



Recombinant GroEL based latex agglutination test (GroEL-LAT) for the serodiagnosis of bovine leptospirosis

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Leptospirosis continues to be a universal foremost zoonotic disease caused by *Leptospira interrogans* group that affects multi-organ systems including kidney, liver and lungs (Vinetz 2001, Levett 2001). Leptospirosis is reported in human, sheep, goats, dogs, swine, horses, zebra fish, pigs, raccoons, rodents, equine, buffalo and cattle (Desvars *et al.* 2011). The cattle is one of the most important reservoirs for receiving infection and it can also transmit to human beings (Faine *et al.* 1999). Microscopic agglutination test (MAT) is the gold standard serological test for diagnosis but is difficult for the early and rapid diagnosis (Cumberland *et al.* 1999). Enzyme linked immunosorbent assay (ELISA) is more sensitive than the MAT but is expensive and time consuming (Ramadass *et al.* 1999). Commercial kits were found less sensitive and specific compared to MAT and ELISA during acute phase of leptospiral infection (Shegal *et al.* 1999, Vijayachari *et al.* 2002, Shegal *et al.* 2003). The polymerase chain reaction (PCR) also needs sophisticated laboratory facilities (Ooteman *et al.* 2006). In this study diagnostic capability of leptospiral recombinant GroEL protein as antigen in latex agglutination test was assessed.

A panel of 12 leptospiral reference strains was used which included the following serogroups: Australis (strain Jez-Bratislava), Autumnalis (strain Akiyami A), Ballum (strain Mus 127), Bataviae (strain Swart), Canicola (strain Hond Utrecht IV), Grippotyphosa (strain Moskva V), Icterohaemorrhagiae (strain Wijnberg), Javanica (strain Veldrat Batavia 46), Pomona (strain Pomona), Hebdomadis (strain Hebdomadis), Pyrogenes (strain Salinem) and Manhao (strain L60). The reference strains were obtained from Regional Medical Research Centre, ICMR, Port-Blair, Andaman Island and were maintained in Ellinghausen-McCullough-Johnson-Harris (EMJH, Difco-USA) containing

1% BSA with periodical subculture at the Department of Microbiology, Bharathidasan University.

Blood samples were collected from 121 dairy cattle suspected for leptospirosis attending the Government Veterinary Hospital, Tiruchirappalli. Sera were separated on the same day by centrifugation at 4,000rpm for 30 min and stored at –20°C until used. Blood samples were also collected from 32 apparently healthy cows maintained in the control farm. This study was approved by the Directorate of Animal Husbandry, Government of Tamilnadu (No. 77721/JJ1/09).

Microscopic agglutination test (MAT) was performed according to Faine (1982). One antigen control and two standard (positive and negative) serum controls were used. The recombinant GroEL protein produced in our laboratory (Natarajaseenivasan *et al.* 2011) was utilized. GroEL was used as antigen in ELISA and LAT. The test was performed by mixing equal volumes (10µl) of serum sample and recombinant GroEL sensitized latex particles. PBS and serum samples from healthy animals were used as negative controls (Ramadass *et al.* 1999, Smits *et al.* 2000).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of GroEL-IgG-ELISA and GroEL-LAT were calculated in comparison with MAT. Further the results obtained from the tests were analyzed for the percentage agreement with MAT by using the kappa statistics. A Kappa value greater than 0.81 is the indication for the perfect agreement of the tests.

During the study 121 bovine serum samples of clinically suspected bovines were subjected to MAT. Among them 87 samples were found positive (71.9%) with titres in the range of 1 in 80 to 1280 with a median titre of 1 in 80 (Table 1). Serovars Pomona 66 (75.8%), Pyrogenes 58 (66.6%), Canicola 56 (64.3%), Ballum 44 (50.5%) and Autumnalis 31 (35.6%) were amongst the most prevalent serovars observed among the dairy cattles as single and mixed equals. MAT results of control serum samples from healthy animals (n=32) were found seronegative (<1 in 20).

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Table 1. Microscopic agglutination test for the detection of seropositivity of bovine serum samples

	Total number of samples	MAT negative titre (<80)	MAT positive	
			No. of samples (%)	Titres (≥80) ^a
Suspected animals	121	34 (28.1%)	22 (24.7)	80
Healthy animals	32	32 (100%)	52 (58.4)	160
			13 (14.6)	320

^aMaximum reciprocal MAT titres.

Table 2. Latex agglutination test grading using recombinant GroEL protein in bovine positive serum samples

	Total number of samples	Recomb GroEL-LAT positive			
		Grade ^a			
		+1	+2	+3	+4
Suspected animals	121	24 (26.9) ^b	38(42.6)	22(24.7)	5(5.6)

^a+1, mild agglutination in 2 min; +2, moderate agglutination in 1.5 min; +3, heavy agglutination in 1 min; +4, very heavy agglutination in 30 sec. ^bValues in percentage.

A total of 88 serum samples (72.7%) were positive for IgG GroEL-ELISA. The mean (X) and the standard deviation (SD) of the OD of the serum samples of 32 healthy animals were 0.06 and 0.03 respectively. The mean +2SD (OD 0.12) was used as the cut-off OD for the recombinant GroEL-IgG ELISA. The comparison of MAT with GroEL IgG-ELISA sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and kappa value were 96.5%, 89.4%, 95.4%, 91.8%, 94.4% and 0.867, respectively (Table 3).

The latex agglutination test was done with leptospiral recombinant GroEL protein. Eighty-nine samples (73.5%) were positive by Recomb-GroEL-LAT out of 121 samples. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy and kappa value of LAT were 98.8%, 89.4%, 95.5%, 97.1%, 96.0% and 0.903, respectively (Table 3). The intensity of agglutination was graded to make semi-quantitative evaluation as 1+, 2+, 3+ and 4+, depending upon the intensity and speed of the agglutination reaction (Table 2). In this study MAT was used as standard test and compared with recombinant GroEL-IgG-ELISA and

recombinant GroEL-LAT. The recombinant LAT showed higher sensitivity and specificity in comparison to GroEL-IgG-ELISA and MAT.

Leptospirosis is an economically significant infectious disease hence its diagnosis and serosurveillance is very important for any control program (Jalii 2008). MAT helps to detect the specific serum antibodies (Theirmann 1983), and needs the maintenance of a large number of live *Leptospira* strains of locally circulating serovars as a source of antigens (Faine 1982). In addition to that it is very laborious to perform, time consuming and require sophisticated laboratory facilities with much expertise for reading the results (Vijayachari and Sehgal 2006). Culture in laboratory media like EMJH takes up to 2 months and does not provide an emergency diagnosis. ELISA used to be an alternate genus specific test in laboratories. But ELISA also needs the propagation of the live leptospires for the preparation of the antigen, and the sensitivity may be compromised during diagnosis (Mastsuo *et al.* 2000). These circumstances warrant for the development of conserved leptospiral protein based diagnostic formats to increase the sensitivity without much alteration in its specificity.

Especially the recombinant antigen based serological tests may show better sensitivity and specificity than that of other tests because of the purity of immunodominant antigen and lack of non-specific moieties present in whole cell preparations (Senthilkumar *et al.* 2010). Besides the developed test system must be robust in its performance with easy handling procedures for the rapid screening with less machinery even in the field. This type of formats will have more applicability even in field investigations during outbreak situations. For that reason in this study, the recombinant GroEL produced from *L. interrogans* serovar Autumnalis strain N2 (Natarajaseenivasan *et al.* 2011) was utilized in ELISA and LAT formats for the diagnosis of bovine leptospirosis in comparison with MAT. Both the GroEL-ELISA and the GroEL-LAT showed good sensitivity and specificity among the utilized bovine samples during the evaluation. Further the GroEL-LAT showed increased sensitivity of 2.3% over the GroEL-ELISA. Leptospiral GroEL was reported to be an immunoreactive antigen and it showed an overall sensitivity of 90.6% among human patients suspected for leptospirosis with various clinical manifestations (Natarajaseenivasan *et al.* 2011). In addition the results from the present investigation also have supplemented applicability of GroEL-LAT for routine

Table 3. Statistical analysis of comparison of GroEL ELISA and GroEL LAT with MAT for serodiagnosis bovine leptospirosis

Test	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%	Kappa value
GroEL-ELISA	96.5	89.4	95.4	91.8	94.4	0.867*
GroEL-LAT	98.8	89.4	95.5	97.1	96.0	0.903*

PPV, Positive predictive value; NPV, Negative predictive value. *Significant.

diagnosis of bovine leptospirosis apart from the human samples. In GroEL-LAT the results used to appear within a few minutes, so this test system may be widely applied in the field investigations as a preliminary genus specific screening test for the diagnosis of leptospirosis especially during outbreak situations. In tropical countries like India, leptospirosis outbreak is a common sequel and every year it was reported and so many places it is under reported. In Tamil Nadu \ an organized outbreak investigation was carried out among human and animals in Mepur Village near Chennai to establish leptospirosis (Ratnam *et al.* 1983).

In conclusion the present study was carried out to evaluate the recombinant GroEL-LAT for the diagnosis of bovine leptospirosis. The sensitivity, specificity, accuracy and kappa value were higher in comparison with the gold standard MAT. This test does not require sophisticated equipments for its performance. Therefore the outcome of this study suggested that recombinant GroEL antigen based LAT may be a better rapid and simple genus specific diagnostic method for the preliminary diagnosis of bovine leptospirosis.

SUMMARY

The recombinant GroEL antigen based latex agglutination test (GroEL-LAT) was evaluated for its efficacy for the serodiagnosis of bovine leptospirosis in comparison with the gold standard microscopic agglutination test (MAT). The overall results of the recombinant GroEL-ELISA showed sensitivity, specificity, positive predictive value, negative predictive value, accuracy and kappa values of 96.5%, 89.4%, 95.4%, 91.8%, 94.4%, and 0.867, respectively. The corresponding values for the assay based on recombinant GroEL-LAT were 98.8%, 89.4%, 95.5%, 97.1%, 96%, and 0.903, respectively. These findings suggested that recombinant GroEL based LAT could be a rapid genus specific diagnostic tool for the serodiagnosis of bovine leptospirosis especially during outbreak investigations.

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