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Detection of *Leptospira* Igm Antibody in Bovine Sera

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A total of 680 serum samples collected from both cattle (No. 544) and buffaloes (No. 136) in an organized farm were initially screened in a qualitative MAT using 12 strains of L interrogans and subsequently tested in quantitative MAT. Of which 26 sera (cattle and buffaloes inclusive) in various antibody titres were subjected to following treatments (inactivation at 560C for 30 minutes, inactivation at 65°C for 1h and treatment with 2 ME (Adler, 1965). The titres obtained after treatments and before treatments were compared. Of the 26 samples, 20 (76.92%) sera samples showed reduction in MAT titre after inactivation at 65°C for 1h and 23 (88.46%) sera samples after 2 ME treatment. The reduction in titres of 20 sera due to inactivation at the 65 °C and of 23 sera due to 2 ME treatment indicated the presence of IgM in the sera samples which suggests the recent (acute) infection. Hence this study would be useful to differentiate the acute infection of leptospirosis from the chronic case of leptospirosis.

Key words: Bovines, *Leptospira*, IgM antibody.

World Health Organisation / Food and Agricultural Organisation recognized Microscopic Agglutination Test as a means of Laboratory confirmation of Leptospirosis. Since MAT detects both IgM and IgG, it cannot differentiate the acute infection from chronic infection. To diagnose the recent infection, MAT needs paired sera samples which are not generally submitted for laboratory confirmation. In general, paired sera samples would be collected after 14th day of 1st sera collection. By the time of examination of second sera samples, the animal might have undergone treatment or it might have been under chronic phase shifted from acute state. Hence there is a need to develop a test which differentiates IgM from IgG. Earlier, differentiation of Brucella IgM and IgG antibody was attempted using inactivated serum at 65°C for 1hr and Mercapto ethanol treated serum (Mc Mohan, 1983 and Bejo, *et al.*, 2006). Further recommendations were made for rinderpest antibody detection also (Scott, *et al.*, 1986). Following this method, an attempt was made to differentiate IgM and IgG against Leptospirosis by treating the sera with 2 Mercaptoethanol (2 ME) as well as heat and were used in MAT.

MATERIALS AND METHODS

A total of 680 serum samples collected from both cattle (No. 544) and buffaloes (No. 136) in an organized farm were used in this study. All the serum samples were initially screened in a (australis, autumnalis, ballum, canicola, grippotyphosa, hardjo, hebdomadis, javanica, icterohaemorrhagiae, pomona, pyrogenes and tarassovi) as per OIE, 2004 at a final dilution of 1/100. Positive serum samples reacting to one or more serogroups / serovar were subsequently tested in quantitative MAT in doubling dilution from 50 - 51600. These specimens were stored at -30°C in Sanyo Biomedical deep freezer until further use. 26 sera (cattle and buffaloes inclusive) in various antibody titres were subjected to following treatments (inactivation at 56°C for 30 minutes,

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inactivation at 65°C for 1h and treatment with 2 ME (Adler, 1965). The titres obtained after treatments and before treatments were compared.

RESULTSAND DISCUSSION

Of the 26 samples, 20 (76.92%) sera samples showed reduction in MAT titre after inactivation at 65°C for 1h when compared with treatment at 56°C for ½ h. In 6 (23.08%) sera samples, the titres remained same in both heat treatments. Out of 26 sera samples, reduction in MAT titres was observed in 23 (88.46%) sera samples after 2 ME treatment when compared with treatment at 56°C. No significant reduction in titres was observed in 3 (11.54%) sera samples. The reduction in titres of 20 sera due to inactivation at the 65°C and of 23 sera due to 2 ME treatment indicated the presence of IgM which suggests the recent (acute) infection. The 2 ME removes the membrane immunoglobulins (IgM) alone by destroying the disulfide bonds linking the pentamer structure of the IgM and rendering it inactive. The absence of reduction in titres observed in 6 and 3 sera samples after inactivation at 65°C and 2 ME respectively indicates the presence of heat and 2 ME resistant antibodies (IgG) only which is suggestive of chronic infection. The reduction in the titres after treatments was due to the presence of IgM in the sera samples.

This preliminary study has not been previously described for detection of IgM antibody by MAT. Hence this study would be useful for the diagnosis of acute infection of leptospirosis which is timely need to institute the treatment of leptospirosis.

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