IJBAF, July, 2018, 6(7): 82-91

ISSN: 2320 – 0774

International Journal of Biotechnology and Allied Fields (IJBAF)

www.ijbaf.com

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EVALUATION OF ROMP28 PROTEIN BASED ELISA FOR SERO DIAGNOSIS OF BOVINE BRUCELLOSIS

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Received 18th Nov. 2017; Revised 25th Dec. 2017; Accepted 9th Jan. 2018; Available online 1st July 2018

ABSTRACT

Brucellosis, a zoonotic infection is recognized as an emerging public health disease that is endemic in most regions of the developing countries including India. It causes severe economic losses in the form of loss in productivity, abortion, repeat breeding in animals and loss of man days in human beings. In the absence of safe and effective isolation procedure, serological tests like complement fixation test (CFT), rose bengal plate test (RBT), standard tube agglutination test (STAT), milk ring test (MRT) and enzyme-linked immunosorbent assay (ELISA) are relied on for the clinical diagnosis of brucellosis. However, agglutination tests sometimes give false-positive results due to cross-reactions with other pathogenic organisms. There is need to have sensitive and specific diagnostic test. For this purpose the outer membrane proteins of Brucella spp. have been extensively studied for their immunogenicity and serodiagnostic applications. In the present study, cloning and expression of B. abortus Omp28 were accomplished by PCR amplification cloning into a prokaryotic expression system, and purification of a recombinant Omp28 protein. The immunogenicity of rOmp28 was confirmed by Western blot analysis with known Brucella positive bovine serum. On checkerboard titration, the optimum concentration of recombinant antigen which was able to differentiate both known positive and known negative serum was 100 ng per well and serum dilution was standardized, at 1:100 dilution of serum for further analysis. Two hundred seventy six sera from cattle and buffalo collected from different parts of the Uttar Pradesh state were tested by rose bengal plate test and Indirect ELISA against the recombinant Omp28 antigen and commercially available ELISA kit. In case of bovine serum O6p28-ELISA showed (21.13%), while commercial-ELISA and RPT showed (23.55%) and (21.38%), respectively. Concordance between Omp28-ELISA and commercial ELISA was slightly higher than concordance between Omp28-ELISA and RBPT. Kappa statistics between OMP28-ELISA and commercial ELISA showed almost perfect agreement, while RBPT and OMp28-ELISA showed substantial agreement. In conclusion, the recombinant Omp28 protein of Brucella