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Is the microagglutination test (MAT) good for predicting the infecting serogroup for leptospirosis in Brazil?

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Abstract

Leptospirosis is a zoonotic infection caused by pathogenic members of the genus *Leptospira* spp. Knowledge of the prevalent serovars and their maintenance hosts is essential to understand the disease. The aim of this study was to evaluate the ability of serology by the microscopic agglutination test (MAT) to predict the serogroups compared with results of identification of leptospires in São Paulo, Brazil. MAT correctly assigned the serogroup of the infecting isolate in 49/52 cases (94.23%). The serogroup Ictero-haemorrhagiae was the predominant serogroup (88.46%). This study showed the usefulness of the MAT to correctly identify the infecting serogroup with a good overall agreement between the serologically-identified infecting serogroup and by identification of the isolate and can be used in epidemiological surveys in São Paulo. However, it should be complemented by the identification of *Leptospira* isolates.

Keywords

Leptospirosis; Microscopic agglutination test; *Leptospira* spp.; Zoonosis

1. Introduction

Leptospirosis, a zoonotic infection caused by pathogenic members of the genus *Leptospira* spp., is most common in tropical regions where incidence peaks during the rainy season [1–3]. Symptoms range from mild flu-like manifestations to severe, potentially fatal septicemic complications. Some symptoms can mimic other tropical diseases such as dengue fever and malaria. Different animals may be reservoirs of distinct serovars and the specific serovars involved depend largely on the geographic region and the ecology of local maintenance hosts [1]. Furthermore, knowledge of the prevalent serovars and their maintenance hosts is essential to understand the epidemiology of the disease in any area. Previous studies of human leptospirosis in São Paulo, Brazil have largely relied on serological methods to confirm clinical cases and to predict the infecting serogroup [2]. The increasing number of fatal human cases in Brazil [4] has led to growing concern about the risk factors and

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potential infection sources, reinforcing the need for leptospiral identification through culture. Cross-agglutinin absorption test (CAAT), the recognized method of identification of leptospiral isolates based on antigenic relatedness, provides definitive identification of the infecting serovar and is an important technique for the study of outbreaks and epidemiology. However, this method requires the use of hyperimmune sera produced in rabbits to conduct the test. This method is time consuming and takes 6–10 weeks before a suitable hyperimmune titer is achieved. The CAAT is different from microscopic agglutination test (MAT) which is the serological diagnostic tool for identifying cases of the disease in humans and animals [5]. Molecular methods such as polymerase chain reaction assay, using a primer from repetitive DNA elements (iRep1-PCR), multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) are also used to identify the isolates. However, these methods are unable to differentiate all leptospires at the serovar level [6–8] and are sophisticated and expensive to perform. In view of these limitations, an alternative method of predicting the serogroups involved in this disease is serology by the MAT, which only enables the presumptive identification of the etiologic agent. The MAT uses a number of different serovars of leptospires as antigens. The serovars used in the antigen panel depends upon the laboratory that performs the test. Usually, laboratories use at least one serovar from each serogroup that is known to occur within the region in the antigen panel [9]. The aim of this study was to evaluate the ability of serology by MAT to predict the serogroups compared with results of previous studies of identification by serotyping, iRep1-PCR, PFGE and MLST in São Paulo, Brazil.

2. Material and methods

2.1. Samples and isolates

A blinded study was conducted with all culture-confirmed leptospirosis cases during the 16-year period 1995–2010. Laboratory records were reviewed retrospectively to identify all cases of leptospirosis confirmed by isolation of *Leptospira* spp. from blood and that showed a 4-fold rise in titer between acute and convalescent samples of sera by MAT. All serological testing was blinded. All samples for cultures were obtained on average 6.2 days after the onset.

2.2. MAT

The MAT was performed as described elsewhere [9]. Briefly, 20 serovars were added to serially diluted serum specimens in 96-well microtiter plates and incubated at 30 °C for 2 h. The antigen panel contained reference strains that commonly infect patients in São Paulo as well as strains isolated from patients [2,8]: 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 CZ214K, Pomona strain Pomona, Pyrogenes strain Salinem, Shermani strain 1342K, Tarassovi strain Perepelitsin and Wolfii strain 3705. Agglutination was examined by dark-field microscopy at a magnification of 100×. According to the standard criteria the titers were determined as the highest serum dilutions

that agglutinated at least 50% of the cells for each serovar used. The probable predominant serogroup was defined as the serogroup with the maximum titer directed against a single serovar. If two or more serovars showed the same titer it was considered undetermined.

2.3. Identification of isolates

The serogroups have been identified previously using serotyping, iRep1-PCR [6], PFGE [7] and MLST [8].

2.4. Statistical analyses

Cohen's kappa coefficient (κ) and associated 95% CI was used to evaluate the degree of concordance between the serogroup identified by serology and that determined by the identification of the isolate.

3. Results

A total of 52 cases of leptospirosis were confirmed by isolation of *Leptospira* spp. and by serology. Compared to the results of serotyping, MLST, iRep1-PCR and PFGE, MAT correctly assigned the serogroup of the infecting isolate in 49/52 cases (94.23%). MAT failed to assign the serogroup of the infecting isolate in 3/52 cases (5.77%). Table 1 shows the results of serology compared with the identification. One case which was considered undetermined was not included. The correct predictions for each serogroup were as follows: for serogroup Icterohaemorrhagiae 97.82% (45 of 46 isolates); for serogroup Canicola 50% (1 of 2 isolates); for serogroups Autummnalis, Sejroe and Grippotyphosa 100% (1 of 1 isolate of each one). By serology, the distribution of serogroups among the isolates was as follows: 46 cases of serogroup Icterohaemorrhagiae (88.46%), two cases of serogroup Autumnalis (3.84%), one case each of serogroup, Canicola, Grippotyphosa and Sejroe (1.92%). Among the remaining three cases that MAT failed, one case showed equal titer against two or more serovars (Icterohaemorrhagiae and Cynopteri) and was considered undetermined. This isolate was identified as serogroup Icterohaemorrhagiae by molecular methods. One isolate was identified as serogroup Icterohaemorrhagiae and by serology the highest titer (25,600) was against Autumnalis. In the other case, the isolate was identified as Canicola molecularly and by serology the highest titer (1600) was against Icterohaemorrhagiae. The degree of agreement between serogroup prediction using MAT serology and by identification of the isolates was good ($\kappa = 0.787$, 95% CI: 0.501–1.000).

4. Discussion

Leptospirosis has been considered a public health problem of increasing importance in São Paulo, Brazil due to both a high annual incidence rate [2] and the occurrence of fatal cases. Leptospirosis has been studied extensively in São Paulo, with an average of 162 cases of leptospirosis diagnosed annually according to clinical manifestations and laboratory confirmation [2]. Infection sources have to be identified based on the evidence of the same leptospires in both patients and suspected hosts in order to set up control and prevention measures [1]. However, due to the difficulties in the isolation of leptospires and the low contribution of culture to an early diagnosis, MAT is the method of choice. Although the

disease may be detected by serology, cross-reactions between serogroups and paradoxical reactions are common [10], in which the initial immune response is directed toward a heterologous serovar or serogroup, can confound the diagnostic process [11]. So far, knowledge of the causative serogroups in our region has been mainly based on the titers in the MAT [2]. In São Paulo, seropositivity is high in the general population. In this study we showed the usefulness of the MAT to correctly identify the infecting serogroup with a good overall agreement between the serologically-identified infecting serogroup and by identification of the isolate. The majority of the serogroups with the highest titers matched the cultured serogroup. Results of serologic testing were consistent with the culture for 49/52 cases (94.23%). Also, MAT appears to be a good indicator of the infecting serogroup in our region, partly due to the dominance of serogroup Icterohaemorrhagiae in Brazil. The ability to infer the serogroup identity of infecting leptospires from the results of serologic testing by use of the MAT was already evaluated by others [11,12]; however, our study differs remarkably from their results. Since the range of serogroups in São Paulo is limited and well defined [2,6–8,13] the ability to predict the prevalent serogroups in our population is better than are those that might be encountered in other regions. One isolate identified as Icterohaemorrhagiae showed the highest titer of 25,600 against Autumnalis whereas Icterohaemorrhagiae was 6,400. Another isolate identified as Canicola showed the highest titer of 1,600 against Icterohaemorrhagiae whereas the Canicola titer was 400. The remaining one case showed equal titer against two or more serogroups. Our study shows that the majority of human disease was caused by the serogroup Icterohaemorrhagiae. Since this serogroup appeared to be predominant we can infer that rodents are the main natural incriminated source in our region. In conclusion, although our study showed that MAT is good to predict the presumptive serogroup, it should be used only to give an idea of the common serogroups present in a population in São Paulo. The isolation and identification of serovar by CAAT remains the technique of choice.

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Table 1

Results of serology by MAT compared with the isolates identification by serotyping, iRep-PCR, MLST and PFGE.

Serology by MAT	**Isolates identified by serotyping, iRep-PCR, MLST and PFGE**					
	Icterohaemorrhagiae	**Autumnalis**	**Canicola**	**Grippotyphosa**	**Sejroe**	**Total**
Icterohaemorrhagiae	45	0	1	0	0	46
Autumnalis	1	1	0	0	0	2
Canicola	0	0	1	0	0	1
Grippotyphosa	0	0	0	1	0	1
Sejroe	0	0	0	0	1	1
Total	46	1	2	1	1	51 *

* The remaining one case was considered as undetermined.