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HUMAN LEPTOSPIROSIS IN THE CITY OF VASSOURAS, PROVINCE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT

Leptospirosis is a widespread systemic acute infectious disease caused by serovars of the bacteria of the Genus Leptospira. The etiologic agent of the disease is mainly transmitted through infected animals' urine, being domestic and wild animals the reservoir. Humans are accidental hosts within the transmission chain. Some serovars of Leptospira can be harmful to humans. The objective of this research was to estimate the incidence of infection by serovars of the Genus Leptospira through serological analysis of individuals living in the city of Vassouras, Province of Rio de Janeiro, Brazil. Venous blood samples were collected from 51 volunteers inhabitants of Vassouras, and the sera were submitted to microagglutination tests for detection of specific antibodies to Leptospira serovars. Among the 51 volunteer individuals participating in the research, 13 (25.5%) were positive to Leptospira serovars. In most samples, tests reacted to multiple serovars antibodies.

The registered serovars distributed by samples were: Hebdomadis (92%), Patoc (69,23%), Pomona (53,85%), Castellonis (46.15%) Copenhageni (38, 46%), Grippotyphosa (38,46%) and Javanica (38,46%) Panama (30, 77%) Australis (23,08%), Canicola (23,08%), Tarassovi (23,08%), Autumnalis (15,38%), Bataviae (15,38%), Sejroe (15,38%) and Pyrogenes (7,69%). Although pathogenic serovars were not found among Vassouras inhabitants sera, the high prevalence of less pathogenic serovars (25,5%) points to high exposure to rodents' urine and a consequent high risk for acquiring the severe form of leptospirosis if pathogenic serovars arise with migrant infect rodents to the city.

KEYWORDS: Leptospira, leptospirosis, serovars, Vassouras.

INTRODUCTION

Leptospirosis is a widespread systemic acute infectious disease caused by serovars of the bacteria of the Genus Leptospira. The etiologic agent of the disease is mainly transmitted through infected animals' urine, being domestic and wild animals the reservoir. Humans are accidental hosts within the transmission chain. Human infections occur through direct or indirect exposure of the infected animals. The incubation period is usually one to two weeks after the contact with the bacteria.[1,2] Clinical presentations of leptospirosis may vary from mild to severe and can be lethal. First symptoms are non-specific, such as fever, headache, myalgia (mainly in the calves) prostration, vomit, diarrhoea and cough. In the first stages can be misdiagnosed with other diseases as respiratory syndromes, dengue fever, malaria or chikungunya. In a more advanced stage of the infection, jaundice, hemorrhagic and urinary complications may occur, characterizing Weil's Syndrome. Leptospirosis can also lead to complications as pneumonia, meningitis and uveitis, as well as renal, hepatic, pulmonary and vascular failure and, in more severe cases, systemic compromising. It is estimated that approximately 15% of patients diagnosed with leptospirosis evolve to more severe clinical forms.[1,3,4]

Leptospirosis is endemic in Brazil and occurs in all months, mainly in poor communities. The increasing incidence of leptospirosis is associated with extreme wheater events, especially heavy rainfall and flooding.[3,4] Rainfall and floodwater carry the urine of rats deposited in sewers and drains, especially in areas of high rodent infestation and compromised sanitary infrastructure, exposing individuals' skin and mucous membranes to the contact with the urine of rodents.[1,3,6,7]

Laboratory diagnostics involves specific exams and depends on the diseases' phase that the patient is. The exam in the initial stages can be performed through the research of bacteria of the genus Leptospira in the blood by direct examination, culture in proper media, inoculation in laboratory animals or detection of the DNA of the microorganism, by the technique of polymerase chain reaction (PCR). In the late stages, Leptospira can be identified in the urine, cultivated or inoculated. The most commonly used serological methods in Brazil are the ELISA-IgM test and microglutination.[1,6,7]

In Brazil, leptospirosis is an endemic disease, turning epidemic in rainy periods,[8] mainly in urban areas with high agglomeration and slum, where the incidence is linked to poor sanitation infrastructure, poverty, dumps, nearby streams and high infestation of infected rodents. The mimicking of the clinical signs in the milder forms that resemble those of other more common diseases and the difficulty of access to the health system by the poor population lead to an underestimation of the real number of infected people.[9]

Among the measures to prevent leptospirosis are control of rodent's population, improvement of the living conditions, improvement of sanitation infrastructure, avoid contact with floodwater and mud, cisterns disinfection, cleaning and channelling streams, use personal protection equipment in risky activities, proper collection, conditioning and disposal of garbage, sealing of water reservoirs, constant maintenance of urban abandoned land, vaccination of domestic animals against leptospirosis, avoiding the consumption of food that came in contact with flood water.[1,6,7]

The objective of this research was to estimate the incidence of infection by serovars of the genus Leptospira through serological analysis of individuals living in the city of Vassouras, Province of Rio de Janeiro, Brazil.

METHODS

The research has a cross-sectional, quantitative and qualitative design, involving 51 volunteers living in the city of Vassouras, Province of Rio de Janeiro, Brazil. Venous blood samples were collected from the participants at the Teaching Hospital of Severino Sombra Foundation. After coagulation, the samples were centrifuged at 3000 rpm for 5 minutes. After centrifugation, serum samples were frozen until performing microagglutination tests. The participants were submitted to clinical evaluation and presented no signs or symptoms suggestive of leptospirosis. Serologies were performed at the Hermes Pardini Research

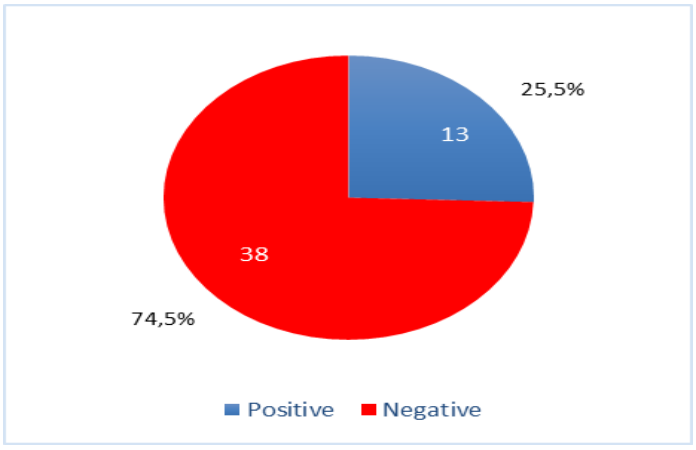
Laboratory in Belo Horizonte, Brazil. The microagglutination technique (MAT) was used because it is considered the best method in the serological diagnosis of leptospirosis. This reaction consists in the detection of specific antibodies to Leptospira serovars and the identification of specific antibodies to those species. The technique used allowed the identification of the group of infective serovars. Drops of samples from each serum were added to suspensions containing the various Leptospira serovars to verify the presence of the antibody against the specific serovar that infected the patient. The interpretation of the exams was performed under a microscope with a darkfield condenser and magnification of 40X.

According to the Brazilian Institute of Geography and Statistics (IBGE), it is estimated that in the year 2019 the city of Vassouras had 36896 inhabitants. The city is located at latitude 22:24:145 and longitude 43:39:45, with a municipal area of 532.4 km² at an altitude of 418 meters above sea level. The city has an average temperature of 23ºC. The average infant mortality rate in the city is 10,22 by 1000 births. Vassouras presents 73,5% of residences with satisfactory sanitary conditions, 63,2% of urban domiciles in the urban area with garden and trees and 32,7% of urban houses with adequate urbanization (presence of rain draining system, sidewalk, pavement and curb). The hydrography of the city is composed of small streams that are inserted in the hydrographic basin of the Paraíba do Sul River. Vassouras is a nationally recognized educational centre. The University of Vassouras (Severino Sombra Foundation), offers 26 undergraduate courses and is the 2nd largest employer in the southern region of the Province of Rio de Janeiro.

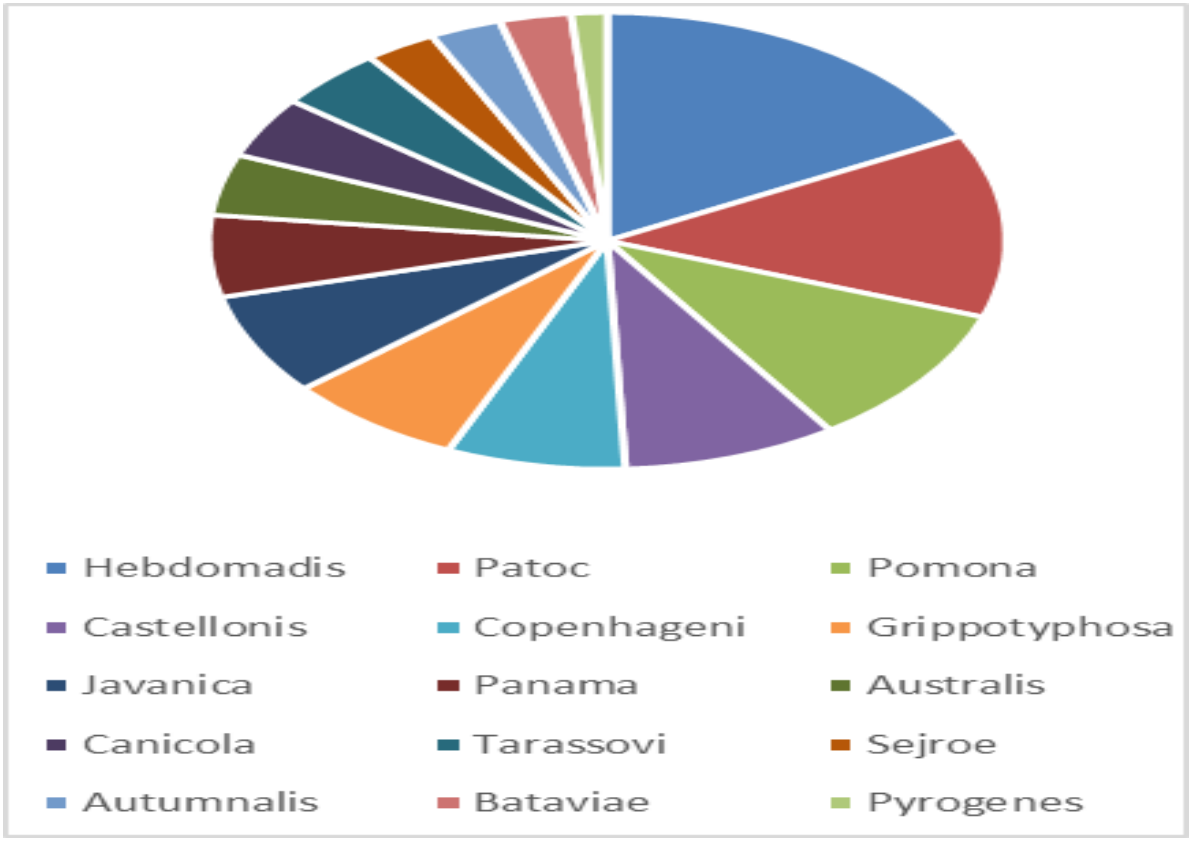
The research was approved and authorized by the Ethics Committee of the University of Vassouras in the Decision 3.368.640.

RESULTS

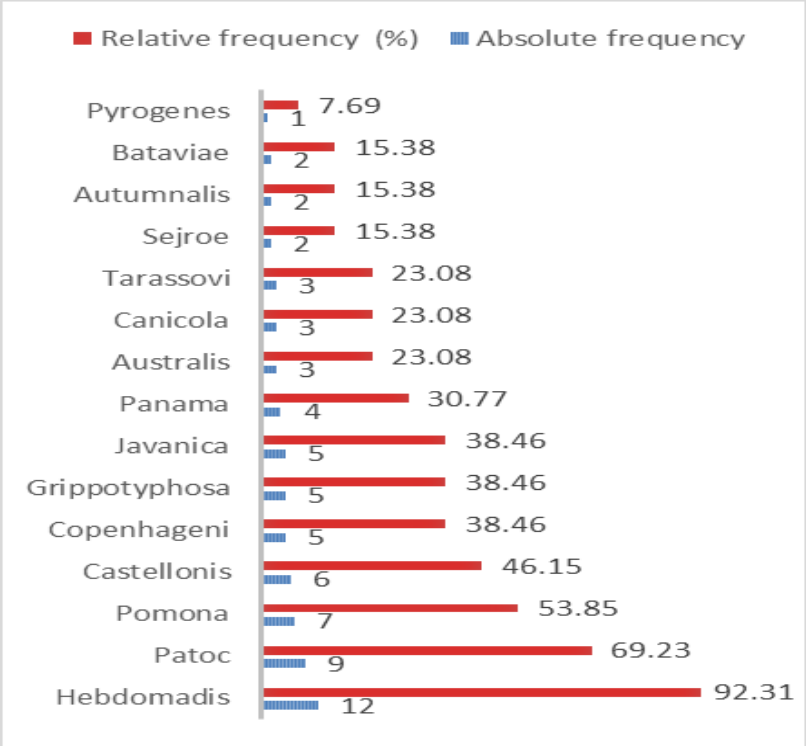
Among the 51 volunteer individuals participating in the research, 13 (25.5%) were positive to Leptospira serovars.



Graph 1: Results of the microagglutination to serovars of the Genus Leptospira in blood samples obtained from volunteer inhabitants of the city of Vassouras.



Graph 2: Frequency distribution of Leptospira spp. serovars in blood samples obtained from volunteer inhabitants of the city of Vassouras.



Graph 3: Absolute and relative frequencies of Leptospira spp. serovars in blood samples obtained from volunteer inhabitants of the city of Vassouras.

Serological reactions revealed that the most incident serovar among the examined volunteers was Hebdomadis in 92% (12 of the 13 examined individuals), followed by Patoc, Pomona and Castellonis serovars in 69, 23% (nine individuals), 53, 85% (seven individuals) and 46.15% (six individuals) respectively. The serovars Copenhageni, Grippotyphosa and Javanica were found in 38, 46% (five individuals); the serovar Panama was found in 30, 77% (four individuals). The serovars Australis, Canicola and Tarassovi were found in 23, 08% (three individuals). In 15, 38% (two individuals) the serovars Autumnalis, Bataviae and Sejroe were recorded, while the serovar Pyrogenes was found in only one volunteer (7, 69%).

DISCUSSION

The identification of serovars has great epidemiological importance as they show a direct relationship with several reservoir animals, besides considerable variations in the geographical distribution.[8] Jara et al.[10] argument that geographical and ecological characteristics influence the distribution of Leptospira serovars, as soil pH, mean annual temperature and animal diversity limit the presence of certain serovars. Ecosystem changes of a region also modify patterns of the geographic distribution of Leptospira vectors, also transforming risk patterns of transmission of the disease.

A research on leptospirosis seroprevalence in the District of Colombo, Sri Lanka, was conducted by Rajapakse et al.[11] Among the 810 examined individuals, 429 were positive for saprophytic Leptospira serovars, 269 were positive for pathogenic serovars, and 221 individuals were positive for saprophytic and pathogenic serovars simultaneously. The prevalences distributed by serovars presented the following profile: Pyrogenes (15.9%), Ratnapura (9.9%), Bankinang (9.6%), Australis (9.3%), Hebdomadis (8.8%), Pomona (5.7%), Icterohaemorrhagiae (5.6%), Cynopteri (5.4%), Canicola (3%), Bataviae (2%), Hardjo (1.1%). The authors recognize that data on the seroprevalence of Leptospira serovars are important sources for the design of control and prevention policies for the disease. We agree with Rajapakse et al.[11] and point out that the determination of Leptospira serovars in the city of Vassouras is essential for the design of prophylaxis measures and environmental, health and infrastructure interventions in order to minimize the possibility of disease transmission in the city.

Sara et al.[12] investigated the seroprevalence of leptospirosis among members of the Army in Northeast Malaysia. Among 616 examined individuals, 100 (16, 2%) had seropositivity for anti-Leptospira antibodies. Terengganu serovar, of local incidence, was the most prevalent, with 70,4%, followed by Patoc (35.2%), Celledoni (5,7%), Hardjobovis (2,6%), Australis (2,1%), Canicola (2,1%), Sarawak (2,1%), Autumnalis (1,6%), Copenhageni (1,6%), Lai (1,6%), Melaka (1,6%), Pyrogenes (1,6%), Bataviae (1%), Hardjoprajitno (1%), Javanica (1%), Icterohaemorrhagiae (0,5%) and Pomona (0,5%) serovars. The distribution profile of serovars in Malaysia, a region far from Brazil, presents relevant differences from those found in the city of Vassouras, confirming the observations on environmental and geographical differences made by Jara et al.[10]

El-Azhari et al.[13] verified the seroprevalence of leptospirosis in workers from meat and fish markets in the city of Casablanca, Morocco. Among the 118 examined individuals, 24 (20, 3%) were positive for the agglutination reaction for leptospirosis. The serovars found were: Icterohaemorrhagiae (29, 1%), Javanica (16, 7%), Australis (8, 3%), Sejroe (4, 2%), Mini (4, 2%) and Panama (4, 2%). Leptospira from an undetermined serogroup were identified in 33, 3% of the samples.

Ataya et al.[14] conducted a study on the serological prevalence of anti-Leptospira antibodies among 186 workers in meat markets of the city of Tunja, Colombia, in the year of 2014.

Total seroprevalence was 43%, and among the positive samples, the distribution by serovars was: Bratislava (30%), Hardjo (21, 25%), Pomona (20%), Icterohaemorrhagiae (17,5%), Grippotyphossa (16,25%), Canicola (16,25%), Tarassovi (11,25%). The profile of Leptospira infection found by Ataya et al.[14] differs substantially from that found in our research, both in the high total prevalence as the distribution patterns of serovars.

Dias et al.[15] conducted a serological survey for leptospirosis in wagoners in Belém, Province of Pará, Brazil. The prevalence among this group was 55%, and the most frequent serovars were: Icterohaemorrhagiae (15m4%), Bratislava (12, 9%), Butembo (10, 25%), Autumnalis (7, 7%) and Copenhageni (7, 7%).

Ferreira-Homem et al.[16] investigated the serological prevalence of human leptospirosis in the eastern Brazilian Amazonia, finding a total prevalence of 32,8%, with at least one individual with positive serum agglutination in each examined family group. The most prevalent serotype by household was Bratislava, with 9%, followed by Hardjo (6%) and Grippotyphosa (4.5%). Although geographically close, the areas studied by Ataya et al.[14] Dias et al.[15] and Ferreira-Homem et al.[16] have different patterns for the distribution of serovars infection, showing that peculiarities of each population and environment are essential even within a macro-region. This fact highlights the need for investigations such as that conducted in the city of Vassouras, as a basis for studies involving the ecological management of reservoir species with higher affinity for specific serovars, to minimize or control transmission between mammalian species occurring in the region.

Souza et al.[17] investigated anti-Leptospira antibodies in patients in the Province of Mato Grosso do Sul, Brazil, with clinical suspicion of dengue fever or viral hepatitis. Serology demonstrated positivity for anti-Leptospira antibodies in 12,3% of the 439 examined patients. The results obtained by these authors showed positive reactions for 11 serovars, the most prevalent was the serovar Hurstbridge with 70,4% of the total positive samples. These researchers also detected antibodies to the following serovars, in descending order: Gripphotyphosa, Australis, Tarassovi, Icterohaemorrhagiae, Cuica, Panama, Hardjo, Canicola, Copenhageni, Bataviae, Javanica and Shermani. The authors indicate that socioeconomic factors and environmental conditions, particularly degraded ecosystems in urban areas, impoverishment of the population and deficiencies in basic sanitation provide the installation and maintenance of leptospirosis foci, allowing the free circulation of Leptospira among the reservoirs and the bacteria transmission to humans.

A seroepidemiological research of leptospirosis among environmental sanitation workers in the city of Pelotas, Province of Rio Grande do Sul, Brazil, conducted by Almeida et al.[18] revealed seropositivity of 10,4% of these individuals for Leptospira serovars. The most prevalent serovars were Castelonis and Australis, with 17, 4% each of total reactive sera.

Other serovars seropositivity in the tested samples were: Djasiman (8,7%), Pomona (8,7%), Icterohaemorrhagiae (8,7%), Copenhageni (8,7%), Sejroe (8,7%), Panama (4,3%), Pyrogenes (4,3%), Shermani (4,3%), Cynopteri (4,3%) and Autumnalis (4,3%). These authors aimed that environmental sanitation workers were exposed to risk of contamination by continuous contact with the material that could be contaminated by rodent urine. In the city of Vassouras the seroractivity for Leptospira varieties was 25, 5%, a rate considerably higher than the positivity of 10,4% found among workers at risk of contamination in the city of Pelotas. This fact indicates that, besides the level of infection by serovars reputable as less pathogenics already verified in our research, the potential for transmission of pathogenic serovars that eventually reach the region is extremely high.

In a serological study with data obtained from the Zoonotic Diseases Control Center of the Federal University of Pelotas, Province of Rio Grande do Sul, Brazil, Jorge et al.[19] tested 997 blood samples from patients suspected of being infected with Leptospira spp. The seroagglutination result revealed that Leptospira kirschneri serovar Butembo (Autumnalis serogroup) had the highest prevalence rates among pathogenic serovars, followed by Leptospira interrogans serovar Sentol (Djasiman serogroup) with 11,7% prevalence. Seroprevalence for saprophytic varieties of Leptospira in the studied population was 69,41%. Our results in the population of the city of Vassouras registered only low pathogenic strains of Leptospira. However, as the transmission chain for the various serovars of Leptospira is the same, the potential for transmission of pathogenic serovars is considerable, as rodents migrate between the various regions, often following food loads, contaminating the entire murine population of other areas. In our research, the set of individuals examined was not selective like the group examined by Jorge et al.[19] who only examined people with suspects of leptospirosis. Nevertheless, 25,5% of the patients examined in Vassouras showed positivity for serovars of Leptospira spp., projecting that a quarter of the population may be exposed to the possibility of contact with pathogenic serovars of the genus Leptospira.

Oliveira et al.[20] examined 4654 sera obtained from patients suspicious of leptospirosis in the Province of Minas Gerais, Brazil, between the years 2008 and 2012. Serum agglutination

reaction was positive in 273 patients and the most frequent serovars, in descending order, were: Icterohaemorrhagiae, Andamana, Patoc, Tarassovi, Copenhageni, Hardjo and Australis.

Castro et al.[21] researched Leptospira serovars in the city of Uberlândia, Province of Minas Gerais, Brazil. These researchers found between 2007 and 2009 serum reactivity in the individuals examined for the following serovars: Canicola, Hardjo, Tarassovi, Wolffi, Grippotyphosa, Bataviae, Copenhageni, Djasiman, Icterohaemorrhagiae, Panama and Patoc. We highlight that in the areas studied by Oliveira et al.[20] and Castro et al.[21] the serovar Icterohaemorrhagiae, which has recognized human pathogenicity, was found. This serovar was not found in our research, but the geographical vicinity between the Province of Minas Gerais and the city of Vassouras, where the infection rates were 25,5% for serovars of low pathogenicity, indicate the risk of introduction of Icterohaemorrhagiae serovar in possible migrations of rodents from surrounding regions to the city.

A serological survey for the detection of anti-Leptospira antibodies involving 32 rural workers in the micro-region of Itaperuna, Province of Rio de Janeiro, Brazil, was conducted by Silva.[22] Only five individuals were positive for hemoglutination reactions, representing a prevalence of 15,62%. The reactive serovars were: Icterohaemorrhagiae (6,25%), Australis (6,25%), Djasiman (3,125%), Hardjo (3,125%) and Hebdomadis (3,125%). Despite the total prevalence found by Silva (2007) is lower than that found in the city of Vassouras, there is also the possibility of migration of rodents or animals hosts of the serovar Icterohaemorrhagiae, incident in the micro-region of Itaperuna. The city of Itaperuna, as well as the city of Vassouras, is inserted in the basin of the Paraíba do Sul river, which can be an easing factor for the migration of rodents that are the reservoir of the human pathogenic strain.

CONCLUSION

Leptospirosis is present in the city of Vassouras, with a prevalence of 25, 5% among the examined volunteers. The registered serovars distributed by samples were: Hebdomadis (92%), Patoc (69, 23%), Pomona (53, 85%), Castellonis (46.15%) Copenhageni (38, 46%), Grippotyphosa (38, 46%) and Javanica (38, 46%) Panama (30, 77%) Australis (23,08%), Canicola (23,08%), Tarassovi (23,08%), Autumnalis (15,38%), Bataviae (15,38%), Sejroe (15,38%) and Pyrogenes (7,69%). Although pathogenic serovars were not found among Vassouras inhabitants sera, the high prevalence of less pathogenic serovars (25, 5%) points to high exposure to rodents' urine and a consequent high risk for acquiring the severe form of

leptospirosis if pathogenic serovars arise with migrant infect rodents to the city. Preventive measures and sanitary interventions should be performed to avoid an outbreak of severe forms of leptospirosis due to a high level of infection risk and the presence of the serovar Icterohaemorrhagiae verified in nearby regions.

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