

Further evaluation of one-point microcapsule agglutination test for diagnosis of leptospirosis

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SUMMARY

A total of 100 serum samples including 22 acute phase sera and 39 paired sera collected from clinically diagnosed cases of leptospirosis in Ming-shan County, Sichuan Province, China were examined by the one-point microcapsule agglutination test (MCAT), which was developed in Japan, and by conventional microscopic agglutination tests (MAT). The one-point MCAT is more reactive to IgM antibody than MAT and is superior in detecting antibodies in the early stages of the disease.

INTRODUCTION

Human leptospirosis is well recognized in endemic areas in China [1] and in cases with typical presenting features local physicians can make the diagnosis on clinical grounds alone. However, serological confirmation is considered desirable and in some cases necessary.

There are several serological tests available for testing serum for antibodies to leptospirosis. Among them, the conventional microscopic agglutination test (MAT) is currently the most commonly used laboratory procedure. The method is specific and sensitive. However, it is time-consuming and tedious to perform, and requires the use of a battery of live antigens representing all the serovars indigenous to each area.

Recently, we have developed a passive microcapsule agglutination test (MCAT) for the serodiagnosis of leptospirosis [2, 3]. A synthetic polymer of uniform particle size is used as a carrier for sensitization with mixed antigens of sonicated leptospire. It was found that the test could be used reliably for the early diagnosis of leptospirosis. It has since been further simplified [4], so that only one dilution of the test serum is sufficient for the one-point MCAT. A commercial kit form is now on the market in Japan.

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In order to determine whether the kit could be employed outside of Japan, we [5] have tested the kit and found that this simplified technique was reliable in detecting leptospiral antibodies in the serum of leptospirosis patients in China. This paper describes further evaluation of the test in patients suspected of leptospirosis in Ming-shan County, an endemic area in Sichuan Province of China.

MATERIALS AND METHODS

Leptospiral serovars used for the MAT

The following 11 serovars which are the most commonly seen in China, *Leptospira australis*, *L. autumnalis*, *L. bataviae*, *L. canicola*, *L. grippityphosa*, *L. hebdomadis*, *L. javanica*, *L. lai*, *L. manhao*, *L. pomona*, and *L. pyrogenes* were used in the MAT. These strains were all isolated from specimens taken in China and were maintained in Fletcher's medium containing 10% rabbit serum.

Patients' sera

A total of 100 serum specimens, 39 paired sera and 22 single sera were obtained from patients clinically diagnosed as cases of leptospirosis in Ming-shan County. The 39 pairs of sera consisted of acute-phase sera taken within 7 days after the onset of illness, and convalescent sera taken between 2 and 3 weeks after the onset of illness. All sera were inactivated by heating at 56 °C for 30 min and kept under refrigeration until use. Paired sera were tested simultaneously under uniform conditions.

MAT

The test was performed essentially according to the method described by Faine [6]. The titre was expressed as the reciprocal of the highest dilution of the antiserum which showed 50% microscopic agglutination using live antigens. MAT titres of 400 or more are considered significant and a fourfold or greater increase in titres is diagnostic for current infection in the endemic areas.

One-point MCAT

The kit which was supplied by Japan Lyophilization Laboratories, (1-5 Matsuyama, 3-chome Kiyose City, Tokyo 180-04, Japan) consisted of two vials of lyophilized reagents, A and B, and one vial of a diluent (1% bovine serum albumin-PBS, pH 7.2). The reagents A and B are microcapsules sensitized separately with mixed sonicated antigens of *L. australis*, *L. autumnalis*, and *L. hebdomadis* (Reagent A) and *L. canicola*, *L. icterohaemorrhagiae*, and *L. pyrogenes* (Reagent B). A test-tube rack with an obliquely placed mirror and a number of disposable test tubes are also provided with the kit.

Briefly, the test procedure is as follows. After reconstituting the reagents with the designated amount of diluent, 0.3 ml of each reagent is transferred to corresponding tubes marked A or B. A loop of the test serum (c. 1 μ l) is then added to both test tubes. These test sera were consequently diluted about 300-fold in the tube. After mixing well the tubes are allowed to stand in the rack at room temperature for 3 h. The reaction is recorded as 3+, 2+, +, \pm , or - according to settling patterns defined by Seki and colleagues [4]. A reaction of one plus (+) is considered to be positive.

Table 1. Comparison between MAT and one-point MCAT on acute-phase and convalescent sera from leptospirosis patients

	No. of cases	One-point MCAT* positive (%)	MAT† positive (%)
Acute-phase sera	61	60 (98.4)	7 (11.5)
Convalescent sera	39	39 (100)	33 (84.6)

* Over one plus (+) is positive.

† MAT titre of ≥ 400 is significant.

Table 2. Correlation of days after onset and serological results by one-point MCAT and by MAT in 15 cases during the acute stage, as confirmed by the cultural method

Case No.	Day after onset	One-point MCAT		<i>L. aust.</i>	MAT† <i>L. lai</i>	Isolated serovar
		A	B			
P-2§	2	2+	+	—†	—	<i>L. lai</i> *
P-5	2	+	+	—	—	<i>L. lai</i> *
P-25	2	2+	2+	—	—	<i>L. lai</i>
P-30	2	2+	2+	—	50	<i>L. grippotyphosa</i>
S-3	2	2+	2+	—	—	<i>L. hebdomadis</i>
S-15	2	2+	2+	—	—	<i>L. australis</i>
P-4	3	+	+	—	—	<i>L. autumnalis</i>
P-10	3	+	+	—	50	<i>L. hebdomadis</i> *
P-15	4	2+	2+	—	—	<i>L. lai</i>
S-20	4	2+	2+	—	—	<i>L. hebdomadis</i>
P-11	5	3+	3+	100	100	<i>L. hebdomadis</i> *
P-3	7	+	2+	—	—	<i>L. australis</i> *
S-8	?	3+	2+	—	50	<i>L. lai</i>
S-11	?	2+	2+	—	—	<i>L. australis</i>
S-19	?	3+	2+	—	—	<i>L. grippotyphosa</i>

* Confirmed also by the convalescent serum sample in MAT.

† Negative with the remaining nine serovars.

‡ — indicates < 50.

§ Paired sera.

|| Single serum.

Isolation of leptospires from patients

In order to isolate leptospires from blood, cultures were taken during the acute pyrexial stages of illness. Two tubes, each containing 10 ml of Korthof medium, were inoculated with 1 or 2 drops of blood and incubated at 28–32 °C for 4 weeks.

RESULTS

Of the total of 61 acute-phase serum samples, 60 were positive by one-point MCAT (98.4%), whereas only 7 (11.5%) were positive by MAT. All convalescent sera were positive by the MCAT though 2 were only weakly so, but only 33 of the 39 in the paired sera group (84.6%) showed diagnostic rises in titre by the MAT (Table 1).

Fifteen cases were confirmed by successful cultivation. The correlation of the serological results of both tests with the number of days after the onset of the

Table 3. *Perplexity in the interpretation of the MAT results of three cases from 39 paired sera*

Case no.	Serum	MAT					
		<i>australis</i>	<i>autumnalis</i>	<i>hebdomadis</i>	<i>lai</i>	<i>manhao</i>	<i>pomona</i>
14	1st	50	—	—	50	—	—
	2nd	—	—	400	200	—	—
20	1st	—	—	—	50	—	50
	2nd	200	800	—	800	—	—
21	1st	—	199	—	—	100	—
	2nd	100	—	—	400	400	—

disease is shown in Table 2 which also includes the results on the six single serum specimens taken during the acute stage. Isolations were obtained as early as 2 days after the onset, and the one-point MCAT was able to detect antibody even in such early stages of illness. Out of 9 paired serum specimens from cases where leptospire were isolated, 5 serum samples were also positive by MAT, but the remaining 4 cases were not (P-4, P-15, P-25, P-30). Cases P-15 and P-25 were both serovar *lai* but showed antibody titres of 800 to *L. hebdomadis* and *L. javanica*, respectively. Cross reactions with more than two serovars, particularly with *australis*, *autumnalis*, and *lai*, were very often seen in this area (Table 3).

DISCUSSION

Leptospirosis is a zoonotic disease occurring in most parts of China, particularly in the rice growing areas in the southern parts of China where sporadic cases or outbreaks occur frequently among rice farmers during the harvest season. This study attempts to evaluate the one-point MCAT in suspected-leptospirosis patients in Ming-shan County, an endemic area in Sichuan Province of China. Compared with MAT, one-point MCAT showed high sensitivity and was useful both for the confirmation and early diagnosis in human leptospirosis cases which might be due to the ability of the MCAT to detect IgM antibodies [3]. Early post-infection serum samples quite often had high agglutination titres against one or more serovars unrelated to those endemic in Ming-shan County, Sichuan Province, China. Such paradoxical reactions [7] are seen in 10–20% of cases in China. Generally, however, the highest titres were with the serogroup or serovar of the infecting leptospire. Sometimes nearly identical titres to several serovars were found by MAT making interpretation difficult though the possibility exists of double or multiple infections. Furthermore, the widespread incidence of leptospirosis in humans, the annual mass administration of leptospiral vaccine and the early administration of antibiotics may further confuse the interpretation of the serological results. Serum samples with antibody titres of 50 or 100, associated with past or subclinical infections, or with vaccination were detected regularly in farmers living in the countryside. We therefore adopted a single titre of 400 or over, or a four-fold increase in the titres by MAT as the criteria for the diagnosis of current infection though the titre of 400 in a single test can only be regarded as presumptive leptospirosis infection. In contrast, a weakly positive (+) reaction by the one-point MCAT may be strongly suggestive of current infection.

In summary, the present study indicates that the one-point MCAT is the best single test for the demonstration of leptospiral antibodies in early infection. The test covers a broad spectrum while being genus-specific, and is more sensitive and reliable than MAT. Moreover, the test kit is self-contained, requires no special equipment or training, and yields results in less than 4 h. Large volumes of specimens can be processed in small peripheral laboratories where early diagnosis is desirable for immediate initiation of treatment.

It must, however, be emphasized that while the one-point MCAT is suitable for the performance of screening tests for the diagnosis of patients recently infected with leptospires, it is not appropriate for epidemiological research.

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