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Interpretation of microscopic agglutination test for leptospirosis diagnosis and seroprevalence

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PEER REVIEW

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Comments

This work can be as good laboratory medicine paper discussing on diagnostic test evaluation of the tool to investigate and trace the problem of leptospirosis in the endemic area. As noted, the result can be a good data for further laboratory technique to stimulate the way to diagnose and control of the leptospirosis.
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ABSTRACT

Determination of antibody titer by microscopic agglutination test (MAT) has been used as a tool for leptospirosis diagnosis. Four fold or greater rise in antibody titers between acute and convalescent sera suggests recent *Leptospira* infection. In addition, results obtained by MAT have been used to predict infecting serovars. However, cross reactivity among various *Leptospira* serovars have been reported when patient sera were tested with a battery of *Leptospira* serovars. This study demonstrates cross- reactivity among several *Leptospira* serovars when MAT was performed on leptospirosis sera. The data support a role of MAT as a tool for diagnosis. However, for information on infecting serovars, *Leptospira* isolation and molecular identification should be performed.

KEYWORDS

Leptospirosis, Microscopic agglutination test (MAT), Seroprevalence

1. Introduction

Leptospirosis is a widespread zoonosis caused by spirochetes of the genus *Leptospira*. Most leptospirosis patients have mild diseases including fever, headache and myalgia. However, patients with severe illness have been reported. Severe symptoms include liver and kidney failure. Moreover, pulmonary hemorrhage has been increasing reported as a cause of death of leptospirosis patients. *Leptospira* are classified into 24 serogroups and more than 200 serovars according to the difference of their lipopolysaccharide. Currently, *Leptospira* can be genetically classified based on DNA hybridization technique into at least 19 species[1,2].

Cultivation of leptospires requires special media and it takes at least one week before organisms can be observed. Confirmation of leptospirosis diagnosis mostly relies on antibody detection. Microscopic agglutination test (MAT) has been widely used as the reference test for antibody detection. MAT is performed by incubating patient serum with various serovars of leptospires. MAT titer is obtained by testing various serum dilutions with the positive serovar. The serovar that reacts with patient serum is suggested to be the infecting serovar. Information on infecting serovars obtained by MAT has been used for epidemiological study.

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Since performing MAT requires maintaining of live leptopsires, several techniques such as ELISA, indirect immunofluorescent and slide agglutination test have been developed[3-6]. Detection of IgM antibody specific to *Leptospira* by ELISA (IgM-ELISA) has been widely used. There is no need to test the second sample if IgM ELISA is positive; whereas paired sera testing is required for diagnostic confirmation by MAT assay. Four fold rising of MAT titer suggests current *Leptospira* infection.

Although MAT has been used as the reference assay, it has been shown that MAT sensitivity is relatively low[7]. Smythe *et al*, has shown that MAT could correctly predict infecting serovars in only 33% of leptospirosis cases[8]. In addition, sera from some patients can react with more than one serovar.

In this report, we analyzed MAT results of patients who visited King Chulalongkorn Memorial Hospital and were suspected to have *Leptospira* infection. Seventeen pairs of sera were included in this study.

The representative *Leptospira* serovars included in the MAT assay were listed in Table 1. Two-fold dilution of serum starting from 1:50 was performed for each serum. IgM ELISA (Panbio, Sinnamon Park, Australia) was also performed according to manufacturer's protocol. Seventeen paired sera, which showed rising MAT titers and were positive by IgM ELISA, were included in this report. The results of MAT were shown in Table 2.

Table 1

Leptospira used in this study.

Species	Serovars
Leptospira interrogans	Bratislava
Leptospira interrogans	Autumnalis
Leptospira borgpetersenii	Ballum
Leptospira interrogans	Bataviae
Leptospira interrogans	Canicola
Leptospira weilii	Celledoni
Leptospira kirschneri	Cynopteri
Leptospira interrogans	Djasiman
Leptospira kirschneri	Grippotyphosa
Leptospira borgpetersenii	Hebdomadis
Leptospira interrogans	Icterohaemorrhagiae
Leptospira borgpetersenii	Javanica
Leptospira noguchi	Louisiana
Leptospira alexanderi	Manhao
Leptospira borgpetersenii	Mini
Leptospira noguchi	Panama
Leptospira interrogans	Pyrogenes
Leptospira meyeri	Ranarum
Leptospira santarosai	Sarmin
Leptospira interrogans	Sejroe
Leptospira santarosai	Shermani
Leptospira borgpetersenii	Tarassovi
Leptospira biflexa	Patoc

Table 2

MAT titer of 17 pairs of leptospirosis sera.

#	AUS	AUT	BAL	BAT	CAN	CEL	CYN	DJA	GRI	HEB	ICT	JAV	LOU	MAN	MIN	PAN	POM	PYR	RAN	SAR	SEJ	SHE	TAR	PAT
1/1	50	50																				100		
1/2	100																					800		
2/1	50	50																				100		
2/2	100																					800		
3/1																						100		
3/2																						1 600		
4/1																								
4/2																						200		
5/1																								
5/2																					100	3 200		100
6/1																								
6/2														200										
7/1	1 600	100							100			100	400	400	400	100		200				3200		
7/2	3 200	200				100	100		100			200	400	200	800	400		200		100	400	1600		
8/1																						50		
8/2										100											400	6400		
9/1	50	100								50			50		50				25			200		
9/2	400	1 600					100			50			100		50	50			25			200		
101																								
10/2	800	100					100						200						25			100		
11/1																						400		
11/2		100		400											50						400	1 600		100
12/1																								
12/2		100		200			100						200									400		
13/1																								
13/2		400		1 600																		800		
14/1	50	50					50						100						25			100		
14/2	800	400					1 600	100					3200				50		25			400		
15/1												200			100	200		200				400		
15/2	1 600	400					400	200	100		100	200			400	1 600	6 400	400				6 400		
16/1																						200		100
16/2		3 200		3 200			400				400		800	100							200	1 600		400
17/1																								
17/2	3 200	400						200	400			400	800		400	400					800	1 600		

AUS=Bratislava; AUT=Autumnalis; BAL=Ballum; BAT=Bataviae; CAN=Canicola; CEL=Celledoni; CYN=Cynopteri; DJA=Djasiman; GRI=Grippotyphosa; HEB=Hebdomadis; ICT=Icterohaemorrhagiae; JAV=Javanica; LOU=Louisiana; MAN=Manhao; MIN=Mini; PAN=Panama; POM=Pomona; PYR=Pyrogenes; RAN=Ranarum; SAR=Sarmin; SEJ=Sejroe; SHE=Shermani; TAR=Tarrassovi; PAT=Patoc; Underline indicates the serovar that showed at least 4-fold rising titer against tested sera.

Numbers of serovars that showed at least 4-fold rising MAT titers against patient paired sera were analyzed. Sera from 6 (# 1-6) out of 17 patients showed rising titer with only one serovar. There were 4 (# 7-10), 3 (# 11-13) and 1 (# 14) pairs of sera that demonstrated rising titer with 2, 3 and 5 serovars, respectively. In addition, sera from two patients (# 15 and 16) showed rising titer with 8 serovars. Sera from a patient (# 17) demonstrated ≥ 4-fold rising MAT titer with 10 serovars.

The reasons that serum from a patient reacted with various serovars could be 1) cross-reaction among various serovars 2) a patient was infected with more than one serovars. It was recommended that the serovar providing highest antibody titer could be an infecting serovar. However, it is also possible that this patient was previously infected with one serovar and later on, the same patient was infected with another serovar. The newly acquired serovar may have cross-reaction with the former infecting serovar. This leads to the activation of memory response against previous serovar. If this is the case, titer of antibody specific to previous serovar could be higher than of antibody against the new infecting serovar. Four-fold rising antibody titer has been used as an indicator of current infection. All serovars that provide 4-fold rising antibody titer or higher should also be considered. For example, the highest MAT titer of the patient # 14 is the antibody against serovar Louisiana (titer 3200). However, sera from this patient also showed at least 4-fold rising against serovars Bratislava, Autumnalis, Cynopteri, and Shermani. These 4 serovars should not be excluded from a list of suspected infecting serovars. Similar results were observed in other sera that showed rising MAT titer against more than one serovars.

Although several techniques have been developed, MAT is still being used for leptospirosis diagnosis and for seroprevalence survey. These data support that MAT could be used for laboratory diagnosis. Four-fold rising of MAT antibody titer is an evidence of *Leptospira* infection. However, the information on serovars that cause infection in patients or are responsible for outbreaks should be carefully interpreted. Isolation of organisms for serological or molecular typing will give more accurate information for these purposes.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

A diagnostic test to study a new tool to test leptospirosis, an important problem in tropical medicine. The report is from a tropical endemic area, Thailand, which makes this work becomes very interesting.

Research frontiers

Some interesting new data from the tropical endemic area of leptospirosis can be seen in this work. It can be a good report in laboratory medicine and infectious medicine. Future relating citation can be expected

Related reports

There are some related reports but there is no completely similar publication to this work. This work shows some new epidemiological aspect plus the evaluation of the diagnosis test.

Innovations

Although there is no new intervention some new information can be derived from this study. The new insight in the field to study leptospirosis can be a useful point emerged from this article. The work can be a good example for other researchers to follow and cite.

Applications

This work can be applied in the field of clinical microbiology. The result can be a good data for further laboratory technique search and verification to fight an important tropical infection, leptospirosis. Further study on this area can be expected.

Peer review

This work can be as good laboratory medicine paper discussing on diagnostic test evaluation of the tool to investigate and trace the problem of leptospirosis in the endemic area. As noted, the result can be a good data for further laboratory technique to stimulate the way to diagnose and control of the leptospirosis.

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