

## Short Communication

# Seroprevalence of and risk factors for leptospirosis in the City of Manaus, State of Amazonas, Brazil

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### Abstract

**Introduction:** Leptospirosis is caused by a bacterium of the genus *Leptospira*. This study aimed at investigating the seroprevalence of and risk factors for leptospirosis in humans in Manaus, State of Amazonas. **Methods:** Interviews were performed, and 1,000 blood serum samples were examined using a microscopic agglutination test. **Results:** Forty-three cases were positive; there were 10 serotypes, with coagglutination in 8 cases. The most frequently occurring serotypes were *Icterohaemorrhagiae* (20.7%), *Cynopteri* (20.7%), *Australis* (18.8%), and *Copenhageni* (16.9%), and the Midwest (54.7%) and South (23.8%) had the most cases; these areas lack basic sanitation. **Conclusions:** Disease occurrence might be reduced through improved basic infrastructural conditions.

**Keywords:** Leptospirosis. Serovars. Manaus.

Leptospirosis is an acute, infectious, and systemic disease caused by a pathogenic bacterium of the *Leptospiraceae* family, *Leptospira* gender known as *L. interrogans*; more than 200 serovars have been described, with distribution into 19 serogroups<sup>(1)</sup>.

According to the Centers for Disease Control and Prevention, leptospirosis is a globally spread zoonotic disease that affects feral and domestic animals and subsequently human beings who come in contact with the urine of infected animals. In the urban environment, the main *Leptospira* reservoir hosts are dogs and rodents (mainly *Rattus norvegicus* and *Rattus rattus* species), which are regarded as important modes of leptospirosis transmission to humans<sup>(2)(3)</sup>.

Despite its global presence, leptospirosis is endemic in tropical and subtropical countries, particularly in areas that lack basic sanitation and have a high population of rodents. In Brazil, leptospirosis occurrence is closely related with the socioeconomic conditions of the population, humid tropical climate, and long and heavy rainfall seasons<sup>(3)</sup>.

From 1980 to 2005, about 60,000 cases of the disease were reported in Brazil, and in 2008, the Ministry of Health reported 3,306 leptospirosis cases<sup>(4)</sup>. According to the Health Surveillance

report, 114 cases were reported in Amazonas from 2013 to 2014, of which 111 cases were reported in Manaus<sup>(5)</sup>.

The diversity of *Leptospira* serovars can be identified by using a microscopic agglutination test (MAT), which is considered the gold standard for diagnosis in humans by the World Health Organization. These results are mostly used in epidemiological investigations and provide important data for the serogroups in the study area<sup>(6)</sup>. This technique enables not only detection of circulating antibodies in an infected person but also identification of standardized serovars in affected people. The most frequent serovars detected in human beings are *Icterohaemorrhagiae*, *Canicola*, *Copenhageni*, and *Autumnalis*<sup>(7)(8)</sup>.

This study aimed at investigating the seroprevalence of human leptospirosis in high-risk areas for disease spread in Manaus, State of Amazonas and its associations with socioenvironmental factors.

The research was conducted with 1,802,014 residents of Manaus, Amazonas, a state in the North Region of Brazil, which has a humid tropical climate, medium temperatures, and heavy rainfalls that are more frequent in the regional winter, from January to April<sup>(9)</sup>.

In 2011, 1,000 blood serum samples were analyzed from asymptomatic humans in areas in which the disease had been reported and the socioenvironmental conditions were propitious for infection. Twenty neighborhoods were selected from different areas of Manaus, with characteristics that

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have been described previously, avoiding possible population effects on the selected neighborhoods. High-risk blocks were defined based on previously confirmed cases of leptospirosis through data analysis from the Municipal Secretariat of Health [Secretaria Municipal de Saúde (SEMSA)] of Manaus. All of the houses within a 200m radius of a positive case were identified numerically; then, 20 houses were selected using a random number table. Samples were collected from residents of these houses, of either sex and any age, that agreed to participate, with the aim of collecting samples from 50 independent individuals from the 20 randomly selected homes.

Interviews were conducted to collect socioenvironmental data for the residents of houses in which samples had been collected.

For the leptospirosis serological test, blood samples were collected in tubes without anticoagulant and transported to the Leonidas and Maria Deane Institute (ILMD) laboratory for serum separation. Then, the serum samples were transported to the Leptospiriosis Reference Laboratory (FIOCRUZ/ Rio de Janeiro) to undergo MAT. For this technique, 19 live antigens corresponding to globally circulating *Leptospira* serovars were used.

According to the Ministry of Health manual (2014), a confirmed case of leptospirosis using serology was defined using the following criteria: a negative first sample and a second sample at least 14 days later with titers  $\geq 200$ ; 2 samples, collected 14 days apart, with at least a 4-fold increase in titers based on MAT; or a sample with titers  $\geq 800$  based on MAT.

Of the 1,000 blood serum samples that were analyzed using MAT, 43 were positive. Ten types of globally circulating serovars were identified. The most frequently occurring serovars detected in asymptomatic people were *Icterohaemorrhagiae* (20.7%), *Cynopteri* (20.7%), *Australis* (18.8%), and *Copenhageni* (16.9%) (Table 1).

Of the 53 identified serovars, 8 (16.3%) samples showed positive serological reactions to more than one *Leptospira* serovar (Table 2). This could be explained by the existence of paradoxical reactions and cross-reactions between serogroups observed with the MAT<sup>(10)</sup>. Among the main antibodies detected using this methodology are those specific against lipopolysaccharides (LPS), one of the most abundant macromolecules present in the leptospiral outer membrane. It was previously observed that antibodies against LPS in patients with leptospirosis are highly related with *Leptospira* serovars<sup>(11)</sup>.

In the analysis of sociodemographic features, a percentage of the positive samples was distributed in 6 districts of Manaus; the highest serovar concentration rate was observed in the West area (54.7%), followed by the South (23.8%), North (19.2%), and Midwest (2.3%). The West and South areas had the highest seropositive rates (each, 73.7%) (Figure 1).

Of the infected people, 25 (58.1%) were men, and 18 (41.8%) were women. The highest rate was observed for individuals aged 23-44 years old (51.1%), who were mostly men (58.3%). Regarding education, 71.4% had not finished any grade, 9.30% had finished elementary school, 9.3% had not finished high school, 4.6% had finished high school, and 4.6%

TABLE 1

Frequency of *Leptospira* serovars in sera samples of asymptomatic humans living in the City of Manaus, State of Amazonas, in 2011.

Serovar	Absolute frequency	Relative frequency (%)
<i>Icterohaemorrhagiae</i>	11	20.7
<i>Cynopteri</i>	11	20.7
<i>Australis</i>	10	18.8
<i>Copenhageni</i>	09	16.9
<i>Canicola</i>	04	7.5
<i>Tarassovi</i>	03	5.6
<i>Patoc</i>	02	3.7
<i>Panama</i>	01	1.9
<i>Autumalis</i>	01	1.9
<i>Pyrogenes</i>	01	1.9
<b>Total</b>	<b>53</b>	<b>100</b>

TABLE 2

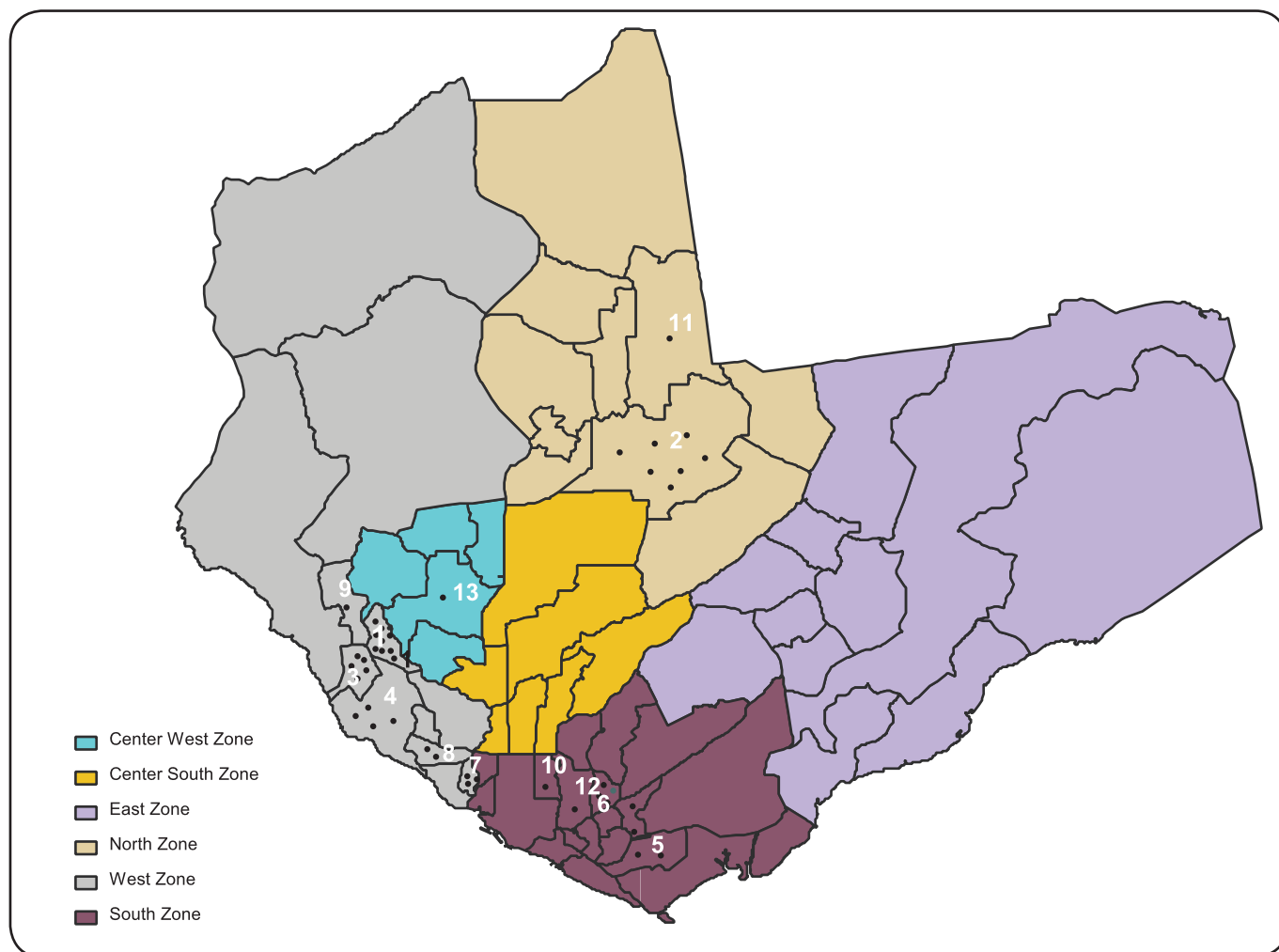
Results of *Leptospira* serovar coagglutination in sera samples of asymptomatic humans living in the City of Manaus, State of Amazonas, in 2011.

Serovar	Absolute frequency	Relative frequency (%)
<i>Icterohaemorrhagiae</i> + <i>Cynopteri</i> + <i>Australis</i>	02	25.0
<i>Icterohaemorrhagiae</i> + <i>Cynopteri</i>	02	25.0
<i>Icterohaemorrhagiae</i> + <i>Copenhageni</i>	02	25.0
<i>Copenhageni</i> + <i>Australis</i>	01	12.0
<i>Copenhageni</i> + <i>Canicola</i>	01	12.0
<b>Total</b>	<b>08</b>	<b>100.0</b>

were illiterate; none of the participants had started undergraduate studies.

The results of the present study indicate an endemic scenario of leptospirosis in the city of Manaus, based on the detection of different serovars in asymptomatic individuals. The prevalence of leptospirosis infection (4.3%) is one of the lowest reported in serological surveys in Brazil, which range from 9.7% to 28.5%. However, those surveys were conducted with high-risk groups, whereas the present study included the general population. The results support another study conducted in the City of Belém, State of Pará, which also had an endemic profile with a few cases during the investigation<sup>(12)</sup>.

Among the analyzed samples, ten different globally circulating serovars were detected, with no significant statistical difference. However, *Icterohaemorrhagiae* (20.7%), *Cynopteri* (20.7%), *Australis* (18.8%), and *Copenhageni* (16.9%) were the most frequently detected serovars. In most of the leptospirosis cases detected in urban areas in Brazil, strains of the serogroup



**FIGURE 1. Distribution of leptospirosis cases in the neighborhoods of Manaus, State of Amazonas.** 1: Nova Esperança; 2: Cidade Nova; 3: Santo Agostinho; 4: Compensa; 5: Crespo; 6: Raiz; 7: Glória; 8: Santo Antônio; 9: Lírio do Vale; 10: Praça 14; 11: Nova Cidade; 12: Cachoeirinha; 13: Alvorada.

*Icterohaemorrhagiae*, whose main host carriers are *Rattus rattus* and *Rattus norvegicus* (domestic rat), have been observed, reinforcing the participation of ordinary rats in the leptospirosis transmission chain<sup>(13)</sup>. Similarly, in the present study, the serogroup *Icterohaemorrhagiae* was the most pathogenic serovar for human beings.

Socioenvironmental conditions in Manaus favor infection by more than one serovar, as identified in several individuals; the diversity of infected reservoirs and favorable environment for bacterial proliferation to which people are exposed might explain these results. Human infections are related with exposure to contaminated water, urine, or other infected animal tissues and to the environmental conditions in countries with tropical and subtropical climates, mainly during seasons of heavy rain<sup>(3)(13)</sup>.

In Manaus, the South and West areas had the highest rate of seropositivity (73.7%); these areas are located around the two river basins for the São Raimundo and Educandos rivers, which are often affected by seasonal floods. The highest rate was present for young men; similarly, previous studies in Manaus

showed that the disease mainly affected young male adults with limited schooling who had been infected while performing strenuous activities either at home or work<sup>(14)</sup>.

The infected individuals lived in high-risk areas, in substandard housing near streams or open sewage, usually exposed to floods. Previous studies also identified that areas with poor sanitation, rodents, and dogs contribute to the natural spread of leptospirosis<sup>(15)</sup>.

Therefore, the risk of leptospirosis transmission in urban areas of Manaus may be reduced through improved basic infrastructural conditions (sewage, pluvial water drainage, proper garbage collection and disposal, and extinction of rodents); in addition, streams and rivers should be cleaned and dredged, which are fundamental measures to mitigate the frequency of floods and consequently reduce disease proliferation.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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