FIELD APPLICATION AND THE EFFICIENCY OF INDIRECT HAEMAGGLUTINATION TEST KIT IN THE DIAGNOSIS OF HUMAN LEPTOSPIROSIS

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Abstract

Human sera with the history of pyrexia of unknown origin (PUO) received from physicians of various hospitals and laboratories in Namakkal of Tamilnadu were screened a developed IHA test kit and the results were compared with that of MAT. Out of a total of 176 sera screened by the developed kit, 95 samples (54.0 per cent) were positive. The sensitivity and specificity of IHA with reference to MAT were found to be 91.2 and 85.9 per cent, respectively, with no significant difference (P>0.05). Hence, the test would be an alternate tool for MAT in the diagnosis of leptospirosis in human and also animal during the active stage of the infection.

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Key words: Leptospira, indirect haemagglutination test, Field kit, MAT, sensitivity, specificity

INTRODUCTION

Leptospirosis is an emerging zoonotic disease caused by a number of different pathogenic bacteria of the genus Leptospira [6] that affects most species of warm-blooded animals and humans. The disease has gained extreme...
public health importance in India, because of huge livestock, rodent and wild life populations, poor sanitary conditions and close association between man and animals [18]. Leptospira infection produces a wide spectrum of clinical manifestations ranging from subclinical infection to a severe syndrome of multiorgan dysfunction with high mortality [6]. Since leptospirosis is confused easily with other febrile diseases clinical diagnosis becomes more difficult.

Leptospirosis is grossly under reported due to its protean clinical manifestations and the lack of simple diagnostic measures for early detection and control of the infection [17]. Hence, accurate and rapid diagnosis is of paramount importance for an effective treatment and in the interest of public health. The present work aimed at the development of indirect haemagglutination test (IHA) kit for an early and accurate serological diagnosis of leptospirosis in humans due its simplicity, safety and suitability to examine a large number of sera in the field [16] and the efficiency of this test was compared with the conventional microscopic agglutination test (MAT) which is recommended by Office International des Epizooties (OIE) as gold standard test.

2. MATERIALS AND METHODS

Human sera with the history of pyrexia of unknown origin (PUO) and febrile illness submitted by medical practitioners and government hospitals in and around Namakkal of Tamilnadu referred to this Department were tested by the developed IHA kit and compared with the application and results of MAT.

Standard leptospiral strains Rachmati, Hond utrecht IV, RGA and Pomona of the serogroups, Autumnalis, Icterohaemorrhagiae, Canicola and Pomona, respectively [8] were obtained from Leptospirosis Research Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai were subcultured in Ellinghausen McCullough Johnson Harris liquid (EMJH) medium and four to eight day old liquid culture of live leptospires incubated at room temperature containing 2x10⁵ leptospires per ml was used as pooled antigen for performing IHA and MAT with known positive and negative human sera as controls.

The antigen was prepared as per the method suggested by Palit and Gulasekharam (1973) with slight modification. Ten millilitre of eight day old liquid culture of live leptospires from each serovar in sterile test tubes were centrifuged at 14000 rpm at 4°C for 30 minutes. The pellet was suspended in 10 ml of PBS (pH 7.2) and washed twice by centrifugation at 1500 rpm for 10 minutes. The pellet was resuspended in 10 ml of PBS and the suspension was added with 50 per cent (v/v) cold absolute alcohol (ethanol), and centrifuged at 10000 rpm for 20 minutes. To the supernatent separated, sufficient quantity of ethanol was added to bring concentration up to 90 per cent (v/v) and stored at 4°C overnight. The precipitate was separated and suspended in PBS and stored at -20°C until use.

Fixed erythrocytes for IHA were prepared as per the method prescribed in the Manual on Diagnosis of Poultry Diseases [11] was followed with slight modification and the RBCs were labeled with the antigen. The IHA was carried out as per the standard procedure and a titre of 1:100 was considered positive for active recent leptospirosis [12].

MAT was carried out as per the procedure suggested by Cole et al. (1973) with slight modification. A titre of 1:100 was considered positive by MAT [14].

3. RESULTS AND DISCUSSION

Out of a total of 176 sera screened by IHA, 95 samples were positive with the seropositivity of 54.0 per cent. In the present study, seropositivity by IHA was lower when compared to the previous findings (73.75 per cent) by Naigowit et al. (2000) and 68.0 per cent for Icterohaemorrhagiae by Effler et al. (2000). However, Agunloye et al. (2001) reported a lower seropositivity of 41.5 per cent and opined that the higher prevalence by the IHA test might be due to non-specific reactions.

The seropositivity by MAT was 51.7% which was in agreement with the findings of Koteeswaran (2006) who reported 57.6 per cent seropositivity by MAT. However, Natarajaseenivasan et al. (2002) reported higher seropositivity of 68.3 per cent.

The sensitivity and specificity of IHA with reference to MAT were found to be 91.2 and 85.9 per cent, respectively (Table 1). The sensitivity of IHA in this study highly correlated with the findings of Ahmad et al. (2005), who reported 92.0 per cent sensitivity. However, Levett and Whittington (1998) reported 100.0 per cent sensitivity. The specificity of IHA in this study corroborated with the findings of Bajani et al. (2003) and Ahmad et al. (2005), who reported specificity of 85.0 to 100.0 per cent. However, differences in sensitivity of IHA may occur when there is time variation between onset of symptoms and the collection of acute phase specimen.

Table 1: Comparative results of IHA with reference to MAT

<table>
<thead>
<tr>
<th>IHA</th>
<th>MAT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>83</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>73</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>85</td>
</tr>
</tbody>
</table>

The testing efficiency of IHA and MAT revealed no significant difference (P>0.05) in diagnosis of leptospirosis, which was in accordance with the findings of Bajani et al. (2003).

4. CONCLUSION

Microscopic agglutination test, though recognized as a “gold standard” test by OIE, is laborious and requires maintenance of live culture organisms which are dangerous to the laboratory workers and its interpretation being relatively
subjective. Since, application of sensitized (with genus-specific antigen), freeze-dried red cells in the IHA is safe, the test would be a useful tool and good alternate for MAT for the diagnosis of leptospirosis in human and also animal sera when collected during the active stage of the infection.

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6. REFERENCES


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