ABSTRACT

Brucellosis is one of the major bacterial zoonosis disease affecting both human and animals. In Indian scenario, this disease poses a major public health problem and hence a proper diagnosis is always necessary to combat this remerging zoonosis. At present, the available diagnostic tests for Brucella infection are based on serology (anti-LPS antibody detection) and PCR (polymerase chain reaction) based genome detection. Though, serology based antibody detection is most preferred test in field conditions, the main drawbacks of this system are poor specificity and sensitivity which leads to false positivity/negativity. Hence, looking on to these limitations, we choose to work on detection of diagnostics protein markers of Brucella abortus using highly specific and sensitive monoclonal antibodies. Based on the literature and our in-silico bioinformatic analysis of membrane proteins of B.abortus, we found that a particular class of outer membrane protein called “porin” was highly conserved among the genus of Brucella and the protein sequence had no homology with other gram negative bacteria. We started the work with two targets in mind. Ace of the aim was to produce highly specific and sensitive monoclonal antibodies to the porins of Brucella abortus and to assess the presence/availability of it as a diagnostic marker in sera of infected human/ bovine subjects using sandwich ELISA. The second objective was aimed to clone and express the recombinant form of the B.abortus porin and then to evaluate its utility in detection of anti-porin antibodies in sera based on indirect ELISA. Among the developed mAb, we found that (BAPM 2) was able to detect the Brucella porin as marker in both human and bovine infected sample and moreover it did not cross react with other bacteria outside the genus Brucella. Thus, our approach based on monoclonal antibody based detection of Brucella porin would serve as a rapid immunoassay for early diagnosis of Brucella infection in India. The recombinant porin used in our study methods was also useful in detecting anti-recombinant porin IgG and IgM antibodies in the infected human/bovine subjects. Thus, the recombinant porin based antibody detection could be an alternate tool towards the existing LPS (lipopolysaccharide) based ELISA methods for the detection of chronic Brucella infection in areas of high prevalence of cattle movement.