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Development of an indirect ELISA based on the recombinant 28 kDa protein Omp28 from Brucella melitensis

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Introduction. Brucellosis remains an important zoonotic infectious disease causing economic losses all over the word. Ukraine is currently free of bovine and swine brucellosis. However, there is risk of recurrence due to the endemic situation in neighboring countries, and by spreading among wild animals. Therefore, there is the need for effective diagnostic tools such as enzyme-linked immunosorbent assay (ELISA) to perform brucellosis surveillance studies. The lipopolysaccharide (LPS) in the cell wall of *Brucella* is considered the strongest and most important antigen for the immune response during infection. To overcome the cross-reactive nature of LPS with other Gram negative bacteria, the outer membrane protein Omp28 of *B. melitensis* was chosen as the recombinant antigen for the development of an indirect ELISA (iELISA). *Brucella* outer membrane proteins (OMPs) have been evaluated as a non-LPS group of immunogens and have received major attention for their use in diagnostic systems for various zoonotic diseases, including brucellosis.
In the framework of the “Ukrainian-German Biosecurity Initiative for Risk Management of Zoonosis close to the European Border” an iELISA for *Brucella* -specific antibodies based on a recombinant antigen is under development.
Methods. The Omp28 protein was overexpressed in E. coli BL21 with an N-terminal Twin-Strep-tag, by cloning into pASG-IBA105 vector. A single step purification was done under native conditions. To ensure intracellular expression of the protein, the sequence coding for the signal peptide was removed. An optimal concentration of the obtained recombinant antigen for iELISA was established by chessboard titration using Nunc MaxiSorp 96-well plates (Thermo Scientific, USA). Coating of the plate with the antigen and titration were performed in carbonate buffer pH 9.5. For testing, a panel of sera from the Bundeswehr Institute of Microbiology was used. Brucella-positive and -negative human serum samples were titrated in the dilution buffer (10% skim milk powder and 1% rabbit serum in PBS containing 0.05% Tween 20) from 1:50 to 1:400. The antigen-antibody complex was detected by a secondary Ig-Poly HRP-coupled antibody (Sigma, USA). TMB SeramunBlau Slow Solution Substrate (Seramun Diagnostica, Germany) was used as substrate for the HRP.
Results. The recombinant protein Omp28 was tested with serum samples positive either against B. melitensis or B. suis for its efficacy as an antigen in an iELISA. By this Omp28 proved to be immunoreactive in the ELISA. No cross-reaction was detected with sera against *Francisella tularensis*.
Conclusions. The recombinant 28 kDa protein of *B. melitensis* Omp28 is validated as a potential antigen for the serodiagnosis of brucellosis. Preliminary results have shown that the immunoreactive Omp28 protein of B. melitensis can be highly expressed in an E. coli system and purified to near homogeneity in a single step. This lends itself to large-scale antigen production in a very short time and also avoids the biological risk involved in handling Brucella to produce LPS antigen. The next steps will be to test a larger number of brucellosis positive sera to address sensitivity as well as sera known to be positive for further known cross-reactive antibodies like *Yersinia* IgG to address specificity in more detail.