Research article

Validity of a commercially available IgM ELISA test for diagnosing acute leptospirosis in high endemic districts of Sri Lanka

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Abstract

Introduction
Lack of point of care diagnostics is a major challenge for control of human leptospirosis. Immunoglobulin M enzyme linked immunosorbent assays (IgM ELISA) have been widely used for the diagnosis of leptospirosis. The purpose of the present study was to determine the validity of IgM ELISA in the diagnosis of leptospirosis in a Sri Lankan context.

Methods
Confirmed cases of leptospirosis from the 2008 Sri Lankan outbreak of leptospirosis and a group of leptospirosis excluded febrile patients were selected for the validation study. Disease confirmation and exclusion was carried out using either paired sample MAT (optimized for the region) or qPCR or both. A commercially available IgM ELISA kit was used and the procedure performed according to the manufacturers' instruction in the Department of Microbiology, Faculty of Medicine, University of Peradeniya.

Results
The study sample included 88 confirmed cases of leptospirosis and a comparison group of 71 acute fever patients. Of the 88 confirmed cases selected, 53 reacted in IgM ELISA and of the comparison group, 38 gave a positive reaction. Sensitivity and specificity of IgM ELISA, as a point of care diagnostic test for patients in this sample with acute leptospirosis, was 60.23% (95% CI 49.78, 69.82) and 46.48% (95% CI (35.36, 57.96) respectively. Diagnostic accuracy of the test was 54.09% (95% CI 46.34, 61.65 %). The ROC (**Receiver-operator characteristic curve)** curve for the IgM ELISA showed a value of .669 for area under the curve. Optimal cut off points were not detected due to the poor test parameters in this sample.

Conclusion
This study shows the limited diagnostic capabilities of IgM ELISA during the acute phase of leptospirosis in high endemic settings

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Introduction

Leptospirosis is the most widespread zoonotic disease in the world.[1] It is transmitted to the human host through direct or indirect contact with infected urine from reservoir animals. The commonest reservoirs of human leptospirosis are rodents that are closely associated with human habitation. These include black and brown rats and domesticated mice. Domesticated large animals such as horses, dogs and farm animals like cattle, pigs, goats, and sheep are potential carriers of the human disease.[2] In rural areas where there is a close association with wild life, any rodent, marsupial or mammal in wildlife could be the source for human disease.[2]

Sri Lanka is identified as a country with one of the highest incidence of leptospirosis.[3] Since 2008, Sri Lanka is experiencing a sustained outbreak of leptospirosis. The worst ever documented outbreak of leptospirosis in Sri Lanka was reported in 2008 with over 7000 cases.[3] The number of cases reported during 2009, 2010 and 2011 were 4980, 4533 and 6633 respectively.[4] Despite all the measures taken by the public health system, leptospirosis continues to threaten the lives of people in Sri Lanka. One major challenge for leptospirosis control in Sri Lanka is the lack of point of care diagnostic facilities. Leptospirosis diagnosis is routinely achieved by using clinical features. The Medical Research Institute (MRI) of Sri Lanka is the only place which provides diagnostic facilities for leptospirosis using an abridged version of the microscopic agglutination test (MAT). The MAT is considered the gold standard for leptospirosis diagnosis. However, the MAT requires maintenance of regionally optimized leptospira culture panels, which needs expertise and resources. At the time of this study, Sri Lanka did not have the resources for gold standard diagnostic facilities, but used the genus specific patoc strain for leptospirosis diagnosis. In addition, the MAT mainly provides a retrospective diagnosis due to the need for paired samples, which is not helpful in patient care services.

Immunoglobulin M enzyme linked immunosorbent assays (IgM ELISA) have been widely used for leptospirosis diagnosis as a point of care diagnostic procedure in recent years.[5] It is cheaper when compared with the MAT and does not require labor intensive procedures. WHO recommends IgM ELISA as a diagnostic tool for leptospirosis in resource poor settings.[6] In Sri Lanka, IgM ELISA based methods were introduced to selected government hospitals during the 2008 outbreak as a short term measure. In the private sector, IgM ELISA based tests are available for leptospirosis diagnosis. As most ELISA based methods use genes specific leptospira[7], the validity of these methods are often questioned. Sensitivity and specificity of these tests may vary in different settings due to varying antigenic properties of infecting serovars and due to background leptospiral antibodies in high endemic areas. The reported sensitivity and specificity of ELISA IgM has a wide variation from <50% to >90%.[7-10] Validation of this test is therefore needed in different settings. The purpose of this study was to evaluate the validity of a commercially available IgM-ELISA method in leptospirosis diagnosis in the Sri Lankan setting.

Methods

Validation was performed using a well characterized collection of febrile patient population studied during the 2008 outbreak of leptospirosis in Sri Lanka. Details of patient recruitment, inclusion exclusion criteria, sample collection and disease confirmation have been published previously.[11,12] The validity of a presumptive diagnosis of leptospirosis has been reported in the previous paper which included IgM ELISA as a part of presumptive case definition.[11] However, based on the retrospective confirmation of additional cases[13], we conducted this study as a new analysis. In summary, the study sample comprised of possible cases of leptospirosis admitted to three tertiary care hospitals in central Sri Lanka during the period of August 2008 to January 2009. All adult (>12 years) febrile patients (fever <15 days) admitted to these hospitals were screened by two physicians and consented patients were recruited. Serum and whole blood samples were taken on admission and a convalescent serum sample was obtained after 10 days. IgM ELISA was carried out on fresh serum samples in the Department of Microbiology, Faculty of Medicine, University of Peradeniya. All other samples were stored at -70°C and the confirmatory tests carried out later.

Of the patients studied during the 2008 outbreak, we selected 88 confirmed cases of leptospirosis in whom IgM ELISA results were available and a comparison group of 71 febrile patients among whom leptospirosis was excluded using gold standard criteria. Case confirmation was carried out as suggested by the WHO Leptospirosis Epidemiology Reference Group (LERG) criteria: (i) sero-conversion (negative first sample and a titer >= 1:100 in the second sample), or a 4-fold rise in MAT titer between acute and convalescent samples; (ii) Leptospira DNA detected by PCR in either whole blood or serum. For the control group we selected patients in whom both paired sample MAT and qPCR were negative. Patients with uncertain results in MAT (reacted in MAT but not fourfold rise) were excluded from the control group. Negative results in qPCR were defined as having negative results in triplicate samples. MAT was performed by the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Western Pacific Region, Queensland, Australia using a regionally optimized panel of serovars. For the detection of leptospira DNA, we used a validated quantitative PCR method[13] in George Palade Laboratories, School of Medicine, University of California, San Diego, USA.

A commercially available Leptospira IgM ELISA was used in this validation study. IgM ELISA assay was performed according to the manufacturer's instructions. Serum samples from patients, and calibrator control sera supplied by the manufacturer were diluted 1:100 in serum diluent, and 100 µL added to antigen-coated micro wells. After the recommended incubation and washing cycles, absorbance of each well was read at a wavelength of 450 nm. Results were interpreted according to the manufacturers' instructions.

Statistical analysis was carried out using SPSS version 13. All test parameters were calculated with 95% confidence intervals. ROC curve was used to determine the optimal cut off point for IgM ELIA.

Results

A total of 88 confirmed cases were selected for this validation study, which included 31 in whom both MAT and qPCR were positive, 41 MAT positive patients and 16 qPCR positive patients. Mean age of confirmed cases and comparison group was 37 years (SD 12 years) and 36 years (SD 14 years) respectively. Of the confirmed cases, 45 (64.3%) were males compared to 48 (82.8%) males in the comparison group. Median duration of fever on collection of acute sample was seven days (Inter- quartile range 5-9) in both groups.

Table 1: Validity of ELISA for diagnosing leptospirosis in Sri Lanka, compared to MAT

ELISA	MAT		Total
	Positive	Negative	
Positive	53	38	91
Negative	35	33	68
Total	**88**	**71**	**159**

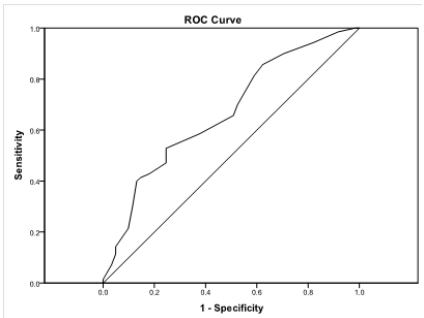
Altogether 91 patients had positive reactions in IgM ELISA (Table 1). These included 38 false positives. Of the 68 patients with negative results, 35 were false negatives. Sensitivity and specificity of IgM ELISA in this sample, as a point of care diagnostic test for patients with acute leptospirosis, was 60.23% (95% CI 49.78, 69.82) and 46.48% (95% CI (35.36, 57.96) respectively. Diagnostic accuracy of the test was 54.09% (95% CI 46.34, 61.65 %) (Table 2).

The ROC curve (figure 1) for the IgM ELISA showed a value of .669 for area under the curve.

Table 2: Test parameters of IgM ELISA test (based on manufacturers' cut off points)

Parameter	Estimate	95% CIs
Sensitivity	60.23%	(49.78, 69.82)
Specificity	46.48%	(35.36, 57.96)
Positive Predictive Value	58.24%	(47.98, 67.84)
Negative Predictive Value	48.53%	(37.05, 60.17)
Diagnostic Accuracy	54.09%	(46.34, 61.65)

Figure 1
Receiver-operator characteristic curve of the leptospira IgM ELISA versus paired sample MAT/qPCR



Discussion

The present study shows that the IgM ELISA has limited diagnostic value in diagnosing acute leptospirosis in high endemic districts of Sri Lanka. The low sensitivity of 60.2% misses a considerable proportion of leptospirosis cases during the early stage of the disease, while low specificity resulted in very high number of false positive results and overestimation of the disease. Low specificity could further affect patient care services by missing other important tropical conditions presenting as leptospirosis, such as dengue[14,15], Hantavirus infections[16,17], rickettsial disease[18,19] and malaria.[20] As previously shown in Sri Lanka, clinical diagnosis of leptospirosis has a low sensitivity and specificity.[21]

As specified by the manufacturer, IgM ELISA does not stand alone as a diagnostic test. It should always be combined with clinical criteria and could be used only among probable (not possible) cases. Nevertheless, IgM ELISA is being used as a confirmatory test in Sri Lanka due to lack of other diagnostic facilities. Independent studies among probable cases of leptospirosis in non-endemic settings have shown that in the acute phase of the disease, among patients with classical symptoms, IgM ELISA is more sensitive than MAT.[7] In those settings, the reported sensitivity was more than 87.5% and specificity more than 93%.[7,9] However, the findings of our study is compatible with studies done in Vietnam[8], Laos[10] and Thailand[22] where in endemic settings, IgM ELISA showed limited diagnostic utility as a rapid diagnostic test. The control sample was from the same endemic setting and we did not have a control from a non endemic setting, which is a limitation of this study.

One reason for the poor sensitivity of IgM ELISA observed in this sample could partly be due to too early testing. The median duration of fever for acute samples was seven days in this study. It is well known that the antibody response in leptospira infections could take up to two weeks[2] or even longer. Our findings are based only on acute samples, because our objective was to assess the validity of IgM ELISA as a point of care diagnostic test in acute leptospirosis. In high endemic settings, where the patients are already exposed to *Leptospira* early in their life, an early IgG response rather than an IgM response during an acute infection will not be detected by the IgM ELISA test. The most plausible explanation for low specificity would be the high background seropositivity. The districts selected for the present study are identified as high endemic districts for leptospirosis in Sri Lanka. Patients included in the comparison group might have had previous infections with *Leptospira* resulting in invalidating the cut off points for IgM ELISA in this setting. In Thailand, a recent study showed that higher cut off points are required in their setting compared to the suggested value by the manufacturers.[22] In addition, the validity of MAT which was used as the gold standard in this study has recently been challenged as an imperfect test, which could have affected the results of the present study.[23]

Our study strengthens the evidence against the poor utility of IgM ELISA as a point of care diagnostic test to confirm acute leptospirosis in high endemic areas. Newer methods are required to fill the gap in diagnostics for acute leptospirosis in resource poor settings. Recent studies on molecular diagnostic techniques have shown promising results[13], but this utility in resource poor settings has to be evaluated carefully. Evaluation of other commercially available screening/ diagnostic test for diagnosis of acute leptospirosis in endemic settings and further studies on cost effective, valid diagnostic tests are of utmost importance for control and prevention of leptospirosis worldwide.

Competing Interests

Authors have no conflict of interest. Leptospira IgM ELISA kits were provided without charge by Panbio Pty, Ltd., Queensland, Australia. The company had no role in the study design, analysis, interpretation of results, and writing or submission of this manuscript.

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Ethical approval

The protocol was reviewed and approved by the Ethical Review Board of the Faculty of Medicine, University of Peradeniya, Sri Lanka.

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