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Human leptospirosis: occurrence of serovars of *Leptospira* spp. in the state of Minas Gerais, Brazil, from 2008 to 2012

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ABSTRACT

Background: Leptospirosis is an infectious and acute disease caused by *Leptospira* spp. that have high epidemic potential. This study verified the main *Leptospira* spp. serovars detected by MAT from serum of patients with suspicion of leptospirosis from 2008 to 2012 in Minas Gerais State.

Methods: The laboratory received sera from 4654 patients. All serum were screened by IgM-ELISA according to the manufacturer's instructions. Each sample reactive or indeterminate were tested against twenty-four serovars of Leptospira by MAT.

Results: In this study, 597 patients were classified as reactive on MAT. Only 301 patients were confirmed by laboratory test. It was not possible confirmation by laboratory diagnosis of 296 patients. Among the samples classified as reactive on MAT, 273 patients exhibited titers bigger than 800 for one or more serovars; seroconversion was detected in 28 cases. Percentage of 85.1% of the samples reactive on MAT corresponded to males, 39.4% corresponded to patients aged between 20 and 39 years old. The most common serovars found were Icterohaemorrhagiae, Andamana, Patoc, Tarassovi, Copenhageni, Hardjo and Australis. Concerning the samples that exhibited titers bigger than 800, serovar Icterohaemorrhagiae was also the most common, followed by Copenhageni, Andamana, Patoc, Tarassovi, Grippotyphosa and Canicola. In this study, 40% of the cases occurred to the metropolitan area, state capital and 34 neighboring towns.

Conclusion: Our results show the possibly spreading serovars in Minas Gerais State and contribute to knowledge of human leptospirosis, aiming at improving the prevention, control of the disease, as well as the treatment of infected patients.

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Introduction

Leptospirosis is an infectious and acute disease that stands out in the world scenario because of its high epidemic potential, especially after long periods of rain.[1] It is caused by bacteria from *Leptospira* genus, further divided into serovars, which were clustered into serogroups, according to their antigenic relations. The advanced molecular techniques allowed the identification of 14 pathogenic and intermediately pathogenic species, and 7 non-pathogenic species, distinguishing three clades in *Leptospira* genus.[2] These clades comprise more than 260 serovars. In the recent years, the classification system based on DNA homology has been used in combination with the classical antigenic classification.[2,3]

Human leptospiral infections result primarily from direct or indirect exposure to the urine of infected animals. *Leptospira* invades the human body through cuts and abrasions in the skin, through intact mucous membranes and through waterlogged skin. Other modes of transmission of this infection are also possible.[4]

Leptospirosis clinical manifestations range from benign aspects to severe forms. Generally, it manifests as an unspecific acute febrile illness, characterized by fever, myalgia and headaches and can be misinterpreted as other diseases, such as influenza, malaria and dengue.[4,5] Indeed, there are many other diseases with similar symptoms, hampering the clinical diagnosis of leptospirosis. Therefore, laboratory and epidemiological approaches are crucial for the conclusion of positive cases.[4] The clinical manifestations and severity of the disease depend on the inoculum size, *Leptospira* strain or serovar involved, as well as the age, health and immune status of the infected individual.[6]

Pathogenic leptospires are widespread in nature, and various animal species, labor activities and territorial occupations have significant importance in maintaining leptospirosis.[1,4] In Brazil, environmental and sanitary factors, such as high temperatures, high rainfall rate and floods, favor rapid spread of the disease, being a serious risk to public health.[1,7]

Bacteriological, microscopic, serological and molecular methods have been used for laboratory diagnosis of leptospirosis.[4,8] The choice of which test to use depends on the evolution phase of the disease, its prevalence and the availability of a qualified laboratory.[9]

Polymerase chain reaction (PCR) has been widely used to diagnose acute leptospirosis, but this method is not always available in reference laboratories of developing countries, where the disease is highly endemic.[9,10]

Culture is rarely used in clinical setting because it demands prolonged incubation and shows low sensitivity.[4,11] Furthermore, this method is not performed in most public and private laboratories in Brazil because of the high costs, besides not being useful for early diagnosis, requiring considerable expertise. However, this technique has an important role in the study of outbreaks and global epidemiology, providing crucial information for the recognition of new patterns of disease presentation and assessing the effectiveness of intervention measures.[12]

The Microscopic Agglutination Test (MAT) is the serological reference test, and mostly reference laboratories are able to perform it. Even with the development of molecular methods, MAT remains the gold standard method, recommended by the Brazilian Health Ministry and worldwide recognized for laboratory confirmation of leptospirosis.[9,12,13] MAT is an indirect diagnosis that detects specific antibodies against *Leptospira*, although serum samples can react with more than one serovar.[4] Information obtained through MAT has been used in epidemiological studies to infer the possible serovars infectious.[10,14,15] The occurrence of serovars detected through MAT must be considered as a general idea of the serogroups/serovars in a population and cannot be used to definitely determine the infecting serovars.[4,15]

The epidemiology of leptospirosis in Minas Gerais, Brazil, is little known. MAT is the only tool available to infer the possible serogroups/serovars that cause the disease, considering the low sensitivity of the isolation method and the limitations of the Central Laboratory of Public Health of Minas Gerais, Brazil, in identifying new strains of *Leptospira* spp. through traditional (*cross-aglutinin absorption test*) or molecular methods.[16,17]

In different epidemiological settings, various animal species can be considered a source of transmission.[18] In Minas Gerais, Brazil, there are insufficient data of the association between different host species and human leptospirosis. Inferring potential infective serovars is very important to evaluate the virulence of these microorganisms. This knowledge contributes to a better understanding of the clinical manifestation of leptospirosis.[1,19] Thus, the objective of the present study was to verify the main *Leptospira* spp. serovars detected by MAT from serum samples of patients with suspicion of leptospirosis in the state of Minas Gerais, Brazil, from 2008 to 2012.

Methods

A descriptive study was conducted from the analysis of data obtained from Ezequiel Dias Foundation, Reference Laboratory for Leptospirosis Diagnosis in Minas Gerais, Brazil.

The Ethics Committee in Research at the Federal University of Minas Gerais, Department of Post Graduate Studies in Infectious Diseases and Tropical Medicine, approved this study, since all data regarding to the patients remained anonymous and unlinked (CAAE:05120113.0.00005149). Internal laboratory records were analyzed without exposing the identities of the patients.

From 2008 to 2012, the laboratory received 5370 serum samples from 4654 patients with leptospirosis suspicion. The samples were received with epidemiological records from the Notifiable Diseases Information System (SINAN). All serum samples were screened through IgM ELISA (PanBio Pty Ltd., Brisbane, Australia) according to the manufacturer's instructions. The cut-off value was assessed using the average absorbance of the calibrator multiplied by the specific calibration factor of each lot. The results were expressed in Pan-Bio units, using an indexed value obtained by dividing the sample absorbance by the cut-off value multiplied by 10. The results were interpreted according to the reference values: reactive sample, Pan Bio unit 11; indeterminate, Pan Bio unit 9–11; and nonreactive, Pan Bio unit <9.

All reactive or indeterminate samples in IgM ELISA were examined by MAT. Each sample was tested against

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