

Anti-*Leptospira* spp. antibodies in meat dealers in Tunja, Boyacá, Colombia

Anticorpos anti-Leptospira spp. em distribuidores de carne na cidade de Tunja, Boyacá, Colômbia

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ABSTRACT

Leptospirosis is a zoonotic disease distributed worldwide, which is emerging as a public health problem in tropical and subtropical countries. Traditionally, it has been considered as an occupational disease. The objective of this study was to determine the serological prevalence of anti-*Leptospira* antibodies in meat dealers in the city of Tunja, Boyacá, Colombia. An observational, cross-sectional and descriptive study was carried out in a population of 186 operators from meat establishments in the city of Tunja. The samples were analyzed using the microagglutination test in order to determine the serovar that causes the infection. Each participant answer a survey with important questions about socio-demographic, epidemiological and occupational factors. A seroprevalence of 43% [$n = 80$; 95% confidence interval (CI): 36%-50%] was found. According to the serovar analysis, the distribution was: *L. bratislava* 30% ($n = 24$; 95%IC: 20%-40%), *L. hardjo* 21.25% ($n = 17$; 95%IC: 12%-30%), *L. pomona* 20% ($n = 16$; 95%IC: 11%-29%), *L. icterohaemorrhagiae* 17.5% ($n = 14$; 95%IC: 9%-26%), *L. grippityphosa* 16.25% ($n = 13$; 95%IC: 8%-24%), *L. canicola* 16.25% ($n = 13$; 95%IC: 8%-24%), and *L. tarassovi* 11.25% ($n = 9$; 95%IC: 4%-18%). The prevalence rate found in this study confirms the circulation of the microorganism in these operators, which may suggest the need to reinforce hygienic-sanitary, biosecurity and prevention measures as well as the control of the disease in this population.

Key words: leptospirosis; leptospira; zoonotic disease; prevalence; epidemiology.

RESUMO

A leptospirose é uma zoonose de distribuição mundial que está emergindo como um problema de saúde pública em países tropicais e subtropicais. Tradicionalmente, tem sido considerada uma doença ocupacional. O objetivo deste estudo foi determinar a prevalência sorológica de anticorpos anti-*Leptospira* em distribuidores de carne na cidade de Tunja, Boyacá, Colômbia. Realizou-se um estudo observacional, transversal e descritivo em uma população de 186 trabalhadores de estabelecimentos de carne na cidade de Tunja. As amostras foram analisadas pela técnica de microaglutinação para determinar o sorovar que causa a infecção. Cada participante respondeu a um questionário com questões importantes sobre fatores sociodemográficos, epidemiológicos e trabalhistas. Foi encontrada soroprevalência de 43% [$n = 80$; intervalo de confiança (IC)95%: 36%-50%]. De acordo com a análise por sorovar, a distribuição foi: *L. bratislava* 30% ($n = 24$; IC95%: 20%-40%), *L. hardjo* 21,25% ($n = 17$; IC95%: 12%-30%), *L. pomona* 20% ($n = 16$; IC95%: 11%-29%), *L. icterohaemorrhagiae* 17,50% ($n = 14$; IC95%: 9%-26%), *L. grippityphosa* 16,25% ($n = 13$; IC95%: 8%-24%), *L. canicola* 16,25% ($n = 13$; IC95%: 8%-24%) e *L. tarassovi* 11,25% ($n = 9$; IC95%: 4%-18%). A prevalência encontrada neste estudo confirma a circulação do microrganismo nesses trabalhadores, o que sugere a necessidade de reforçar medidas higiênicas, de biossegurança e de prevenção e controle da doença nessa população.

Unitermos: leptospirose; leptospira; zoonoses; prevalência; epidemiologia.

RESUMEN

*La leptospirosis es una zoonosis de distribución mundial como un problema de salud pública, en países tropicales y subtropicales. Tradicionalmente ha sido considerada como una enfermedad profesional. El objetivo de este estudio fue determinar la prevalencia serológica de anticuerpos anti-*Leptospira* en expendedores de carne en la ciudad de Tunja, Boyacá, Colombia. Se realizó un estudio observacional, transversal y descriptivo en una población de 186 trabajadores de expendios de carne en la ciudad de Tunja. Las muestras fueron analizadas mediante la técnica de microaglutinación para determinar el serovar que causa la infección. A cada participante se aplicó una encuesta con preguntas importantes respecto a factores sociodemográficos, epidemiológicos y laborales. Se encontró seroprevalencia del 43% [n = 80; intervalo de confianza (IC) 95%: 36%-50%]. Según el análisis por serovar, la distribución fue: L. bratislava 30% (n = 24; IC95%: 20%-40%), L. hardjo 21,25% (n = 17; IC95%: 12%-30%), L. pomona 20% (n = 16; IC95%: 11%-29%), L. icterohaemorrhagiae 17,50% (n = 14; IC95%: 9%-26%), L. grippityphosa 16,25% (n = 13; IC95%: 8%-24%), L. canicola 16,25% (n = 13; IC95%: 8%-24%) y L. tarassovi 11,25% (n = 9; IC95%: 4%-18%). La prevalencia hallada en este estudio confirma la circulación del microorganismo en aquellos trabajadores, lo cual exige reforzar las medidas higiénicas, de bioseguridad y de prevención y control de la enfermedad en esa población.*

Palabras clave: leptospirosis; Leptospira; zoonosis; prevalencia; epidemiología.

INTRODUCTION

Leptospirosis is a zoonosis of global distribution in urban and rural areas that is emerging as a public health problem in tropical and subtropical countries, affecting mainly vulnerable populations. It is caused by a spirochete of the genus *Leptospira*. In humans, symptoms include a wide range from asymptomatic infections to nonspecific febrile symptoms, gastric, muscular, renal and meningeal involvement, and death^(1,2).

According to the World Health Organization (WHO), it is estimated that there are more than 500,000 cases annually worldwide and mortality higher than 10%. In recent years, the Americas reported the highest number of notifications on leptospirosis, with 568 cases between 2007 and 2011. The main countries involved in these notifications are Brazil, Nicaragua, the Dominican Republic and Honduras, due to the weather conditions that favor the permanence and the distribution of *Leptospira*⁽³⁾.

Among the sources of infection that transmit leptospirosis in humans, a wide variety of animal species is found, both wild and peridomestic animals (rats, rodents, etc.), as well as domestic animals (cows, pigs, dogs and horses). Transmission from individual to individual rarely occurs⁽⁴⁾. Many cases were related to occupational hazards – for example, workers from rice field, sewers and slaughterhouse – and other forms of contact with contaminated soil or water – such as consumption or recreation⁽³⁾.

Majd *et al.* (2012)⁽⁵⁾ carried out a study of the prevalence of leptospirosis in groups at risk in Iran, slaughterhouse workers in Zanjan province, they found a prevalence of 34.7% for different *Leptospira* serovars. They concluded that the disease occurs in this group of workers, and preventive screening should be performed on these workers.

In India, a clinical and epidemiological study of human leptospirosis was carried out between 2002 and 2008, near Calcutta. An increasing trend in incidence was detected, rising from 11.7% in 2004 to 20.5% in 2008. From this study, it was possible to observe that cases of leptospirosis increase in patients who are in endemic areas, which causes serious diseases with pulmonary, hepatic or renal signs. They emphasize the importance of further studies in order to make an early diagnosis and offer the patient timely treatment, contributing to the reduction of mortality caused by this disease⁽⁶⁾.

In Paraguay, an 8.6% of *Leptospira* seroprevalence [95% confidence interval (CI): 5.9%-2.2%] was found among workers at urban cleaning services in the city of Asunción; all individuals were male and waste collection worker. Although the prevalence was lower than that expected, it was observed that work and household conditions became a risk factor for the infection transmission⁽⁷⁾.

In Colombia, epidemiological data on the frequency and distribution of leptospirosis are not well-known because of the paucity of extensive research studies in different populations at risk. In addition, the disease is commonly mistaken for other endemic species that present associated symptoms. Case reporting

is mandatory and should be done immediately after diagnosis, but often the record is not released properly – when it is done –, because the health care team is not trained to refer to this event in patients with associated symptoms⁽⁸⁾.

Prevalence studies in the human population present considerably higher numbers, as reported by Astudillo *et al.* (2009)⁽⁹⁾, in which presumptive cases of leptospirosis were evaluated in the Valle del Cauca by serological techniques; prevalence of 20.6% was found. In another study conducted in the department of Córdoba, Colombia, populations at occupational risk of leptospirosis were evaluated, with a prevalence of 13.1% in agricultural workers, butchers and waste collectors⁽¹⁰⁾.

In Villavicencio, the seroprevalence of *Leptospira* spp. was evaluated in the following risk groups: fish workers (48%); pig farm workers (35%); rice fields workers (23%); dairy farmers of dual-purpose cattle breeds (21%); veterinarians and small animal clinic assistants (17%); senior Veterinary Medicine and Zootechnics students (17%); and slaughterhouse workers (7%)⁽¹¹⁾.

Despite its importance, the actual prevalence of leptospirosis in groups of workers at risk has not been accurately determined in Colombia, although there are local studies showing this.

A recent study conducted in the Boyacá department showed a prevalence of 35% in slaughterhouse workers, which indicates the existence of an occupational hazard for this group⁽¹²⁾.

The present study aimed to estimate the serological prevalence of anti-*Leptospira* spp., using the microagglutination test (MAT) in a representative group of butcher in the city Tunja, Colombia.

MATERIALS AND METHOD

An observational, descriptive and cross-sectional study was conducted in the city of Tunja, capital of the Boyacá department, Colombia, at altitudes ranging from 2700 to 3150 meters above sea level, and 13°C of average temperature⁽¹³⁾. Butchers from this city were established as the population. According to the report of the Ministry of Social Protection of Tunja, for the year 2014, 174 meat establishments were registered, of which 186 workers were selected, applying the following data in the statistical calculator StatCalc of the Epi Info software version 7: n = total of the population (522); $Z\alpha = 1.96$; p = expected proportion (0.25); e d = precision (5%).

Sample

All participants previously signed the informed consent, and venous blood samples were collected in a tube containing no anticoagulant. The samples were taken to the Laboratory of

Molecular Epidemiology of the University of Boyacá, Colombia, and centrifuged at 3500 revolutions per minute (rpm) for 10 minutes. The serum was separated into flasks and stored at -80°C. After processing the 186 sera, they were sent to a specialized laboratory in the city of Bogotá D.C. to undergo a procedure that uses the microagglutination technique for seven *Leptospira* spp. serovars.

In addition to the blood sample, a survey was carried out in each of the meat shops and important sociodemographic, labor and epidemiological data were assessed, which could be associated with the seropositivity status.

Bioethical aspects

From an ethical point of view, this work was considered a minimal risk study, since the blood samples were obtained by venipuncture. These procedures and the acquisition of relevant information were preceded by the formulation of ethical guarantees of confidentiality, beneficence and non-maleficence established in Resolution 8430 of 1993, issued by the Colombia Ministry of Health. Authorization was obtained from the Committee of Animal and Human Research Ethics of the University of Boyacá by means of a CB107 memorandum on July 22, 2014. When the result was delivered to the positive cases, individuals were instructed to seek medical assistance. The report of these cases was sent to the Colombia Ministry of Health, which is responsible for their capture through epidemiological surveillance.

Microagglutination test

The MAT technique consists in comparing the serum of the patient with live antigens from a group of serovars selected from *Leptospira* spp. For the present study, serum dilutions were made for each worker using *L. bratislava*, *L. ardjo prajitno*, *L. canicola*, *L. icterohaemorrhagiae*, *L. grippityphosa*, *L. Pomona* and *L. tarassovi*, which after incubation for 60 minutes at 37°C, were observed in a dark-field microscope. Dilution was considered positive with 50% or more microscopy reading of agglutinated leptospire, taking a positive control as reference. The performance and interpretation of the test were conducted under the parameters established in the protocol of the veterinary laboratory where the test was performed.

Statistical analysis

The data tabulation was performed in Excel spreadsheet. The data were analyzed using a reliability parameter of 95% ($\alpha = 0.05$) in SPSS software version 20.0, licensed by the University of Boyacá, Colombia. A descriptive analysis was performed to obtain

the absolute and relative frequencies for categorical variables, and the dispersion and central tendency for quantitative variables. The chi-square test was applied to determine significant differences between the seropositivity condition for anti-*Leptospira* antibodies and sociodemographic, epidemiological and labor variables.

RESULTS

The study population consisted of 186 workers from meat shops in the city of Tunja, Colombia, in 2014. The sociodemographic variables were evaluated and distributed in 56.4% ($n = 105$) men and 43.5% ($n = 81$) women, mean age 39 years [standard deviation (SD) = 11.5]; urban origin 91.4% ($n = 170$); socioeconomic stratum 2 (within a scale of 0 to 6); and education – the majority of workers have completed high school. Among the epidemiological and labor variables of interest evaluated, the presence of animals at home and the occurrence of occupational accidents, such as cuts stand out (**Figure 1** and **Table 1**).

Regarding the working conditions evaluated, we found a mean of 13 years length of service (SD = 11.8). All participants ($n = 186$) reported using personal protective equipment, but none of them use the complete equipment. The use of gloves was evident in 76.3% ($n = 142$) of the workers; 57% ($n = 106$) use steel glove; and 45.7% ($n = 85$) use mask. Failure to use these accessories makes the individuals more susceptible to any type of contamination or accident. From the study population, 81.7% ($n = 152$) had some kind of accident (cut, scratch, laceration, etc.), and 10.2% ($n = 19$) reported contact with rodents in the workplace (**Table 2**).

From the 186 sera analyzed, 43% ($n = 80$; CI 95% 36%-50%) were positive for one, two or more *Leptospira* serovars (Figure 1). According to the analysis performed by serovar, *L. bratislava* [(30%) – $n = 24$; CI 95% 20%-40%], *L. hardjo* [(21.25%) –

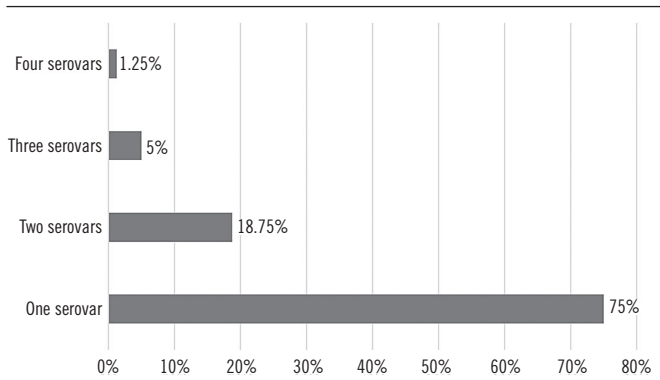


FIGURE 1 – Percentage distribution of positivity for one, two, three or four serovars

TABLE 1 – Description of sociodemographic and epidemiological variables of the study population

Variables		
Socio-demographic		
Age		
Mean	39	
Mode	29	
Median	40	
Standard deviation	11.5	
Sex	n	%
Male	105	56.5
Female	81	43.5
Socioeconomic strata		
Strata 1	43	23.1
Strata 2	98	52.7
Strata 3	42	22.6
Strata 4	2	1.1
Strata 5	1	0.5
Strata 6	0	0
Education		
Primary	59	31.7
Secondary	109	58.6
Technician	7	3.8
Tecnologist	1	0.5
Undergraduation	8	4.3
None	2	1.1
Origin		
Urban	170	91.4
Rural	16	8.6
Epidemiological		
Household pets		
Yes	58	31.2
No	128	68.8
Rodents in the house		
Yes	142	76.3
No	44	23.7
Water fountain in the house		
Aqueduct	185	99.5
Shared well	1	0.5
River	0	0
Reservoir	0	0
Uncovered sewer near the house		
Yes	19	10.2
No	167	89.8

TABLE 2 – Values of the chi-square test for sociodemographic, epidemiological and labor variables of interest in the transmission of *Leptospira* spp.

Variables	X ²	p* value
Sex	0.417	0.519
Origin	2.713	0.1
Household pets	2.501	0.114
Rodents in the house	1.039	0.308
Uncovered sewer near the house	0.164	0.686
Work-related accidents	0.278	0.598
Presence of rodents in the work environment	0.329	0.567

*Fisher exact test.

$n = 17$; CI 95% 12%-30%] and *L. pomona* [(20%) – $n = 16$; CI 95% 11%-29%] were more frequent (**Figure 2**).

The high frequency of *L. bratislava* and *L. hardjo* is explained by the origin of the population and exposure to meat products. The third most frequent serovar was *L. pomona*, probably because it was found more frequently in cattle.

A chi-square test was performed to determine significant differences between the seropositivity status and sociodemographic, epidemiological and labor factors of importance for the disease, which were investigated in the study applied to the population that participated in the study ($p < 0.05$). The p value for all variables evaluated indicates that there are no significant differences in the presence of anti-*Leptospira* antibodies (Figure 2).

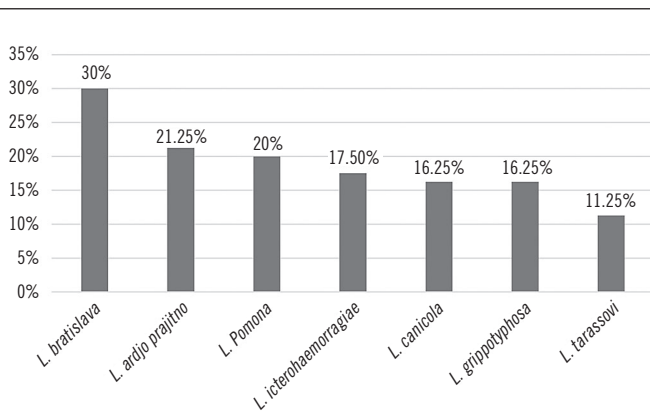


FIGURE 2 – Percentage of positivity for *Leptospira* spp. serovar

DISCUSSION

This is the first study conducted in the meat dealers' population in Tunja and Boyacá, Colombia. Leptospirosis is a zoonosis of great relevance worldwide, but it is under-diagnosed, both in this municipality and throughout the country, even though it is a mandatory notification event. The results obtained in this study confirm that butchers are a population exposed to *Leptospira*, which can be demonstrated by the high prevalence of anti-*Leptospira* antibodies (43%). These data are equivalent to those reported in the study conducted by Pedraza *et al.* (2012)⁽¹²⁾ in a population of slaughterhouse workers, in which seroprevalence of 35% was found, and the most frequent serovars were *L. hardjo* (38.89%) and *L. bratislava* (8.33%). The overall prevalence is similar but it varies in serovars, which can be explained by the origin of the population in the two studies and the degree of exposure to meat products⁽¹¹⁾.

The same situation can be observed when comparing the present research with another one performed in the city of Tunja, which evaluates the canine population, whose prevalent serovar was *L. icterohaemorrhagiae*. This prevalence originates from the high probability of exposure to elements contaminated by rat bodily fluids⁽¹⁴⁾. In the city of Bogotá, 30 canines with renal disease were analyzed, 10 positive (33.33%) and MAT titers for serovars *L. icterohaemorrhagiae*, *L. canicola* and *L. grippityphosa*⁽¹⁵⁾. In another study that evaluated the ownership of pets in Tunja slums, a prevalence of 67.2% was found in dog samples and 21.7% in human samples. These data show that these animals are at high risk of infection due to frequent contact with waste and wastewater runoff, which are common in these neighborhoods⁽¹⁶⁾.

Díaz *et al.* (2008)⁽¹⁷⁾ carried out a study in Villavicencio, Colombia, in a population at occupational risk – slaughterhouse workers – and found a seroprevalence of 7%, which is lower than that reported in the present study. As the study population belonged to a single meat company, in which biosecurity measures were appropriate, it is believed that this value is due to this behavior.

According to Nájera (2005)⁽¹⁰⁾, in a study conducted in Córdoba, Colombia, the seroprevalence of *Leptospira* in workers that practice animal slaughter itself was 34%, an expected number for regions with temperate climates, but lower than as reported in this article (43%). However, it must be taken into account that Tunja is a cold climate city, which precludes better conditions for the *Leptospira* survival.

Minor values of seroprevalence were recorded in studies that did not consider the occupational risk factor, since records between 6% and 20% were reported, such as in investigations conducted in Tolima, where the population was randomly selected, and in the Valle del Cauca, where the selection was due to clinical suspicion^(9, 18, 19). In other studies that considered both the population at occupational risk and the population in general, the reported seroprevalence decreased due to the fraction of inhabitants who do not are at occupational risk^(19, 20).

In relation to the serovar most frequently reported in other studies, *L. icterohaemorrhagiae* ranks first with 88.5%, affecting individuals at occupational risk, whose professions are employees of veterinary clinics, waste collectors and landfill workers⁽¹⁷⁾. For the present study, the most prevalent serovar was *L. bratislava* with 30%, a value compatible with a study performed in a similar population of the same area, in which the prevalence for serovar *L. bratislava* was 38.9%^(12, 21). The serovar *L. hardjo* was the second most observed in frequency, probably because it is the most reported in cattle⁽²²⁾, a scenario similar to that of national and international studies⁽²³⁻²⁵⁾.

Among the risk factors that have been reported are pets, rurality and work; such factors were also related in a study conducted in Lima, Peru, in which seropositivity was 10.1%⁽²⁶⁾. Environmental factors are also important in the seroconversion of individuals exposed to phenomena such as floods. Cases like this were reported in some countries, including Mexico, where the seropositivity of residents of the city of Tabasco after a flood was 21.28%⁽²⁷⁾. Although the highest prevalence rates against *Leptospira* spp. occur in rural areas or in individuals who are in contact with animals, the socioeconomic conditions of those living in urban settlements also predispose them to bacterial infection. This situation is reported in Guatemala, where seropositivity for a low-income urban settlement was 30.3%⁽²⁸⁾. The importance of work as a risk factor for leptospirosis is still observed in studies such as that published by Vado-Solis *et al.* (2014)⁽²⁵⁾, which includes individuals who are in frequent contact with cattle, with prevalence of 12.16%.

The epidemiological situation in Central America was studied by Pulido *et al.* (2014)⁽²⁹⁾, revealing that in Mexico the rate of cases was 8.2% in an exposed population. On the other hand, Guyana is the country with the highest rate of cases of leptospirosis, confirmed by two reports, whose prevalence's were 37% and 60%. However, it is important to emphasize that these two studies were performed at different times, and there may be variability of the results according to the risk factors of each population studied. In contrast, Brazil is one of the countries of South America that presents lower seroprevalence, which do not exceed 8% of positive cases for *Leptospira* spp.⁽²⁹⁾.

Considering the above, it is important to optimize prevention and promotion measures that allow the reduction and control of the distribution of this zoonosis, especially in occupations that are considered at high risk for leptospirosis.

The epidemiology of the disease in humans depends on the direct exposure of the individual to the infected animal,

the conditions in which they carry out their work daily and the environment that surrounds it. In this study, 81.7% of the participants reported having suffered some kind of accident during work, such as lacerations, cuts or scratches, and these are the predisposing factors to contract the disease, since the lesion is considered a direct entering for the *Leptospira*.

One limitation of this study was the inability to carry paired samples to assess seroconversion and confirm if the individuals were infected at that time. However, it was possible to determine that the positive participants were in contact, at some point in their life, with the microorganism.

CONCLUSION

The present study demonstrates the high prevalence of anti-*Leptospira* antibodies found in the population of meat dealers in the city of Tunja, confirming the circulation of the microorganism in this environment. In the identification process through the MAT test, seven serovars pathogenic for humans were found, with a predominance of 30% of *L. bratislava*, which suggests the optimization of prevention, promotion and control actions that allow the reduction of this zoonosis. For this purpose, it is necessary to train the medical team, based on the leptospirosis management protocol of the Colombia National Health Institute, for the appropriate notification and collection of cases, especially in individuals from groups at risk, as well as promoting community outreach actions on disease and prevention strategies.

It is important to reinforce the epidemiological knowledge of leptospirosis through investigations that determine the seroprevalence in different populations at risk in Boyacá, in addition to the factors attributed to the presence of the disease.

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