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Fifteen years of human leptospirosis in São Paulo, Brazil

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ABSTRACT

Objective: Leptospirosis is a worldwide zoonosis caused by pathogenic *Leptospira* spp. The aim of this study was to report the incidence of leptospirosis from 1998 to 2012 in the state of São Paulo, Brazil to show the importance of human leptospirosis and to describe some epidemiological characteristics.

Methods: From January 1998 to December 2012, sera from patients with suspected leptospirosis were analyzed. The microscopic agglutination test (MAT) was used for serological investigations and MLST, serotyping and PFGE methods for the identification of leptospires. The descriptive seasonal analysis was performed with Excel Microsoft version 2007. Pearson's correlation was used to assess the association between rainfall and the number of cases.

Results: Among 22,795 serum samples, 2,430 cases of leptospirosis were laboratory confirmed, giving an average incidence rate of 1.35/100,000 inhabitants. Of these patients, 2,032 (83.62%) were male with a predominance in the age groups of 21-50 years. The highest incidence and rainfall were from December to April. There was correlation between the rainfall and the number of cases. Icterohaemorrhagiae was the predominant serogroup.

Conclusions: This study shows that leptospirosis is a seasonal disease in São Paulo with most cases occurring during the rainy season, and thus, will continue to be a disease of public health importance.

Key Words: Leptospirosis, Serology, Zoonosis, Epidemiology, Microagglutination test

1. INTRODUCTION

Leptospirosis is a worldwide zoonosis caused by pathogenic Leptospira spp. Outbreaks accounting for deaths worldwide underline the importance of leptospirosis as a re-emerging, yet a neglected infectious disease. It is estimated that about half a million severe cases occur annually around the world with an average case fatality rate of 10% .[1]

Climate conditions, socio-economical factors, cultural habits, seasonal variation, and warm seasons, may increase the risk of leptospirosis. Outbreaks of leptospirosis are associated with heavy seasonal rainfall and extreme climatic events.[2–7] Diseases such as chikungunya[8] and the excess cases of campylobacter enteritis and leptospirosis after flooding[9] are examples of the kind of new health threat that will increasingly confront the public health services.

In the city of São Paulo, Brazil located in South America leptospirosis is considered an endemic disease. The region has a population of approximately 11,500,000[10] with warm and wet climatic conditions ideal for the transmission of *Leptospira* spp. Leptospirosis has become an urban health problem as slum settlements have grown worldwide and outbreaks in these communities have increased.[1,4,11] However,

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its incidence is still underestimated due to difficult, poorly standardized diagnosis, low awareness among the medical community and unspecific clinical presentations. Although the disease occurs in developing countries it is becoming an urban health problem also in developed countries.[12–14]

The clinical manifestations vary in terms of severity and symptomatology that makes the diagnosis of human leptospirosis difficult. Because of the difficulties associated with isolating leptospires, laboratory diagnosis of human leptospirosis relies mainly on serological assays to detect antileptospiral antibodies in serum samples. The microscopic agglutination test (MAT) is the reference standard test for serological diagnosis of leptospirosis[15] although the isolation of leptospires from a large number of human cases and molecular identification allow a more precise knowledge of the disease. However, culturing *Leptospira* is difficult and growth success is diminished in patients already initiated on antimicrobial therapy.

Since the last study of leptospirosis in São Paulo, Brazil was during the period of 1969-2007[3] there is a limited knowledge of the seroprevalence of infection and circulation of pathogenic *Leptospira* among the general urban population. After this work no studies have been conducted over the last years to determine if there was changing in the epidemiological patterns of this disease in São Paulo. In order to fill this period gap, the aim of this study was to report the incidence of leptospirosis from 1998 to 2012 in the state of São Paulo to show the importance of human leptospirosis and to describe some epidemiological characteristics.

2. METHODS

2.1 Samples

From January 1998 to December 2012, sera from patients with suspected leptospirosis were analyzed by MAT. Blood culture in appropriate media for *Leptospira* spp. was also performed. A suspected case of leptospirosis was defined as a patient with signs and/or symptoms consistent with leptospirosis as determined by the healthcare provider.

2.2 MAT

Specific antibodies were detected by the reference method[15] using the representative serovars from different serogroups known to be prevalent in São Paulo, Brazil:[3, 16] Australis strain Ballico, Autumnalis strain Akiyami, Bataviae strain Van Tienen, Butembo strain Butembo, Canicola strain Hond Utrecht IV, Castellonis strain Castellon 3, Celledoni strain Celledoni, Copenhageni strain M20, Cynopteri strain 3522C, Djasiman strain Djasiman, Grippotyphosa strain Moska V, Hebdomadis strain Hebdomadis, Icterohaemorrhagiae strain Icterohaemorrhagiae, Javanica strain Veldrat Batavia 46, Panama strain CZ214 K, Pomona strain Pomona, Pyrogenes strain Salinem, Shermani strain 1342 K, Tarassovi strain Perepelitsin and Wolfii strain 3705. All the strains were maintained in Elinghausen McCullough Johnson Harris (EMJH) liquid medium (Difco, Sparks, MD, USA) at 30°C. Titers equal to or higher than 1:100 were considered as positive. Leptospiral serogroup predominant was considered the titer which was the highest sample dilution with 50% of agglutination and cross-agglutination when the highest titer was the same with two or more serogroups. Confirmed case of leptospirosis was considered when the blood culture was positive or when two samples had a titer rise of > 2 dilutions between acute and convalescent samples in MAT or when a single sample showed a minimum titer of 1:800.

2.3 Culture and identification of strains

Blood specimens collected during the first week of illness were cultured in semisolid Fletcher medium at 28°C-30 °C and examined weekly for the presence of leptospires by dark-field microscopy. The cultures were incubated for 12 weeks and then discarded if no growth had then been detected. Cultures that grew fungal colonies were also discarded. Cultures that showed growth of other bacteria were passed through sterile, 0.2 μm pore membrane filters before being sub-cultured into fresh medium. The identification of isolated *Leptospira* was performed by serogrouping, pulsed-field gel electrophoresis (PFGE)[17] and multilocus sequence typing (MLST).[18]

2.4 Epidemiological study

The information was collected on all patients with suspected leptospirosis who were admitted to the several hospitals. These hospitals serve as major providers of care for residents of the city of São Paulo and surrounding areas. A suspected case of leptospirosis was defined as a patient with history and clinical manifestations suggestive of leptospirosis, such as fever, headache, arthromyalgia, jaundice, sub-conjunctival haemorrhage, rash, neurological abnormalities, neck stiffness, oliguria, anuria, nausea, asthenia, vomiting, diarrhoea, abdominal pain, hepatomegaly and/or splenomegaly.

Sera from these patients were regularly sent to Adolfo Lutz Institute. Reports were completed by patient interview, and collected information on age, sex, date of onset, date of lab tests, date of admission and outcome. The collection of case reports were compiled into a database for analysis of the variables of interest. All daily rainfall measurements over the last 15 years were obtained from Department of Atmospheric Sciences – Institute of Astronomy, Geophysics and Atmospheric Science, University of São Paulo. This study was approved by the Ethics Board of the Instituto Adolfo

Lutz, São Paulo, Brazil.

2.5 Data Analysis

The descriptive seasonal analysis was performed with Excel Microsoft version 2007. Pearson's correlation (r) was used to assess the association between rainfall and the number of cases. *P* value of .05 or lower was considered to be significant.

3. RESULTS

Between 1998 to 2012, a total of 22,795 sera were analyzed. On the basis of previous criteria, 2,430 patients were considered confirmed cases of leptospirosis. The monthly distribution was similar in all years with the lowest number of cases from May to December, an increase from January to April and a peak recorded in March. The highest rainfall average occurred in January and February. There was correlation between the rainfall and the number of cases according to Pearson's correlation (r= 0.606, p< .05) (see Figure 1).

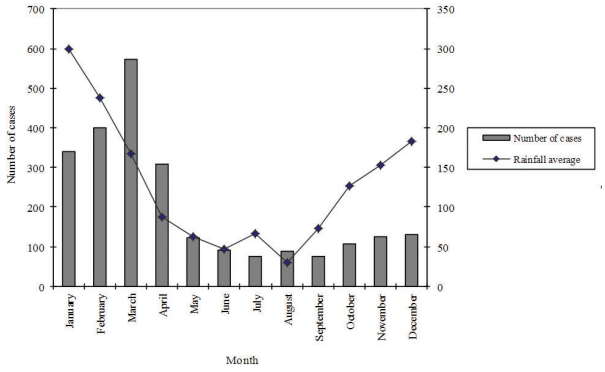

Figure 1. Number of cases of leptospirosis per months and rainfall average (1998-2012) in Sao Paulo, Brazil

The lowest and the highest rainfall average occurred in 2003 and 2010, respectively (see Figure 2). Table 1 shows the mean annual incidence rate in the period of 1998-2012. In the whole period the average incidence rate was 1.35/100,000 inhabitants. Over a period of 15 years, the annual incidence decreased from 3.45/100,000 inhabitants in 1998 to 0.59/100,000 inhabitants in 2012.

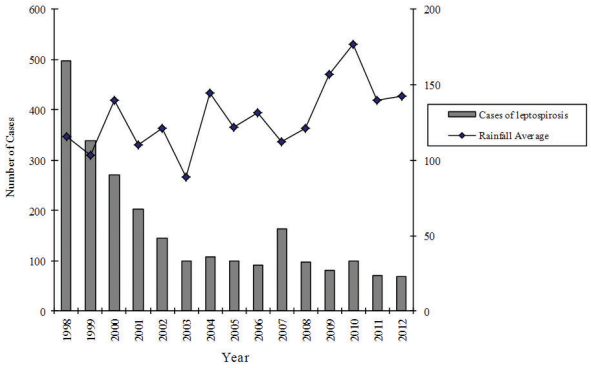

Figure 2. Number of cases of leptospirosis per year and rainfall average (1998-2012) in Sao Paulo, Brazil

By serology, the serogroups were identified in 2,017 cases (83%). The predominant serogroups identified by serology are shown in Table 2. Icterohaemorrhagiae was the predominant serogroup (1,287 cases, 52.96%) followed by Autumnalis (155 cases, 6.37%), Cynopteri (106 cases, 4.36%) and Canicola (90 cases, 3.70%). Cross-agglutination with at least two serogroups occurred in 413 cases (16.99%). The highest number of serogroup Icterohaemorrhagiae occurred in 2003 and 2011 with 71.71% and 67.14%, respectively. The serogroup Icterohaemorrhagiae occurred more frequently in January to April in all years. Eight-nine blood samples were sent to culture. Of these, 60 of the cultured samples yielded leptospiral isolates. By culture, Icterohaemorrhagiae was the predominant serogroup (54 cases, 90.0%) followed by Canicola (3 cases, 5.0%), Autumnalis (2 cases 3.33%) and Sejroe (1 case, 1.67%).

Table 1. The mean annual incidence rate in the period of 1998-2012 in São Paulo, Brazil

Year	Cases	Incidence rate
1998	497	3.45
1999	339	3.14
2000	270	2.40
2001	202	1.70
2002	145	1.30
2003	99	0.94
2004	107	1.05
2005	100	0.95
2006	92	0.81
2007	162	1.12
2008	97	0.77
2009	82	0.77
2010	100	0.77
2011	70	0.58
2012	68	0.59

Complete data on age was available for 2,267 (93.29%) patients diagnosed with leptospirosis. Table 3 shows the distribution of cases of leptospirosis according to age groups and sex. There was a predominance of males in all years. Of the 2,430 confirmed cases, 2,032 (83.62%) were male. Among female and male subjects, the age distribution of the patients showed a predominance in the age groups of 21-50 years.

4. DISCUSSION

The results of this study show that leptospirosis is a seasonal disease in São Paulo, Brazil with most cases occurring during the rainy season. Heavy rains drive rodents from burrows creating conditions conducive to outbreaks of leptospirosis. The prevention and control of leptospirosis require a permanent surveillance system. However, leptospires cannot usually be eradicated as rodents form major natural reservoirs.[1] In tropical urban environments, increased transmission and seasonal outbreaks occur during periods of heavy rainfall and flooding. During the rainy season, the populations in flood areas generally do not use adequate protection and also experience contact with urine of rats diluted in the water.

Table 2. Distribution of Serogroups diagnosed by MAT in São Paulo, Brazil during 1998-2012

Serogroup	No. of cases (%)
Icterohaemorrhagiae	1.287 (52.96)
Autumnalis	155 (6.37)
Australis	41 (1.68)
Ballum	76 (3.12)
Bataviae	29 (1.19)
Canicola	90 (3.70)
Celledoni	2 (0.08)
Cynopteri	106 (4.36)
Djasiman	6 (0.24)
Grippotyphosa	38 (1.56)
Hebdomadis	38 (1.56)
Panama	42 (1.72)
Pomona	4 (0.16)
Pyrogenes	19 (0.78)
Sejroe	68 (2.79)
Shermani	11 (0.45)
Tarassovi	5 (0.20)
Cross-agglutination	413 (16.99)

The weather in São Paulo is tropical and a rainfall occurs during the hot season with several floods.[19] Our study demonstrate that the number of cases and rainfall had statistically significant correlation, once Pearson's correlation of these two variables showed a positive strength ($r = 0.606$; $p < .05$). The largest number of cases was closely related to rainfall which occurred during the rainy season (December to April) just after the occurrence of flood-prone areas. These climatic conditions explain the evolution of the incidence of leptospirosis during the year characterized by a clear seasonal pattern. We observed that the largest number of cases was reported 2-3 weeks after the heavy rainfall (data not shown) suggesting that contact with flood water contaminated by rat urine was the probable mode of transmission. This is supported by the large proportion of patients who had their highest MAT titers against strains belonging to serogroup Icterohaemorrhagiae, which is the most associated with the domestic rat reservoir.[13,20] Our results are consistent with the results from other studies conducted in both temperate and tropical regions of the world that have also shown correlation with the rainy season.[3,21–26] In addition, when data from the present study are compared with the previous study[3] in São Paulo during the period of 1969-2007, the average number of cases per month was similar. Therefore, there is limited knowledge of the seroprevalence of infection and circulation of pathogenic *Leptospira* among the general urban population. The results of all isolates were in agreement with the results of serology.

Table 3. Cases of leptospirosis by age and sex distribution (São Paulo, Brazil, 1998-2012)

Age Range	Number of Cases		Total (%)
	Male (%)	Female (%)	
01-10	47 (1.93)	18 (0.74)	65 (2.67)
11-20	336 (13.82)	55 (2.26)	391 (16.09)
21-30	396 (16.29)	70 (2.88)	466 (19.17)
31-40	447 (18.39)	75 (3.08)	522 (21.48)
41-50	364 (14.97)	67 (2.75)	431 (17.73)
51-60	205 (8.43)	42 (1.72)	247 (10.16)
61-70	67 (2.75)	29 (1.19)	96 (3.95)
> 71	37 (1.52)	12 (0.49)	49 (2.01)
No information	133 (5.47)	30 (1.23)	163 (6.70)

There was a predominance of males in all years. Among 2,430 patients with leptospirosis 2,033 male patients and 397 female patients were reported. In the other countries the difference in male and female patients are explained by sex-related occupations;[12,27,28] however, in Brazil it is unlikely that the observed differences in number of cases are related to different sex-specific occupational risk factors. Since the majority of the patients are hospitalized when have symptoms of severe leptospirosis such as jaundice, haemorrhage, kidney or liver failure, we can infer that male patients were more likely than female patients to be hospitalized and to exhibit symptoms of severe leptospirosis. The mean age of the tested population was unaltered compared to previous study of 1969-1997.[3] However, there was a high incidence

in younger populations corresponding to people aged 1-9 years in 1969-1997.

Identification of factors that take part in the dynamics of the circulation of *Leptospira* may contribute towards improving current knowledge of the epidemiology of urban leptospirosis and towards drawing up policies and interventions aimed at controlling this emerging health problem.

In conclusion, the results of this study show that leptospirosis is a seasonal disease in São Paulo with most cases occurring during the rainy season, and thus, will continue to be a disease of public health importance.

5. Conflicts of interest disclosure

The authors declare no conflict of interest.

6. Acknowledgements

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