

# EVALUATION OF LATEX AGGLUTINATION TEST FOR SERODIAGNOSIS OF LEPTOSPIROSIS IN DOGS

T. M. A. Senthilkumar<sup>1</sup>, M. Subathra<sup>2</sup>, Stella Esther<sup>3</sup> and P. Ramadass<sup>4</sup>

Department of Animal Biotechnology  
Madras Veterinary College  
Chennai 600 007

## ABSTRACT

*A rapid semi-quantitative latex agglutination test (LAT) has been standardized for the detection of leptospiral antibodies in serum samples of dogs. The efficacy of the LAT was compared with the standard MAT. A total of 170 canine serum samples were analysed using LAT with 85.2% sensitivity and 89.1% specificity. Latex agglutination test is simple, rapid, uses stabilized components, and can be done with less expertise and inexpensive equipment. The diagnostic sensitivity (Dsn) and specificity (Dsp) are concordant with that of MAT, especially for samples collected during early infection.*

**Key Words :** Canine leptospirosis, Diagnosis, Latex agglutination test, Microscopic agglutination test.

## INTRODUCTION

Leptospirosis, caused by spirochetes belonging to the genus *Leptospira*, is a re-emerging worldwide zoonotic disease that affects virtually all mammals (Bharti *et al.*, 2003). The disease incidence is highest in dogs, cattle, pigs and horses leading to chronic tubulointerstitial nephritis, mastitis, uveitis, myocarditis and hemolysis (Levett, 2001). Organisms are shed primarily through urine, and transmission to animals and people occurs through urine contaminated water, soil and vegetation. Leptospirosis affects mostly people living in tropical countries who lack hospital facilities. The microscopic agglutination test (MAT) for leptospirosis antibodies (Cole *et al.*, 1973) has been used for the diagnosis for many years both in medical and veterinary fields (Faine *et al.*, 1999). It is simple, and requires little expensive equipment, apart from a dark field microscope, broadly differentiates between antibodies directed against different leptospiral serogroups and also requires knowledge of the local strains. Additionally, it is laborious and potentially hazardous to laboratory staff. Latex agglutination test

(LAT) is simple, rapid, uses stabilized components, and can be performed with less expertise or less expensive equipments and can be applied for large scale screening (Smits *et al.*, 2000). In the present study, a rapid, semi-quantitative LAT has been standardized using whole cell (WC) antigen for the detection of leptospiral antibodies in serum samples from dogs.

## MATERIALS AND METHODS

### Blood samples

Blood samples (n=165) were collected from clinically suspected dogs irrespective of sex, age, and breed, brought to the out patient ward of Madras Veterinary College Hospital, Chennai. The serum was separated by clarification at 500x g for 20 min, and stored at -20°C. Blood samples (n=5) from apparently healthy dogs served as negative controls.

### Leptospiral cultures

The leptospiral cultures were grown and maintained in liquid and semisolid EMJH medium. Five to eight day old cultures, having approximately  $2 \times 10^8$

<sup>1</sup>Associate Professor, <sup>2</sup>Senior Research Fellow, <sup>4</sup>Professor and Head, Department of Animal Biotechnology, Madras Veterinary College, Chennai-7. <sup>3</sup>Research Assistant, CARI, Andaman and Nicobar.

leptospire/ml were used in MAT. A panel of 8 leptospiral serovars viz., Australis, Autumnalis, Canicola, Javanica, Pomona, Icterohaemorrhagiae, Grippityphosa and Pyrogenes were included in MAT.

#### MICROSCOPIC AGGLUTINATION TEST (MAT)

Microscopic agglutination test was carried out as per Cole *et al.* (1973) with modifications. Initially, each serum sample was diluted to 1:50 and 100 µl was added in 8 wells of 96 well 'U' bottom plates and an equal volume of each culture in the panel were mixed with each serum sample. Phosphate buffered saline (PBS), pH 7.2 plus the culture was the antigen control. The plates were incubated at 37°C for 2 hr. Samples showing more than 50% agglutination were considered as positive. Positive serum samples were further subjected to quantitative MAT to determine the titre of the samples. Positive serum samples were serially diluted in PBS, pH 7.2 from 1:100 to 1:3,200 and an equal volume of the reacting leptospiral culture was added to all the wells and incubated for 2 hr at 37°C. Ten ml of the mixture of antigen and serum were placed on a clean, grease free glass slide and examined under dark field microscope. Reciprocal agglutination titres of 100 and above were considered as positive reactions.

#### ANTIGEN PREPARATION FOR LATEX AGGLUTINATION TEST (LAT)

*Leptospira interrogans* serovar Canicola was cultured at 28°C-30°C in EMJH liquid medium to log phase of growth (5-7 days), clarified at 10000x g for 30 min at 4°C, washed twice in PBS and finally resuspended in 0.06 M carbonate-bicarbonate buffer, pH 9.6. Then the cells were sonicated at constant pulse for 2 cycles of 30 sec each. Cellular debris was sedimented at 2000 x g for 10 min at 4°C. The supernatant was harvested and stored at -20°C. The protein concentration was measured spectrophotometrically at 280 nm.

#### SENSITIZATION OF LATEX BEADS

A 10% suspension of dyed latex particles

(0.8 µm dia, Sigma, USA) were coated with sonicated WC antigen (25 mg/ml) in 0.06 M carbonate-bicarbonate buffer, pH 9.6, kept at 37°C for 6 hr with constant shaking. The sensitized beads were centrifuged at 6,800x g for 3 min and the pellet was resuspended as a 1% suspension in PBS containing 5 mg/ml of bovine serum albumin (BSA). The latex beads were left at 37°C overnight with constant shaking. Then, the beads were centrifuged as before and the pellet was resuspended in PBS containing 0.5 mg/ml of BSA and 0.1% sodium azide. The sensitized latex beads were stored at 4°C until use. The LAT was performed on cavity glass slides (VDRL slides) by mixing equal volume of serum samples and sensitized beads (20 µl each). The slide was rotated briefly for mixing the reagent and the serum samples. The test was declared positive if agglutination occurred with formation of fine granular particles and tend to settle at the edge of the droplet within 2 min. A score of 3+ was given when agglutination was observed within 30 s, 2+ when it was observed between 30s to 1 min and 1+ when, it became visible between 1-2 min. Where as homogenous suspension was declared negative.

#### COMPARATIVE EFFICACY OF LAT WITH MAT

The relative diagnostic sensitivity (Dsn), diagnostic specificity (Dsp) and accuracy of LAT for the detection of leptospiral antibodies in dog serum samples with MAT were calculated according to Jacobson *et al.* (1998).

Sensitivity =  $a/(a+c) \times 100$ , where 'a' is the number of serum samples positive in both the tests and 'c' is the number of serum samples positive in MAT but negative in LAT.

Specificity =  $d/(b+d) \times 100$  where 'd' is the number of serum samples negative in both the tests and 'b' is the number of serum samples negative in MAT but positive in LAT.

$$\text{Accuracy} = a+d/(a+b+c+d) \times 100$$

## RESULTS AND DISCUSSION

Laboratory diagnosis of leptospirosis in humans and animals is done either by isolation of agent or demonstration of specific serum antibody. Whereas, the time needed to culture and identify the infective organism from culture of undoubted epidemiological importance permits only a retrospective diagnosis. For detection of specific antibody, the MAT is still the standard test. MAT results delay in establishing the cause of outbreaks, as reported elsewhere (Centre for Disease Control, 1997).

This is high time to develop a rapid, sensitive and specific diagnostic test that could be used for routine diagnosis of leptospirosis. In this study, a rapid, semi-quantitative Latex agglutination test (LAT) has been standardized using whole cell sonicated antigen for the detection of leptospiral antibodies in dog serum samples. The rapidity, simplicity and economics of the LAT were found to fulfill the requirements of a rapid screening test for leptospiral antibodies. A total of 170 canine serum samples were tested using MAT and LAT, in which 104 samples were positive in LAT. The sensitivity and specificity of the LAT is shown in Table 1.

The LAT has been proved a useful rapid test for serodiagnosis in epidemiological studies. It is simple, economical and could be carried out in most of the laboratories. It does not require sophisticated equipments and expertise. Hence, LAT could be used for the detection of leptospiral antibodies in lieu of MAT (Ramadass *et al.*, 1999). The present results are in agreement with the earlier reports (Smits *et al.*, 2000).

## REFERENCES

- Bharti, A. R., Nally, J.E., Ricardi, J.N., Matthias, M.A., Diaz, M., Lovett, M.A, Levett. P. N., Gilman, R.H., Willig, M.R., Gotuzzo, E. and Vinetz. J.M. (2003). Peru-United states Leptospirosis consortium, Leptospirosis: A zoonotic disease of global importance. *Lancet Infectious Disease*, 3: 757-771.
- Centers for Disease Control. (1997). Outbreak of leptospirosis among white-water rafters-Costa Rica, 1996. *Morbidity and Mortality Weekly Report*, 46: 577-579.
- Cole, J.R., Sulzer, C.R. and Pursell, A.R. (1973). Improved microtechnique for leptospiral Microscopic Agglutination Test. *Applied Microbiology*, 25: 976-980.
- Levett, P.N. (2000). Leptospirosis. *Clinical Microbiology Review*, 14: 296-326.
- Faine, S. (1982). Guidelines for the control of leptospirosis. World Health Organization. Geneva. pp. 76-79.
- Faine, S., Adler, B., Bolin, C. and Perolat, P. (1999). *Leptospira and Leptospirosis*, 2<sup>nd</sup> Ed. Medsci., Melbourne, Australia.
- Jacobson, R.H. (1998). Serological assays for diagnosis of infectious diseases. *Revue Scientifique et Office International des Epizooties*, 17: 469-486.
- Ramadass, P., Samuel, B. and Nachimuthu, K. (1999). A rapid latex agglutination test for detection of leptospiral antibodies. *Veterinary Microbiology*, 70: 137-140.
- Smits, H.L., Hartskeel, R.A. and Terpstra, W.J. (2000). International multi-centre evaluation of a dipstick assay for human leptospirosis. *Journal of Clinical Microbiology*, 38: 1272-1275.

**Table 1. Comparison of Latex agglutination test and microscopic agglutination test**

		MAT		Total
		Positive	Negative	
LAT	Positive	98 (a)	6 (b)	104
	Negative	17 (c)	49 (d)	66
Total		115	55	170

$$\chi^2 = 86.50^{**}$$

$$K = 0.70$$

Sensitivity: 85.22%; Specificity: 89.09%; Accuracy: 86.47%

\*\*Highly significant  $P \leq 0.01$