**LETTER TO THE EDITOR**
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# Immunogenicity Assessment of *Brucella mellitensis* HSP and TF Proteins by Immunized Rabbit Serum

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Bacteria of the genus *Brucella* are facultative intracellular pathogens which have developed the capacity to survive and multiply in professional and nonprofessional phagocytes. Due to drawbacks of live attenuated vaccines, much attention has been focused on screening *Brucella*-protective antigens as subunit vaccine candidates. In order to screen immunogenic candidate antigens for the development of a *Brucella* subunit vaccine, we cloned, expressed and purified Heat Shock Protein (HSP) and Trigger Factor (TF) from *Brucella melitensis*. These recombinant antigens were then evaluated by serum from a *B. mellitensis*-vaccinated rabbit using ELISA and Western blot. Our results showed that the immunized rabbit serum reacted with recombinant HSP and TF in ELISA and Western blot. These results may suggest that *B.melitensis* rTF and rHSP may serve as candidate subunit vaccine components for protection against the infection.

*Brucella* spp. are Gram-negative and facultative intracellular bacteria which cause brucellosis, a worldwide zoonotic disease causing abortion in domestic animals and Malta fever in humans.[1] *Brucella melitensis* can invade macrophage-monocyte lineage cells and replicate within the phagosomes by inhibiting phagosome-lysosome fusion.[2] In intracellular environment of macrophages, bacteria are subjected to hard conditions[3] thus, the identification of bacterial proteins essential for intracellular survival in this niche is critical in understanding the protective mechanisms and pathogenesis of the disease. Expression of heat shock proteins in this situation, causes bacteria to adapt not only to thermal but also to various other environmental stresses, and the accumulation of heat-shock proteins (HSPs) is thought to preserve bacterial cellular functions.[4] The heat shock protein is a small HSP (sHSP) (Accession No. 1197813). sHSPs are molecular chaperones that suppress protein aggregation and protect against cell stress.[5] Trigger Factor (TF) protein (Accession No. 1196780) is an ATP independent chaperone[6] and has also been reported to act as a protective antigen against *B.melitensis* infection.[7] In view of the immunological importance of HSP and TF, we decided to clone, express in *E. coli* and purify the HSP and TF proteins from *B. melitensis* and study the antibody response to this protein in sera from *B. melitensis*- vaccinated rabbit by ELISA and

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Western blot. The Gateway cloning system (Invitrogen, NY, USA) was used for cloning of genes. Primers used for synthesis of the two genes were designed (Table 1). Cloning procedure was described previously.[8] rHSP and rTF were successfully expressed in the insoluble and soluble fractions of *E. coli* cells. Purification of rHSP and rTF were done as described previously.[7] Purity was assessed by SDS-PAGE and Coomassie blue staining. Sera were obtained from a Newzealand White Rabbit before and after immunization with *B.melitensis Rev.1*. To study the recognition of recombinant proteins by immunized rabbit serum, Western blot and ELISA were used.

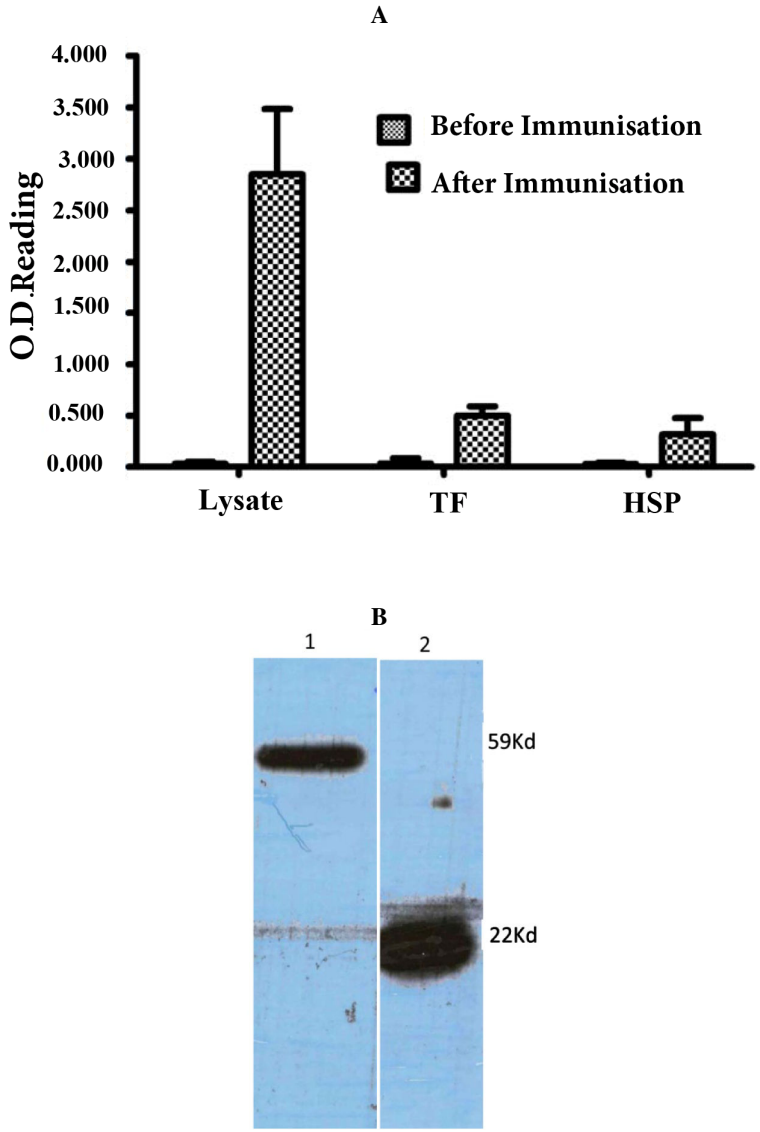


**Figure 1. Analysis of *B. mellitensis* recombinant proteins and lysate reactivity with immunized rabbit serum**
**A.ELISA analysis of immunized rabbit serum with *B. melitensis* lysate, rTF and rHSP by ELISA**
**B.Western blot analysis of immune reactivity of immunized rabbit serum with rTF (Lane 1) and rHSP (lane 2)**

**Table 1. Primers used for amplification of *hsp* and *tf* genes.**

| Gene | Forward | Reverse |
|---|---|---|
| *hsp* | 5´- CACC ATGCGTCACG TAGATTTTTCC | 5´- ATGCGCGGTCTTCGCCTCAATCG |
| *tf* | 5´- CACCATGACAAGAA G TGAAGG TTTGAAC | 5´- AAAAGCCTCTTCGGACTTGCCTTCTTC |

*B. melitensis Rev.1*, an attenuated smooth strain used to control *B. melitensis* infection, induces heterologous protection against other *Brucella spp.* and is currently considered as the best vaccine for the prophylaxis of caprine brucellosis.[9] However, due to different problems caused by administration of live attenuated vaccine, a subunit vaccine that is protective against *B. melitensis* is desirable.[10] The immunized rabbit serum reacted with both recombinant proteins in ELISA and Western blot that suggests the immunogenic nature of these subunits. These data are also confirmed by Yang et al. that immunization with rTF protein could protect mice against infection with *B. melitensis.*[7] To our knowledge, HSP from *B. melitensis* has not been reported as an immunogenic subunit of this organism. Our data may also suggest this protein as a vaccine candidate subunit. Much work is needed to be performed to establish this notion which is the theme of our future research.

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