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RESEARCH ARTICLE

Outer membrane Protein based Latex Agglutination Test for diagnosis of canine leptospirosis in Kerala, India

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Abstract

Leptospirosis is an emerging zoonotic disease transmitted to human beings from a variety of animals including dogs. Kerala, a southern state of India is endemic for the disease. However, canine leptospirosis often goes undiagnosed due to lack of awareness of the disease, non specific clinical signs and also paucity of well equipped laboratories. In these circumstances, the development of a rapid diagnostic tool for this disease is inevitable. The study deals with the screening of canine leptospirosis using Latex Agglutination Test (LAT). Two hundred and eighty six canine serum samples were collected from suspected cases of acute leptospirosis. The LAT was carried out using the outer membrane proteins (OMP) extracted from Leptospira interrogans serovar Australis as antigen. The test results were compared with that of the gold standard test, Microscopic Agglutination Test (MAT). The LAT was found to be 96.48 % sensitive and 91.53 % specific compared to MAT in diagnosing leptospirosis. The advantages of LAT over MAT are that it is simple and rapid and can be employed as a field level diagnostic tool with much lesser technical expertise.
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Introduction

Leptospirosis, a zoonosis with ubiquitous distribution, is gaining importance as a re-emerging disease especially in developing countries like India causing threatening complications in human beings and animals. This was reported to be the most fatal communicable disease that created havoc to the public health system of the state of Kerala during last few years causing the highest case fatality rate in humans. The human beings usually acquire infection from the environment contaminated with the urine of carrier rodents. The infection is also transmitted to human beings from a variety of wild and domestic animals (Levett, 2001; Jobbins et al., 2014) including dogs (Ellis, 2010; Fontaine, 2013). Besides acting as the reservoirs of infection, the dogs may get severely affected (Goldstein, 2010). Complete cure is assured if treated promptly with appropriate antibiotics. However, the clinical manifestations are erratic and indistinguishable from other febrile illnesses which make the laboratory confirmation of the disease inevitable. The conventional methods of diagnosis like isolation and molecular techniques like Polymerase Chain reaction (PCR) although available, are either highly time consuming or can be performed only in well equipped laboratories (Levett, 2003). Microscopic Agglutination Test (MAT), the gold standard reference test is serogroup specific and requires paired sera collected at acute and convalescent phase of the disease for the confirmatory diagnosis which is almost impractical in routine veterinary diagnosis. Moreover the specificity of the test depends on the panel of serovars used as antigens, restricting its use to specialized laboratories. Thus, in an endemic area like Kerala (WHO, 2003), the need for rapid and appropriate diagnostic tests has become ever more urgent for clinical case identification, seroprevalence studies and to instigate suitable medical measures in the risk population. Various whole cell antigen based rapid serological tests described previously, were proved to have less efficiency due to the broadly reactive immunodominant moieties. The outer membrane proteins (OMP) conserved

among pathogenic leptospires can be used as antigens. The present study involves screening of canine serum samples for leptospirosis using OMP based latex agglutination test (LAT).

Materials and Methods

Samples

A total of 286 canine serum samples collected from clinical cases presented at District Veterinary Centres and Veterinary Hospitals of three different districts in central and North Kerala were used in the study. The samples included those collected from 30 healthy unvaccinated animals. The rest of the samples were taken from cases suspected for acute leptospirosis.

Microscopic agglutination test

The MAT was carried out as described previously (Faine et al., 1999) using reference strains of Leptospira interrogans serovars Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Javanica, Pomona and Pyrogenes and Leptospira biflexa serovar Patoc maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala. Reciprocal of agglutination titers greater than or equal to 100 were considered as positive reactions (OIE, 2008).

Outer membrane protein extraction

The OMP of L. interrogans serovar Australis strain Ballico was extracted using sarcosyl detergent as described by Nicholson and Prescott (1993). Protein estimation in extracted samples was carried out using the commercial protein estimation kit (Merck GeNei). The specificity of OMP was tested by immunoblotting with the serum from known cases of acute canine leptospirosis as per the method described by Doungchawee et al. (2008).

Latex agglutination test

The test was performed as per the techniques described by Smits et al. (2000), with relevant modifications. The latex beads (0.80µm diameter, Sigma Aldrich) were used coated with OMP and the test was performed by mixing 10µl of serum sample on a clean grease free glass slide with an equal volume of the sensitized latex beads. The serum samples were also mixed with the latex beads without antigen. The glass slide was then rotated gently against a light source for one minute. Samples were considered as negative, if no agglutination was observed within one minute. The relative sensitivity, specificity, accuracy and predictive values of LAT for the serodiagnosis of canine leptospirosis were evaluated in comparison to MAT as described in Veterinary Epidemiology by Thrusfield (2005).

Results

Among the 286 suspected samples, 227 were found to be MAT positive. Out of 227 MAT positive cases, 219 cases were diagnosed as positive and 8 cases as negative by LAT. The 30 healthy unvaccinated animals were found to be negative by both MAT and LAT. Among 59 MAT negative cases, 54 cases were shown as negative by LAT, but 5 cases were diagnosed as positive by LAT (Table 1). Statistical analysis was done with Fisher's exact test. The two-tailed P value was less than 0.0001. Therefore, the association between LAT and MAT was considered to be extremely statistically significant. The relative sensitivity, specificity, accuracy, likelihood ratios and predictive values of LAT for the serodiagnosis of canine leptospirosis were given in Table 1.

Table 1. Relative measures of diagnostic efficacy of LAT compared to MAT

	MAT	
LAT	Positive	Negative
Positive	219 (a)	5(b)
Negative	8(c)	54 (d)

Sensitivity=a/a+c x 100=96.48%
Specificity=d/b+d x 100=91.53%
Accuracy = a+d/ a+b+c+d x 100=95.45%
Positive Likelihood Ratio=Sensitivity/100-specificity=11.38
Negative Likelihood Ratio=100-sensitivity/specificity=0.04
Disease prevalence=a+c/a+b+c+d x 100=79.37%
Positive Predictive Value=a/a+b x 100=97.77 %
Negative Predictive Value=d/c+d=87.10 %

Discussion

Leptospirosis being an important zoonotic disease of global distribution requires serious attention of the researchers for achieving its effective diagnosis. Definitive diagnosis of leptospirosis is usually provided by culture of the infecting organism (Agudelo-Florez et al., 2009). However, the procedures are highly expensive and time consuming for routine use, because fresh samples are necessary and several months may be required for conclusive results (Rahim et al., 2005). Polymerase chain reaction assay is employed as an alternative for this and is considered as a sensitive and specific diagnostic tool providing significantly faster results (Obregon et al., 2004). However, whole blood PCR results are only positive early in infection and urinary shedding can be intermittent therefore, if leptospirosis is still suspected, MAT testing is the suitable method to be adopted, thus limiting its diagnostic utility in an endemic area.

The MAT is considered the standard serological test which is serovar-specific and provides useful epidemiological data. However, this assay is not suitable for routine laboratories since it is technically demanding, costly and requires the maintenance of live, hazardous stock cultures (Rodriguez et al., 2011). The test should be performed on paired sera collected during acute and convalescent stage of the disease to find out sero-conversion or four-fold rise in titre, which is the evidence of current infection. However, this is impractical in routine veterinary practice. Preferably, a diagnostic test should be easy to perform, rapid and employ only a single specimen from the clinical case presented. Therefore, current research on leptospirosis diagnosis is focused on to evolve a test which is cost effective, rapid and sensitive and preferably genus specific. Genus specific tests like ELISA (Adler and de la Pena Moctezuma, 2010) were tested and proved to be useful diagnostic approaches, but require costly equipment and technical expertise, making them unfit for routine field level diagnosis. The simplified versions of these tests like dot ELISA and dip stick ELISA (Pappas et al., 1985) require a minimum of two to three hours to interpret the result. Thus a rapid field level diagnostic tool is highly essential.

Latex agglutination test is an extremely simple diagnostic test which requires no sophisticated equipment and specific expertise (Levett et al., 2001). The antigen coated latex beads can be stored effectively at 4°C. In the present study, LAT was tested to obtain a sensitivity, specificity and accuracy of 96.48 %, 91.53 % and 95.45 % respectively compared to MAT. The positive predictive value (97.77 %) and negative predictive value (87.10 %) were found to be satisfactory. Hence, LAT could be proved as convenient screening test as reported in certain other works (Dey et al., 2007; Senthilkumar et al., 2007). Five MAT negative samples were diagnosed as positive by LAT, which could be explained by the fact that LAT is genus specific and suggests the possibility of presence of more infecting serovars prevalent in the region other than the nine serovars employed for MAT in the study.

Conclusion

In conclusion, the OMP based LAT mentioned in the study, would be a promising tool in diagnosing canine leptospirosis. This test enables faster treatment and optimal strategies to be applied, to control the spread of leptospirosis.

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