

Presence of Pathogenic *Leptospira* spp. in an Urban Slum of the Colombian Caribbean: A One Health Approach

Presencia de *Leptospira* spp. patógenas en un sector marginal urbano del Caribe colombiano: un enfoque *One-Health*

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ABSTRACT

Objective: This study aims to describe the presence of pathogenic *Leptospira* and potential risk factors for leptospirosis in a marginal sector of the Colombian Caribbean, with a One Health approach.

Methods: an exploratory study was carried out in a marginal sector of Soledad, a municipality located in the north coast of Colombia. Blood samples were taken from 83 subjects, who were also questioned about sociodemographic, clinical and ecological variables related to leptospirosis. The presence of IgM Anti-*Leptospira* antibodies in human serum was performed by ELISA. A total 53 synanthropic rodents were also captured using Sherman traps. Renal tissue samples were taken from rodents to identify pathogen *Leptospira* spp. by conventional PCR using specific primers.

Results: IgM-anti-*Leptospira* was present in 30.12% of study subjects and pathogenic *Leptospira* spp. was identified in 7.55% of captured rodents. In the selected sample we found an association of positive cases with being a merchant, housewife and being in contact with pigs. Suboptimal hygienic-sanitary conditions were also evident in the study area.

Conclusions: Our results show the circulation of pathogenic *Leptospira* spp. and exposure to human and ecological risk factors in a marginal sector (slum) of the Colombian Caribbean. We suggest to direct interventions in the human-animal-environment interface according to the One Health paradigm, considering extreme poverty as a determining factor for Leptospirosis occurrence.

Keywords: Leptospirosis; Rodentia; urban slum; One Health; Colombia.

RESUMEN

Introducción: La leptopiosis es una zoonosis que tiene alto impacto en la salud de las personas y los animales, especialmente en áreas tropicales y subtropicales. Esta enfermedad es causada por el patógeno *Leptospira* spp. y es transmitida principalmente por los roedores.

Objetivo: Describir la presencia de *Leptospira* patógena y los posibles factores de riesgo de leptospirosis en un sector marginal de Colombia, con un enfoque *One-Health*.

Métodos: Se llevó a cabo un estudio exploratorio en un sector marginal de Soledad, municipio situado en la costa norte de Colombia. Se tomaron muestras de sangre de 83 sujetos. Se analizaron variables sociodemográficas, clínicas y ecológicas relacionadas con la leptospirosis. La presencia de anticuerpos IgM anti-leptospira en el suero humano fue detectado mediante la prueba ELISA. También, se tomaron muestras de tejido renal de 53 roedores sinantrópicos para identificar *Leptospira* spp. patógenas mediante PCR convencional a través del uso de cebadores específicos.

Resultados: Se detectaron anticuerpos IgM-anti-leptospira en el 30,12 % de los sujetos de estudio. La *Leptospira* spp. patógena fue identificada en el 7,55 % de los roedores analizados. En la muestra seleccionada se encontró asociación de casos seropositivos con ser comerciantes, ama de casa y estar en contacto con cerdos. Las condiciones higiénico-sanitarias subóptimas también fueron evidentes en el área de estudio.

Conclusiones: La circulación de *Leptospira* spp. patógena y la exposición a factores de riesgo humanos y ecológicos es elevada en el sector marginal (área de pobreza) del Caribe colombiano. Se recomienda dirigir las intervenciones en la interfaz hombre-animal-

ambiente de acuerdo con el paradigma *One-Health*; se debe considerar la extrema pobreza como un factor determinante para la ocurrencia de la leptospirosis.

Palabras clave: leptospirosis; roedores; áreas de pobreza; *One-Health*; Colombia.

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Introduction

Leptospirosis is a worldwide distributed zoonosis with high impact on human and animal health, especially in tropical and subtropical areas. This disease is caused by a spirochete of the genus *Leptospira* and is transmitted mainly by rodents. *Leptospira* is a multi-host pathogen affecting both wild and domestic animals and humans.⁽¹⁾ Each year, an estimated of 1.03 million cases and 58,900 deaths in humans from leptospirosis are reported around the world.⁽²⁾ The effects on animal health also cause significant economic losses.^(3,4)

However, leptospirosis remains an unattended disease, as other infectious diseases, it usually occurs in the acute phase as a nonspecific febrile syndrome, which leads to difficult diagnosis. Additionally, lack of awareness about the importance of leptospirosis as a differential diagnosis of febrile illness is common. Underdiagnosis and gaps in knowledge about epidemiological aspects of leptospirosis contribute to underestimating the true disease vastness and its lack of control.^(5,6,7)

The integral management of leptospirosis can be approached from a One Health perspective, a global initiative that aims to focus intervention actions on the human-animal-environment interface. The usefulness of this approach is since leptospirosis in humans is acquired almost invariably by contact with reservoirs or vehicles such as water, soils and contaminated food by excreta from infected animals. The implementation of strategies with a One Health approach requires integrating collaborative work between the human, animal and environmental health sectors. Likewise, it implies directing efforts to know the particularities of epidemiology in each region to guide the necessary prevention and control measures.^(8,9,10)

Epidemiological behavior of leptospirosis varies from place to place due to factors that include climatic, ecological and sociodemographic characteristics. It has been shown that

leptospirosis is more prevalent in areas with tropical climates, heavy rains, presence of reservoirs and communities in slums.^(6,11) The Colombian Caribbean is characterized by a tropical climate with abundant rainfall and encompasses numerous water sources, where the municipality Soledad is located, which includes part of the Magdalena River in the department of Atlántico. Soledad covers most of the population of the department, mainly displaced communities with low economic income.

Although new emerging cases of leptospirosis are permanently reported from Soledad, there are no published studies exploring *Leptospira* exposure in human and animal populations in that location. A study conducted in Barranquilla, a city next to Soledad, found seroprevalences of *Leptospira* spp. of 18.84%, 22.9%, and 12.4% in rodents, dogs and humans respectively.⁽¹²⁾ Our study provides evidence of the presence of *Leptospira* spp. in synanthropic rodents and in the human population that trades and / or lives in an area of Soledad known as “Caño de Soledad”, which integrates a residential area and the public market of Soledad. Likewise, the exposure to potential risk factors for transmission of leptospirosis is described and intervention measures for the disease are discussed according to the One Health paradigm.

Methods

Subjects and sampling

This research was carried out in the so called "Caño de Soledad" sector, located in the east of Soledad (Atlántico) on the banks of the Magdalena River, Atlántico department in the Colombian Caribbean. This sector, which integrates a residential zone and the public market of Soledad, has an area of approximately 65 km² and an estimated population of 1700 inhabitants (<https://www.instantstreetview.com/@10.915277,-74.761326,101.24h,-16.58p,1z>).

Humans: Inhabitants and merchants were selected from the study site by non-probabilistic sampling. A total of 83 volunteers of both genders between 12 and 65 years were enrolled in the study, with prior sign of an informed consent. Participants were given a survey to inquire about a history of common symptoms related to leptospirosis in the last five months, and exposure to risk factors for the disease, including age, sex, occupation and contact with animals potentially carrying *Leptospira*.

Each participant was transferred to a hospital near the study area and 5 mL of peripheral blood was taken, this procedure was performed by a trained professional. Samples were collected and stored in sample tubes with no anticoagulant at 4 °C during transport. Once in laboratory, blood serum was obtained by centrifugation and stored in microcentrifuge tubes at -30 °C until processing.

Rodents: Synanthropic rodents were captured at the study site. For this, Sherman-type traps were primed and installed inside residences and commercial establishments. The captured rodents were anesthetized with 100 mg / kg of tiletamine / zolacepam (Zoletil 50, Laboratorios Virbac S.A., Colombia), and the rodent species were identified according to phenotypic characteristics.⁽¹³⁾ Next, a dissection at the abdominal level of the rodents was carried out and the kidneys were removed. Samples were deposited in sterile eppendorf tubes identified with codes and stored at -30 °C until processing.

Detection of IgM-Anti-*Leptospira* in human serum

An ELISA was performed for the qualitative detection of anti-*Leptospira* IgM antibodies in blood serum. In the procedure the Panbio® *Leptospira* IgM ELISA Kit (Panbio diagnostics) was used and the manufacturer's instructions were followed.

Detection of pathogenic *Leptospira* spp. in rodents

Genomic DNA extraction was performed using the QIamp DNA Mini kit® (Qiagen, Hilden, Germany) One kidney from each rodent, according to the manufacturer's instructions. Conventional PCR was performed for the detection of pathogenic *Leptospira* spp. A set of previously reported primers was used to amplify a region located between positions 270 and 692 of the lipL32 gene (270F-CGCTGAAATGGGAGTTCGTATGATT and 692R-CCAACAGATGCAACGAAAGATCCTTT).^(12,14) The components of the PCR reaction mixture were supplied by the GoTaq® Flexi DNA Polymerase kit (Promega). The mixture was prepared with a final volume of 50 µL, containing 5 µL of DNA, 0.2 mM dNTPs, 1.25 IU of GoTag® DNA polymerase, 3.0 mM MgCl₂ and 0.1 µM of each primer. The PCR conditions included an initial cycle at 95 °C for 5 min, followed by 34 cycles at 94 °C for 1 minute; 55 °C for 1 min and 72 °C for 2 min; with a final extension stage at 72 °C for 5 min. The amplification products were developed with 1.5% agarose gel electrophoresis. A band of approximately 423 bp was considered positive for pathogenic *Leptospira* spp.

Statistical analysis

The Statistical Package for Social Studies (SPSS) version 25.0 was used to analyze data. The results obtained are shown as absolute and percentage frequencies. To measure the statistical associations between the presence of anti-*Leptospira* antibodies and the other study variables, the Chi-Square test was applied a 95% confidence level was used and a $p < 0.05$ was considered statistically significant.

Ethical declaration

This study was approved by the Ethics Committee of the Universidad del Atlántico in accordance with current Colombian regulations. Informed consent was obtained from the adult participants, as well as the consent of the minors and the consent of the respective parents.

Results

Altogether, 83 people participated in the study, of which 30.12% (25/83) were positive for IgM-anti-*Leptospira*. Among sociodemographic variables, occupation as a merchant ($p = 0.018$) and housewife ($p = 0.032$), showed statistically significant association with positive IgM cases against *Leptospira* spp. (Table).

Table - Sociodemographic and clinical characteristics, exposure to potential reservoirs of the population, and association analysis with IgM cases positive for *Leptospira* spp.

Variable	Category	Total frequency n= 83 [n (%)]	IgM		p-value
			Positive n= 25 [n(%)]	Negative n= 58 [n (%)]	
Sex	F	46 (55,4)	15 (60)	31 (53,4)	0,582
	M	37 (44,6)	10 (40)	27 (46,6)	
Age (years)	10-17	16 (19,3)	2 (8)	14 (24,1)	0,131
	18-30	21 (25,3)	10 (40)	11 (19,0)	
	31-50	21 (25,3)	5 (20)	16 (27,6)	
	> 50	25 (30,1)	8 (32)	17 (29,3)	
Occupation	Housewife	32 (38,6)	14 (56)	18 (31,0)	0,032
	Merchant	29 (34,9)	4 (16)	25 (43,1)	0,018
	Student	15 (18,1)	2 (8)	13 (22,4)	0,117
	Other	7 (8,4)	5 (20,0)	2 (3,4)	-
Symptoms related to leptospirosis	Fever	19 (22,9)	5 (26,3)	14 (73,7)	0,681
	Headache	41 (49,4)	12 (29,3)	29 (70,7)	0,867
	Runny nose	49 (59)	15 (30,6)	34 (69,4)	0,907
	General malaise	18 (21,7)	6 (33,3)	12 (66,7)	0,737
Contact with animals	Pig	9 (10,9)	7 (77,8)	2 (22,2)	0,001
	Dog	42 (50,6)	16 (38,1)	26 (61,9)	0,109
	Cat	31 (37,3)	11 (35,5)	20 (64,5)	0,411
	At least one domestic animal	57 (68,7)	22 (38,6)	35 (61,4)	0,013
	Rodents in the residence	67 (80,7)	21 (31,3)	46 (68,7)	0,619

On the other hand, no association was found with the presence of antibodies and any common cold symptoms presented in the last five months. None of the subjects said they had other symptoms such as jaundice or kidney damage, or previous diagnosis of leptospirosis. Regarding exposure to animals, contact with pigs and having at least one pet in the home (cat, dog or pig) showed an association with the presence of IgM Anti-*Leptospira* antibodies (Table).

A total of 53 specimens were obtained from the rodent capture, including 39 of *Mus musculus* species, 13 *Rattus rattus* and 1 *Rattus norvegicus*. A proportion of 7.55% (4/53) of the rodents were identified as carriers of pathogenic *Leptospira* spp. by PCR analysis (Fig. 1). Among the pathogenic *Leptospira* spp. carriers' rodents, two were *Mus musculus* species, one *Rattus rattus* and one *Rattus norvegicus*.

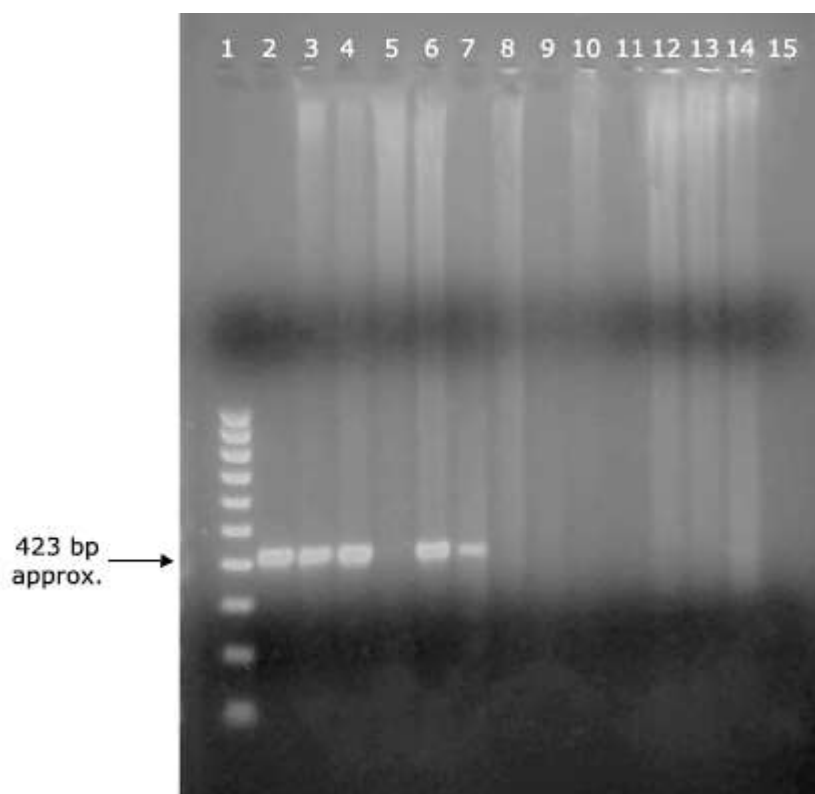


Fig. 1 - Agarose gel electrophoresis of PCR products. DNA was extracted from kidney tissue samples of the rodents and subsequently a sequence of the lipL32 gene was amplified by PCR. The result was observed with 1.5% agarose gel electrophoresis. A band of approximately 423 bp indicates the presence of pathogenic *Leptospira*. Line 1: molecular Ruler 100 bp molecular weight marker (BioRad); line 2: positive control; lines 3, 4, 6 and 7: positive samples; lines 5 and 8-14: negative samples; line 15: negative control.

In the study site, sub-optimal sanitation conditions were evidenced, characterized by the presence of solid waste and wastewater flow in the streets. An abundance of potential *Leptospira* reservoirs such as dogs, cats and pigs were also evident in this place (Fig. 2).



Fig. 2 - Environmental conditions of the Caño de Soledad Sector: a) commercial area, inadequate disposal of solid waste and direct contact of people and animals with these materials; b) - c) residential area, sewage and solid waste streams are observed; d) Interior of one of the houses where rodent capture was carried out.

Discussion

Our research presents evidence of recent exposure to *Leptospira* spp. in a human subpopulation of the Colombian municipality Soledad. In addition, we report the presence of rodents carrying pathogenic *Leptospira* spp. and describe sociodemographic and ecological risk factors that could favor the transmission of leptospirosis in this population. It should be noted that to our knowledge, this is the first investigation that actively explores the exposure of this population to leptospira spp. and it is added to the few studies reported so far in urban slums of the Colombian Caribbean. The results on serological evidence in humans are presented based on the results of the IgM ELISA test. The reason for selecting this test is because the IgM ELISA is mainly used and recommended as a screening test due to its higher sensitivity compared to the Microagglutination Test (MAT) established as a reference test. Furthermore, IgM ELISA compared to MAT has been shown to detect antibodies earlier.^(15,16,17) It is also well known that MAT could fail to detect antibodies

against circulating serogroups that have not been identified and are therefore not included in the antigen panel of the test. In this regard, the IgM ELISA also shows advantages for being a gender specific test.⁽¹⁸⁾ Likewise, due to its excellent characteristics in terms of predictive values, low cost and simplicity in application, the World Health Organization recommends IgM ELISA in epidemiological studies to determine seroincidence / seroprevalence of leptospirosis.⁽¹⁹⁾ In this sense, this test has been suggested for the definitive diagnosis of leptospirosis in places where laboratories are not available to perform MAT.^(15,19) Due to the high specificity of MAT and the preliminary evidence reported in this study, it is suggested to conduct more research in the study population using MAT as a complementary test to the IgM ELISA test.

The results of this study show that a high proportion of the participants presented IgM antibodies against *Leptospira* (30.12%). IgM antibodies remain detectable in serum for up to five months post-infection, so their presence is interpreted as a recent exposure.⁽²⁰⁾ 72% of seropositive individuals had at least one common cold symptom in the last five months. However, these symptoms were not associated with the presence of IgM Anti-*Leptospira* antibodies. This result can be explained by the presentation of asymptomatic forms of leptospirosis and / or a possible diverse composition of pathogens causing these symptoms in the study population.^(21,22) It is well known that other endemic infectious diseases in tropical areas cause symptoms similar to leptospirosis, especially in the acute phase..⁽²³⁾

Otherwise, association of seropositive cases with occupations as merchant and housewife was observed. This could indicate a relationship of *Leptospira* infection with factors present at the study site. 7.55% (4/53) of the captured rodents were identified as carriers of pathogenic *Leptospira*, which confirms the circulation of this infectious agent at the study site. This becomes more important because mostly rodents were captured inside residences and commercial establishments in the market. This in turn explains the fact that most of the captured rodents (39/53) were of the *Mus musculus* species, recognized for being the most abundant species in the interior of the houses compared to the other species of synanthropic rodents. Other studies have reported a high proportion of *Mus musculus* rodents infected with pathogenic *Leptospiras* and have identified that species as transmitters of the infection.^(24,25)

The presence of pigs and having at least one pet in the home was significantly associated with recent exposure to *Leptospira spp.* in humans. Other similar studies have demonstrated the importance of pigs and other domestic animals as reservoirs of *Leptospira*.^(21,25,26) This

suggests the need to conduct studies that include the detection of the bacteria in other potential reservoirs besides rodents.

In the place of study suboptimal hygienic-sanitary conditions were observed inside the residences and public space related to extreme poverty. Particularly, accumulations of solid waste, sewage, presence of rodents, and domestic animals such as dogs, cats, poultry and pigs were observed. These characteristics constitute a favorable environment for the transmission of leptospirosis.^(1,27) Many cases of leptospirosis reported in Colombia have been related to environments which abundance of peridomiciliary waste, uncovered sewers and contact with *Leptospira* reservoirs.^(28,29) The pathogenic species of *Leptospira* manage to survive and perpetuate themselves in an epidemiological chain through a cycle that involves reservoirs and vehicles such as water. Water currents play an important role in the transmission of pathogenic *Leptospira* where reservoirs that release the bacteria to the environment continuously circulate. Likewise, the storage of significant amounts of food and the inadequate disposal of solid waste promotes rodent infestation and exposes other animals to the bacteria, which in turn can serve as reservoirs and give continuity to the transmission chain.^(1,27,30)

On the other hand, because the study subjects were selected by non-probability sampling, it was not possible to extrapolate the data on seroprevalence and associated variables to the general population. However, our data reflects high exposure at the study site to leptospira spp. and to factors that have been reported to increase the risk of leptospirosis. Likewise, the evidence reported here suggests that cases of recent exposure identified at the study site could be related to a possible circulation of the bacteria among reservoirs, and contaminated elements in the environment such as food, solid waste and wastewater. Populations with low socio-economic status have frequently been associated with these factors. Another aspect that would explain this relationship is the low levels of education that typically characterize these populations. It has been reported that poor knowledge about the importance of preventive hygiene measures represents an increased risk of contracting leptospirosis.^(7,31)

The present findings also exhibit the exposure of other communities to *Leptospira* infection, because the site of study covers the marketplace of Soledad. This place is the center of storage, distribution and commercialization of food to the other areas of the municipality, whereby exposure to food potentially contaminated by bacteria is extended to other areas. Another aspect to consider is the presence of favorable elements for the propagation of *Leptospira*, which could facilitate the introduction and diffusion of new strains of the

bacterium with greater pathogenicity, so that this could promote the emergence of epidemics of leptospirosis in the population.⁽³²⁾

To intervene this problem, it is appropriate to implement actions on the human-animal-environment interface as the One Health approach puts it. To achieve this, it is required the prior identification of the main sources of *Leptospira* infection in Soledad and the determination of possible routes of transmission. In this sense, we suggest carrying out additional studies in which the subtypes of *Leptospira* present in humans, in wild and domestic animals, and in potential inert sources of infection such as water and food are characterized.

Finally, the interventions that would be implemented should be aimed at controlling *Leptospira* from its sources of infection. Based on the results of this research, it is possible to design and implement some measures as a first approach to intervene the transmission of leptospirosis in Soledad. These measures may include the development of activities to improve the conditions of peridomiciliary environments and inside homes; apply deratization strategies; development of health education programs aimed at this community (inhabitants and merchants); strengthening occupational hygiene measures in high-risk workers; follow up on food handling practices; awareness raising in the health personnel of hospitals and clinics of the municipality about leptospirosis as a differential diagnosis of febrile illness; among others.⁽⁹⁾

Ideally, these strategies should be part of programs with national coverage for the control and prevention of leptospirosis in humans and animals, in which the human, animal and environmental health sectors collaborate. Currently, Colombia does not have an integrated system for the detection, control and prevention of leptospirosis that articulates these sectors; in addition, collaborative work of these systems is limited. In this sense, efficient communication and collaboration among research institutes, universities, public and private entities, non-governmental entities and the community in general should be activated. It is essential to assume this challenge to mitigate the impact of leptospirosis in other sectors of the Colombian Caribbean and the country in general.

Conclusions

To our knowledge, this is the first publication to present evidence on recent exposure to *Leptospira* in humans and the circulation of rodents carrying pathogen *Leptospira* spp. in a sector of Soledad. The exposure to human and ecological risk factors related to leptospirosis

is also described. The results suggest a possible interaction among human factors, especially socio-economic ones, such as extreme poverty, domestic reservoirs and favorable environmental conditions for the transmission of pathogenic *Leptospira* spp. at the study site.

It is necessary to intervene the transmission of leptospirosis, especially in exposed populations such as Soledad. Through a One Health approach, it is possible to effectively reduce the impact of leptospirosis in places where this disease is endemic. To implement this approach, Colombia needs to improve collaboration among the human, animal and environmental health sectors.

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Conflict of interest

The authors have no conflict of interest to declare.

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Xilene Mendoza Sánchez: Study execution, statistical analysis of results, and preparation, review and final approval of the manuscript..

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