

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**Ada Agustina Sandoval-Carrillo¹,
José Manuel Salas-Pacheco¹,
Elizabeth Irasema Antuna-Salcido¹,
Karla Sujey Castro-Martínez²,
Diana Stephanie Ortiz-Montaño²,
Isabel Beristain-García²,
Hilda Mariela Alvarado-Retana¹,
Agar Ramos-Nevarez³,
Sergio Manuel Salas-Pacheco¹,
Antonio Sifuentes-Alvarez⁴,
Elizabeth Rábago-Sánchez⁴,
Sandra Margarita Cerrillo-Soto³,
Francisco Xavier Castellanos-Juárez¹,
Edith Contreras-Cisneros³ and
Cosme Alvarado-Esquivel⁴ **

Abstract

Objective: To determine the seroprevalence of *Leptospira* immunoglobulin (Ig)G and IgM antibodies and its association with the characteristics of the study population from the northern Mexican city of Durango, Mexico.

Methods: Through a cross-sectional study design, inhabitants of Durango City, Mexico were surveyed between June 2018 and November 2018. Serum samples from the subjects were

¹Institute for Scientific Research “Dr. Roberto Rivera Damm”, Juárez University of Durango State, Durango, Mexico

²Faculty of Nursing, Juárez University of Durango State, Durango, Mexico

³Clínica de Medicina Familiar, Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, Durango, Mexico

⁴Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State, Durango, Mexico

Corresponding author:

Cosme Alvarado-Esquivel, Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Avenida Universidad S/N, 34000 Durango, Mexico.
Email: alvaradocosme@yahoo.com



analysed for anti-*Leptospira* IgG and IgM antibodies using commercially available enzyme-linked immunosorbent assays. Sociodemographic, clinical, behavioural and housing characteristics were recorded. Data were analysed by bivariate and multivariate analyses.

Results: The study enrolled 413 people, of which 124 (30.0%) and 137 (33.2%) were positive for anti-*Leptospira* IgG antibodies and anti-*Leptospira* IgM antibodies, respectively. Multivariate analysis showed that *Leptospira* seropositivity was associated with professional occupation, alcohol consumption, ill clinical status, memory impairment and a history of surgery.

Conclusions: This is the first study to report the seroepidemiology of *Leptospira* infection in an urban general population in the north of Mexico. The seroprevalence of *Leptospira* infection found was higher than those previously reported in Mexican studies.

Keywords

Leptospira, epidemiology, seroprevalence, urban, Mexico

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Introduction

Leptospirosis is the disease caused by the pathogenic bacteria of the genus *Leptospira*.¹ Leptospirosis has the highest burden in low-income populations both in urban and in rural environments.² Typical reservoirs of *Leptospira* are mice and rats, whereas humans are accidental hosts of pathogenic *Leptospira*.³ Human infections can vary from asymptomatic to severe disease with life-threatening consequences.⁴ Some *Leptospira*-infected individuals develop a disease comprising jaundice and renal failure.⁵ In its severe form, leptospirosis can cause multiorgan dysfunction and death in a matter of days.⁶ Despite efforts for the development of an effective vaccine against leptospirosis, few advances have been made.⁷

Knowledge about the seroepidemiology of *Leptospira* infection in Mexico is limited. In a study of inhabitants of the southern Mexican state of Veracruz, researchers found a 4% seroprevalence of *Leptospira* infection.⁸ Whereas in a study of the general population in rural communities in the

northern Mexican state of Durango, a 15.6% seroprevalence of *Leptospira* infection was found.⁹ This current study determined the seroprevalence of anti-*Leptospira* immunoglobulin (Ig)G and IgM antibodies and its association with the sociodemographic, clinical, behavioural and housing characteristics of the study population from the northern Mexican city of Durango, Mexico.

Materials and methods

Study design and population

This cross-sectional study surveyed inhabitants of Durango City, Mexico between June 2018 and November 2018. This study was undertaken by Juárez University of Durango State, Durango, Mexico. Inclusion criteria for enrolment were as follows: (i) inhabitants of Durango City, Mexico; (ii) aged ≥ 14 years; (iii) those that voluntarily agreed to participate in the study. Subjects were recruited in factories, educational and health facilities, businesses and a government agency.

The Ethics Committee of the Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, Durango, Mexico approved this Project. Participation was voluntary and written informed consent was taken from each participant.

Sociodemographic, clinical, housing and behavioural characteristics

Birthplace (Durango or other Mexican state), residence (Durango or other Mexican state), age, sex, education (1–6, 7–12, >12 years), socioeconomic status (low, medium, high) and occupation (housewife, business, construction, employees, student, factory worker, professional, none or other) of participants was recorded. Clinical data included health status (ill or healthy), history of blood transfusions, transplants, surgeries, hepatitis, lymph node enlargement, frequent headache or abdominal pain, and impairments of vision, hearing, memory and reflexes. Housing conditions include information about crowding, form of elimination of excretes, type of floors, availability of drinkable water and education of the head of the family. Behavioural characteristics included consumption of untreated water, unwashed raw fruits or vegetables, contact with soil, consumption of unpasteurized milk, type of meat consumed, raising animals and traveling.

*Detection of anti-*Leptospira* IgG and IgM antibodies and *Leptospira* DNA*

Phlebotomy was performed at the antecubital fossa. Venous blood (5 ml) was collected from each participant. Blood samples were left at room temperature for 1–4 h to allow clot formation and then centrifuged at 2500 *g* for 10 min at room temperature using a Thermo Scientific™ Sorvall™ Legend™ X1R Centrifuge (Thermo Fisher Scientific,

Waltham, MA, USA). Serum samples were obtained and stored at –20 °C until tested. Sera were analysed for anti-*Leptospira* IgG and IgM antibodies using the commercially available enzyme-linked immunosorbent assays (ELISAs) AccuDiag™ *Leptospira* IgG ELISA Kit (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA) and AccuDiag™ *Leptospira* IgM ELISA Kit (Diagnostic Automation/Cortez Diagnostics Inc.), respectively.

Detection of *Leptospira* DNA was performed by end-point multiplex polymerase chain reaction (PCR) following the protocol previously described.¹⁰ DNA was extracted from 0.5 ml of whole blood as previously described.¹¹ PCR was performed using a C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). The primer sequences were as follows: LG1, 5'-CGGTGAAATGCGTAGATATC-3'; LG2, 5'-CGGTTTGTCACCGGCAGTTC-3'; LP1, 5'-TCGTTTGTAGAATCGATAG-3'; LP2, 5'-ATACCTCCATTATGTA-3'. In addition, β -globin was detected using the following primers: Dir1, 5'-ACACAACCTGTGTTCACTAGC-3'; and Rev1, 5'-CAACTTCATCCACGTTCCACC-3'. The reaction mix consisted of 1× PCR buffer (Promega, Madison, WI, USA), 1.5 mM MgCl₂ (Promega), 200 μ M of dNTP (Promega), 60 pmol of each primer (LG1, LG2, LP1, LP2, Dir1 and Rev1; Integrated DNA Technologies, Coralville, IA, USA), 1U Taq DNA polymerase (Promega) and 5 μ l of genomic DNA (5 ng/ μ l). The cycling programme involved preliminary denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and elongation at 72 °C for 2 min, followed by a final elongation step at 72 °C for 5 min. The amplified products were analysed by electrophoresis using 3% agarose gel stained with ethidium bromide followed by visualization with UV light. For the LG1/LG2 primers, the

expected amplicon sizes ranged from 479 to 483 base pairs. For the LP1/LP2 primers, the expected amplicon sizes ranged from 192 to 282 base pairs. For the Dir1/Rev1 primers, the expected amplicon size was of 113 base pairs.

Statistical analyses

All statistical analyses were performed using Epi Info™ for Windows version 7 (The Centers for Disease Control and Prevention, Atlanta, GA, USA) and IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). A sample size of 316 people was required, which was calculated using a 95% confidence level, a reference seroprevalence of 15.6%,⁹ a population size of 500 000 and 4% of confidence limits. Pearson's χ^2 -test was used to compare seroprevalences among the groups. As a strategy to find variables associated with infection, only variables with a P -value ≤ 0.05 obtained in the bivariate analysis were further analysed by logistic regression analysis with the Enter method. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was set at a P -value < 0.05 .

Results

This cross-sectional study surveyed 413 inhabitants of Durango City, Mexico. Of the 413 people studied, 124 (30.0%) and 137 (33.2%) were positive for anti-*Leptospira* IgG antibodies and anti-*Leptospira* IgM antibodies, respectively. The age in participants ranged from 14 to 78 years (mean \pm SD: 35.38 \pm 13.23 years). None of the participants was positive for *Leptospira* DNA by PCR. Of the sociodemographic characteristics studied, only the variable occupation had a P -value ≤ 0.05 by bivariate analysis. With respect to behavioural and housing characteristics,

bivariate analysis showed that only the variables national trips, consumption of untreated water and alcohol consumption were associated ($P < 0.05$) with seropositivity to *Leptospira* (Table 1). With regard to clinical characteristics, bivariate analysis showed that *Leptospira* IgG seropositivity was associated ($P < 0.05$) only with an ill clinical status, memory impairment and a history of surgery. Multivariate analysis of sociodemographic, behavioural and clinical characteristics with P -values ≤ 0.05 obtained by bivariate analysis showed that *Leptospira* IgG seropositivity was associated only with professional occupation (OR = 1.11; 95% CI 1.01, 1.21; $P = 0.01$), alcohol consumption (OR = 2.08; 95% CI 1.14, 3.80; $P = 0.01$), ill clinical status (OR = 1.88; 95% CI 1.06, 3.35; $P = 0.03$), memory impairment (OR = 1.85; 95% CI 1.07, 3.18; $P = 0.02$) and a history of surgery (OR = 1.69; 95% CI 1.06, 2.69; $P = 0.02$) (Table 2).

Discussion

This current cross-sectional study found that 30.0% of the study population had anti-*Leptospira* IgG antibodies. To the best of our knowledge, this is the first report of *Leptospira* seroprevalence in an urban general population in northern Mexico. In a study in rural Durango, 15.6% seroprevalence of *Leptospira* infection was found.⁹ The seroprevalence of *Leptospira* infection found in the present study was higher than those reported in several population groups in Mexico including patients with liver diseases (22.7%),¹² meat workers (17.7%),¹³ gardeners (6%)¹⁴ and waste pickers (4.4%).¹⁵ However, the seroprevalence found in the current study was lower than the 61% seroprevalence found in dog owners in the east central Mexican state of Veracruz (61%)¹⁶ and veterinarians in Mexico (38.8%).¹⁷ Differences in the *Leptospira* seroprevalence among groups

Table 1. Bivariate analysis of selected behavioural and housing characteristics and *Leptospira* seroprevalence in 413 inhabitants of Durango City, Mexico.

Characteristic	Subjects tested*	Prevalence of <i>Leptospira</i> infection		Odds ratio	95% confidence interval	Statistical significance
		n	%			
Dogs at home						
Yes	336	96	28.6	0.70	0.41, 1.17	NS
No	77	28	36.4	1.00		
National trips						
Yes	326	107	32.8	1.98	1.11, 3.53	P = 0.01
No	86	17	19.8	1.00		
Pork meat consumption						
Yes	372	116	31.2	1.86	0.83, 4.17	NS
No	41	8	19.5	1.00		
Pigeon meat consumption						
Yes	12	6	50.0	2.38	0.75, 7.56	NS
No	400	118	29.5	1.00		
Horse meat consumption						
Yes	17	2	11.8	0.29	0.06, 1.32	NS
No	396	122	30.8	1.00		
Raw dried meat of snake						
Yes	2	2	100.0	—	—	NS
No	411	122	29.7	1.00		
Raw milk consumption						
Yes	118	38	32.2	1.15	0.72, 1.82	NS
No	295	86	29.2	1.00		
Untreated water						
Yes	93	20	21.5	0.56	0.32, 0.98	P = 0.04
No	320	104	32.5	1.00		
Alcohol consumption						
Yes	315	105	33.3	2.02	1.16, 3.52	P = 0.01
No	96	19	19.8	1.00		
Washing hands before eating						
Yes	388	116	29.9	0.90	0.38, 2.15	NS
No	25	8	32.0	1.00		
Floor at home						
Ceramic or wood	291	95	32.6	1.00		
Concrete	119	28	23.5	0.63	0.38, 1.03	NS
Soil	3	1	33.3	1.03	0.09, 11.51	NS

*Some values do not total 413 because of missing data.

NS, no significant association ($P \geq 0.05$).

can be explained by differences in the characteristics of the study populations. In the present study, seropositivity was associated with professional occupation; a high seroprevalence of *Leptospira* infection has been

found in several occupational groups including garbage collectors,¹⁸ miners¹⁹ and wet market workers.²⁰ Therefore, these current findings further confirm that *Leptospira* infection is an occupational

Table 2. Multivariate analysis of selected characteristics of 413 inhabitants of Durango City, Mexico and their association with *Leptospira* infection.

Characteristic	Odds ratio	95% confidence interval	Statistical significance
Professional occupation	1.11	1.01, 1.21	$P = 0.01$
National trips	1.33	0.70, 2.51	NS
Untreated water consumption	0.61	0.34, 1.09	NS
Alcohol consumption	2.08	1.14, 3.80	$P = 0.01$
Ill clinical status	1.88	1.06, 3.35	$P = 0.03$
Memory impairment	1.85	1.07, 3.18	$P = 0.02$
History of surgery	1.69	1.06, 2.69	$P = 0.02$

NS, no significant association ($P \geq 0.05$).

disease. *Leptospira* IgG seropositivity was also associated with alcohol consumption in the current study. To the best of our knowledge no previous report describes this association. In the current study, seropositivity to *Leptospira* was associated with an ill clinical status, memory impairment and a history of surgery. *Leptospira* may cause central nervous system involvement.²¹ Therefore, a memory impairment might occur during infection. On the other hand, *Leptospira* infection has been linked to several clinical conditions leading to surgery. For example, leptospirosis mimicking acute cholecystitis has been reported.²² Further research on the association between *Leptospira* infection and a history of surgery and memory impairment should be conducted.

None of the samples in the current study was positive for *Leptospira* DNA. False-positive cases identified via the ELISAs were ruled out by the use of positive and negative controls. It is thought that the PCR was unable to detect the *Leptospira* DNA in the blood samples as a result of a low concentration of bacterial DNA in the samples. A previous study reported that at least 10 pg of bacterial DNA are necessary

for detection with this method.²³ The efficacy of the PCR procedure was confirmed by using the β -globin gene as a positive internal control.

In conclusion, this is the first study to describe the seroepidemiology of *Leptospira* infection in an urban general population in the north of Mexico. The seroprevalence of *Leptospira* infection found in the general population in Durango City was higher than those previously reported in Mexican studies.


Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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ORCID iD

Cosme Alvarado-Esquivel  <https://orcid.org/0000-0002-0367-6052>

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