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COMPARISON OF THREE RAPID DIAGNOSTIC ASSAYS FOR DIAGNOSIS OF LEPTOSPIROSIS IN A RESOURCE POOR SETTING

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ABSTRACT

Rapid serological diagnostic tests are easy to perform, cost effective and useful as a point-of-care diagnostic in resource-poor areas. This study was conducted to compare the performance of three rapid serodiagnostic tests for detection of leptospirosis in Sri Lanka. Serum of 75 clinically suspected leptospirosis patients between January to December 2013 were tested by three commercial immunochromatographic assays; Leptocheck–WB test, SD Leptospira IgM and SD Leptospira IgG/IgM. Leptospirosis was confirmed by MAT titre ≥400 or by a positive PCR. While 75% patients were clinically suspected of leptospirosis according to the Modified Faine's Criteria (2012), leptospirosis was confirmed in 65.3% by MAT or PCR. Of the 75 serum specimens tested, IgM was positive by Leptocheck –70.6%, SD IgM – 50.6% and SD IgG/IgM– 53.3%. Leptospirosis was confirmed by MAT in 54.6% and by PCR in 13.3%. The sensitivity, specificity, PPV and NPV based on Bayesian latent class analysis were Leptocheck – 99.1%, 68.1%, 84.1%, 97.9%, SD IgM – 80%, 93.9%, 95.8%, 73.3% and SD IgG/IgM – 98.3%, 88.9%, 89.8%, 98.2%. The kappa values (Leptocheck-0.49, SD IgM–0.46, SD IgG/IgM–

0.45) showed satisfactory agreement with MAT and PCR. False positives and false negatives were seen in all three kits.

KEYWORDS: Leptospirosis, Immunochromatographic assay, MAT, PCR.

INTRODUCTION

Leptospirosis is an important public health problem in developing countries having a wide range of clinical manifestations, from mild fever to potentially fatal complications such as renal failure, pulmonary haemorrhage, liver derangement and multiple organ failure[1]. Early and prompt diagnosis of leptospirosis is essential to enable prompt initiation of antibiotic treatment, prevention of severe clinical complications and reduce case mortality. Due to the limited availability of specific diagnostic tests in resource-poor settings, physicians often rely on clinical features to make a probable diagnosis. However in tropical countries leptospirosis may often be misdiagnosed as the symptoms may mimic other tropical infections such as Dengue and Hanta virus infections[2]. Therefore early diagnosis of leptospira is a challenge to clinicians working in the developing countries.

Leptospirosis can be confirmed using laboratory diagnostic tests; culture, polymerase chain reaction, histopathological techniques or microscopic agglutination test[3]. However these tests require specialized laboratory facilities which are not available in most health care facilities. The Microscopic Agglutination Test (MAT) serves as the gold standard in serodiagnosis of leptospirosis. In Sri Lanka MAT is done at the National Leptospirosis Reference laboratory, Medical Research Institute, which serves the hospitals all over the country. Therefore, MAT is not easily accessible to the practicing clinicians. Further the turnover time required for obtaining MAT results and the technical expertise required to carry out MAT, limit its usefulness as the gold standard and as an early diagnostic test [4]. Molecular diagnostic tools such as Polymerase Chain Reaction (PCR) are also unavailable in most developing countries. Serodiagnostic tests to detect leptospira specific Ig M antibodies have been shown to be a useful alternative to identify leptospirosis. Therefore simple, rapid, affordable tests with high sensitivity and specificity are of utmost importance for disease diagnosis and subsequent initiation of specific and prompt treatment to ensure a favorable clinical outcome[4, 5].

Currently several commercial diagnostic methods for early diagnosis of leptospirosis are available including Enzyme Linked Immunosorbent Assays (ELISA), Latex agglutination

tests, dipstick assays and lateral flow assays. However these test kits are manufactured in other countries, possibly targeting the circulating leptospira strains in those geographical locations. Several research groups have reported geographic variation of the circulating Leptospira strains within the country[6, 7]. In this context it is very important to determine the performance of these rapid immune assays in the local settings. This study was aimed to evaluate the performance of three commercially available rapid serological kits in the presumptive diagnosis of leptospirosis among a group of clinically suspected leptospirosis patients in Sri Lanka, a developing country in the South Asia Region.

MATERIALS AND METHODS

Study setting and study population

This was a prospective study conducted in two selected hospitals in the Western and Southern provinces in Sri Lanka in 2013. Clinically suspected Leptospirosis patients, based on the World Health Organization (WHO) guideline (2010) [1] were enrolled in the study. Data was collected using a pre tested questionnaire to record clinical features. Ethical approval for the study was obtained from the Ethical Review Committee of University of Sri Jayewardenepura (Application no. 702/12).

IgM immunochromatographic assay and microscopic agglutination test (MAT)

Blood samples were collected on admission from febrile patients clinically suspected of leptospirosis. Whole blood (3ml) was collected into a plain tube to obtain serum for IgM immunochromatography assay and MAT. All samples were transported at 4 ºC to the Department of Microbiology, University of Sri Jayewardenepura within 24 hours. Three rapid immunochromatographic assays were tested following the manufacturer's instructions. Leptocheck WB (Zephyr biomedicals, India) which detects Leptospira genus specific IgM, SD Leptospira IgG/IgM (Standard diagnostics, Korea) and SD leptospira IgM kit (Standard diagnostics, Korea) which detects antibodies to *Leptospira interrogans* species was used for screening 75 of the patient serum samples. Further the Microscopic agglutination test using the genus specific *Leptospira biflexa* serovar Patoc strain was done to confirm leptospirosis using single MAT titres, at the local Leptospirosis reference laboratory, Medical Research Institute, Sri Lanka. A MAT titre of ≥ 400 was considered as confirmatory for Leptospirosis based on the WHO LERG criteria[8].

Polymerase Chain Reaction (PCR)

Two ml of EDTA blood was collected and transported on ice for molecular diagnosis. Leptospira DNA was extracted from 200 µl of EDTA blood samples by using QIAamp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany) and eluted in 50 µl of Tris EDTA. Positive and negative controls were included in each PCR assays and genomic DNA of *Leptospira interrogans* Serovar Canicola was taken as the positive control.

Single tube nested PCR assay was used to amplify 16S ribosomal DNA gene specific for pathogenic and intermediate Leptospira species as described previously by Bandara et al (6). PCR Amplification was carried out with rrs-outer-F(5'-CTCAGAACTAACGCTGGCGGCGCG-3'), rrs–outer-R (5'-GGTTCGTTACTGAGGGTTA AAACCCCC-3'), rrs-inner-F (5'-CTGGCGGCGCGTCTTA-3'), rrs-inner-R (5'-GTTTTCACACCTGAC TTACA-3') primers and followed the previously published protocol (6). The resulting amplicon was 547 bp. Amplicons were visualize by gel electrophoresis using a 1.5% agarose gel with ethidium bromide.

Leptospirosis was confirmed among these patients based on the WHO LERG criteria[8]. A definitive case was classified by a single MAT titre of ≥ 400 or by detection of Leptospira DNA by polymerase chain reaction. Presumptive diagnosis of leptospirosis was made according to the Faine's criteria with amendment[1]. Patients who had a definitive diagnosis other than leptospirosis were used as negative controls.

Data analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of three rapid immunochromatographic assays were calculated using standard equations[4] in data analysis software. A serum sample giving a MAT titre ≥ 400 or positive PCR was considered as a true positive. True negative was defined as MAT titre ≤ 400 or by negative PCR. Agreement of the immunochromatographic test with MAT or PCR was calculated using the kappa test. Kappa test was interpreted as follows; <0: agreement equivalent to chance, 0.21-0.4: fair agreement, 0.41- 0.60: moderate agreement, 0.61- 0.80: substantial agreement, 0.81-0.99: perfect agreement[9]. Bayesian latent class model analysis for imperfect gold standards was carried out for the same population using The MICE tool (Modelling for Infectious Disease Centre, Mahidol-Oxford Research Unit) as described previously[10].

RESULTS

Among the 75 leptospirosis suspected patients, 81% (n=61) presented between 5 – 9 days of fever. On admission to the hospital median duration of fever in this patient group was 6 days (±2.5). According to the Modified Faine's Criteria with amendment 74.6% (n=56) of patients were presumptively identified as Leptospirosis. Overall test positivity for each test is shown in Table 1. Only 45.3% (34/75) were positive by all three immunodiagnostic tests. In the studied population 54.6% (41/75) were positive for leptospirosis by a single MAT titre and among them majority (22/41, 53%) had MAT titre above 3200. MAT results of the population are depicted in Table 2. Leptospiral genomic DNA was detected in 13.3% of patients by nested PCR assay. MAT or PCR confirmed Leptospirosis in 65% (49/75).

Table 1: The percentage of Leptospira IgM positive patients by the rapid Immunochromatographic assay, Microscopic agglutination test and Polymerase chain reaction.

Test	Positive percentage % *(n)*
Leptocheck WB	70.6 *(53/75)*
SD IgM	50.6 *(38/75)*
SD IgG/IgM	53.3 *(40/75)*
Leptocheck WB + SD IgM + SD IgG/IgM	45.3*(34/75)*
MAT	54.6*(41/75)*
PCR	13.3*(10/75)*

Table 2: Results of single titre Microscopic Agglutination Test for patient serum. A MAT titre of ≥400 is considered as a positive for Leptospirosis[8]

MAT titre	percentage of samples (n=75)
≤400	45 %
≥400	6.6 %
≥800	5.3 %
≥1600	13.3 %
≥3200	29.3 %

The kappa values for agreement (with MAT or PCR) in Leptocheck WB, SD IgM and SD IgG/IgM were 0.49, 0.46, and 0.45 respectively which indicated moderate agreement. Further when the efficiency of the three tests were calculated they were similar. Leptocheck had an efficiency of 75.57% while both rapid tests of Standard Diagnostics were 72.97%. The sensitivity, specificity, PPV, NPV and efficiency of the three immunochromatographic assays

considering MAT or PCR as the perfect gold standard are given in Table 3. By this method Leptocheck had the highest sensitivity (91.7%) but low specificity (57.7%), whereas in the SD kits sensitivity and specificity were comparable, with SD Ig M having a higher specificity (80.8%). PPV was higher than Leptocheck for the SD IgM and IgM/IgG kits (80% and 87.2% respectively) and the NPV was lower (60%).(Table 3)

Table 03: Prevalence, sensitivities and specificities, positive and negative predictive values (PPV and NPV) estimated by using gold standard model and Bayesian latent class model (LCM).

Parameter	MAT or PCR (positive) was assumed as a perfect gold standard (%)*	Bayesian latent class model (%) **
Prevalence	64.9 (52.8 - 75.4)	62.9 (46.1 - 77.3)
MAT or PCR positive		
Sensitivity	100	89.9 (76.2 - 99.1)
Specificity	100	77.4 (55.7 - 93.5)
PPV	100	87.2 (67.4 - 96.9)
NPV	100	81.9 (54.8 - 98.7)
Leptocheck WB		
Sensitivity	91.7 (79.1 - 97.3)	99.1 (91.4 - 100)
Specificity	57.7 (37.2 - 76.0)	68.1 (45.6 - 94.3)
PPV	80.0 (66.6 - 89.1)	84.1 (64.0 - 97.9)
NPV	78.9 (53.9 - 93.0)	97.9 (78.6 - 100)
Standard Diagnostics Leptospira IgM		
Sensitivity	70.8 (55.7 - 82.6)	80.0 (63.7 - 97.0)
Specificity	80.8 (60.0 - 92.7)	93.9 (76.3 - 99.9)
PPV	87.2 (71.8 - 95.2)	95.8 (80.6 - 99.9)
NPV	60.0 (42.2 - 75.6)	73.3 (47.2 - 96.8)
Standard Diagnostics Leptospira IgM / IgG		
Sensitivity	72.3 (57.1 - 83.9)	98.3 (87.5 - 100)
Specificity	76.9 (55.9 - 90.2)	88.9 (75.1 - 97.7)
PPV	85.0 (69.5 - 93.8)	89.8 (76.3 - 98.0)
NPV	60.6 (42.2 - 76.6)	98.2 (86.1 - 100)

* Gold standard model assumed that test A is perfect (100% sensitivity and 100% specificity; all patients with gold standard test positive are diseased and all patients with gold standard test negative are non-diseased). Values shown are estimated means with 95% confidence interval.

** Bayesian latent class model assumed that all tests evaluated are imperfect. Values shown are estimated median with 95% credible interval.

In this analysis Leptocheck had the highest sensitivity (99.1%) but lowest specificity(68.1%). The SD Leptospira IgM/IgG test had a higher sensitivity than the SD Leptospira IgM test. However the SD Leptospira IgM test had a higher specificity. The sensitivity of our gold standard (MAT or PCR positive) was 89.9% and the specificity was 77.4%.

DISCUSSION

The performances of three locally available commercial, immunochromatographic assays were evaluated for the detection of leptospira specific antibodies. The assays were rapid and easy to carry out requiring only basic laboratory facilities and therefore can be easily accommodated as point of care diagnostics in resource-poor settings. The two rapid immunochromatographic assays having different target antigens, which detected Leptospira genus specific IgM antibodies by Leptocheck WB and *Leptospira interrogans* species specific IgM antibodies by Standard Diagnostics were both useful in diagnosis in the acute phase of illness. Antibodies for leptospira start to appear within few days of onset of symptoms and usually persist for several months [11]. Genus specific antibodies appear earlier than the serovar specific microscopic agglutinating antibodies. Therefore genus specific tests specially IgM immunoassays are expected to be positive in early stage of the disease although serovar specific MAT test may not be able to detect presence of antibodies due to absence of or low immune response[4].

In this study Leptocheck-WB gave the highest positive test percentage (70.6%). A false positive percentage of 21% with this test compared with the MAT was observed. False negatives were high for the two Standard Diagnostics kits (SD IgG/IgM and SD IgM). One important reason for this finding could be due to differences between circulating strains targeted by the manufacturer and circulating strains available in Sri Lanka. Previous studies done in Sri Lanka had found that *Leptospira interrogans* as the predominant strain causing infection in the country. However, other species, *L. borgpetersenii*, *L. kirshneri* and *L. weilli* have also been identified [6, 7] in Sri Lanka. Performance of various rapid diagnostic tests may differ based on the geographical location and the circulating strain type[12]. It is therefore necessary to validate the rapid Ig M assays in the local setting.

Sensitivity and specificity of Leptocheck WB has been reported by several groups (Table 4). Our results show an even higher sensitivity but a much lower specificity than reported. This can be explained by the higher percentage of false positives in this study. Further Sri Lanka being a country endemic for leptospirosis, high back ground seropositivity may also be a cause for lower specificity observed in this study. The agreement between Leptocheck WB and MAT was very good (kappa =0.81) in Panwala's study, whereas in our study the kappa agreement between MAT or PCR with Leptocheck WB was 0.49 which showed a moderate agreement[4]. A study done by Niloofa et al[10] in Sri Lanka, revealed a lesser sensitivity for Leptocheck WB, however the specificity (and the Kappa value was higher. Unfortunately studies evaluating the commercial kits, SD IgG/IgM or SD IgM is scarce. Panwala et al reports sensitivity, specificity, PPV and NPV of 72.7%, 70.1%, 54.5% and 83.9% respectively for the Standard Diagnostics Leptospira IgM/IgG rapid test. In our study the sensitivity and specificity were comparable assuming that the gold standard was perfect. However in the imperfect gold standard model both SD rapid tests had higher sensitivity and specificity indicating that they are useful in diagnosing infection in acute phase of the illness. Considering the performance characters of three diagnostic kits, the diagnostic efficacy (72% - 75%) and the kappa values (0.45- 0.49) of the three tests were comparable.

Table 4: Results of Leptocheck WB (LCT) in our study in comparison with other studies

Performance character	Panwala et al 2011[4]	Niloofa et al 2015[10]	Current study
Sensitivity	93.36	84.5	99.1%
Specificity	86.95	73.3	68.1%
Kappa value	0.81	0.616	0.49

The variability among screening test among various studies could be attributed to differences in the case definition or case recruitment, prevalence of the infecting serogroups and laboratory error[9]. In this study we used a single serum sample collected after six days of fever for presumptive diagnosis. When the serum sample is taken early during infection it is possible to have yet undetectable levels of antibodies resulting in false negatives. Sensitivity of the tests can be increased by testing paired serum samples in patients suspected of Leptospirosis. However in the hospital setting collecting a convalescent serum specimen is difficult to achieve as the patient may be discharged prior to collection of the convalescent specimen. According to the WHO guidelines we have used a single MAT titre of more than 400 as confirmatory for leptospirosis. Although MAT is the gold standard sensitivity of MAT

varies from 30% in single titre to 76 % for paired serum samples[13]. In the current study the sensitivity of MAT when considered as an imperfect gold standard was 89.9%. Hence it is possible to under diagnose cases based on a single MAT titre. One limitation of this study is that currently we are unaware of the baseline MAT antibody titre among the population as Sri Lanka is a highly endemic area for leptospirosis. It is important to determine the base line antibody tires in a larger population in the local setting. Another limitation of this study was the sample size, hence further studies with greater sample size is required to extrapolate the above results.

The three rapid tests showed comparable results indicating similar efficiency. False positives and false negatives were seen in all the three kits. Serology had a higher diagnostic utility than PCR.

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Conflicts of interest: None declared by all authors

REFERENCES

1. Kumar, S., Indian Guidelines for the Diagnosis and Management of Human Leptospirosis, in Medicine Update 2013. 2013, The Association of Physicians of India. Available at <http://www.apiindia.org/medicine_update_2013/chap07.pdf> Accessed February 1, 2015.
2. Haake, D.A. and P.N. Levett, Leptospirosis in humans. Curr Top Microbiol Immunol, 2015; 387: 65-97.
3. Gamage, C.D., et al., Epidemiology, surveillance and laboratory diagnosis of leptospirosis in the WHO South-East Asia region. 2012: INTECH Open Access Publisher. Available at < http://cdn.intechopen.com/pdfs/34772.pdf> Accessed February 1, 2015.

4. Panwala, T., Mulla, S. and Patel, P. Seroprevalence of leptospirosis in south gujat region by evaluvating the two rapid commercial diagnostic kits against the MAT test for detection of antibodies to leptospira interrogans. Nat J Com Med, 2011; 2(1): 64-70.
5. Suputtamongkol, Y., et al., Strategies for Diagnosis and Treatment of Suspected Leptospirosis: A Cost-Benefit Analysis. PLoS Negl Trop Dis, 2010; 4(2): e610.
6. Bandara, K.K., et al., Molecular characterisation and disease severity of leptospirosis in Sri Lanka. Mem Inst Oswaldo Cruz, 2015; 110(4): 485-91.
7. Agampodi, S.B., et al., Leptospirosis outbreak in Sri Lanka in 2008: lessons for assessing the global burden of disease. Am J Trop Med Hyg, 2011; 85(3): 471-8.
8. WHO, Report of the First Meeting of the Leptospirosis Burden Epidemiology Reference Group. 2010, World Health Organization: Geneva. p. 13. Available at < http://apps.who.int/iris/bitstream/10665/44382/1/9789241599894_eng.pdf> Accessed February 1, 2015.
9. Viera, A.J. and J.M. Garrette, Understanding interobserver agreement: The kappa statistics. Fam Med, 2005; 37(5): 360-363.
10. Niloofa, R., et al., Diagnosis of Leptospirosis: Comparison between Microscopic Agglutination Test, IgM-ELISA and IgM Rapid Immunochromatography Test. PLoS One, 2015; 10(6): e0129236.
11. Silva, M.V., et al., Behaviour of specific IgM, IgG and IgA class antibodies in human leptospirosis during the acute phase of the disease and during convalescence. J Trop Med Hyg, 1995; 98(4): 268-72.
12. Effler, P.V., et al., Evaluation of eight rapid screening tests for acute leptospirosis in Hawaii. J Clin Microbiol, 2002; 40(4): 1464-9.
13. Cumberland, P., C.O. Everard, and P.N. Levett, Assessment of the efficacy of an IgM-elisa and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg, 1999; 61(5): 731-4.