

Proteomic Analysis of Cytoplasmic Antigen from *Brucella canis* to Characterize Immunogenic Proteins Responded with Naturally Infected Dogs

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Abstract : Canine brucellosis is a critical problem in dogs leading to reproductive diseases which are mainly caused by *Brucella canis*. There are, nonetheless, not clear symptoms so that it may go unnoticed in most of the cases. Serodiagnosis for canine brucellosis has not been confirmed. Moreover, it has substantial difficulties due to broad cross-reactivity between the rough cell wall antigens of *B. canis* and heterospecific antibodies present in normal, uninfected dogs. Thus, this study was conducted to characterize the immunogenic proteins in cytoplasmic antigen (CPAg) of *B. canis*, which defined the antigenic sensitivity of the humoral antibody responses to *B. canis*-infected dogs. In analysis of *B. canis* CPAg, first, we extracted and purified the cytoplasmic proteins from cultured *B. canis* by hot-saline inactivation, ultrafiltration, sonication, and ultracentrifugation step by step according to the sonicated antigen extract method. For characterization of this antigen, we checked the sort and range of each protein on SDS-PAGE and verified the immunogenic proteins leading to reaction with antisera of *B. canis*-infected dogs. Selected immunodominant proteins were identified using MALDI-MS/MS. As a result, in an immunoproteomic assay, several polypeptides in CPAg on one or two-dimensional electrophoresis (DE) were specifically reacted to antisera from *B. canis*-infected dogs but not from non-infected dogs. The polypeptides with approximate 150, 80, 60, 52, 33, 26, 17, 15, 13, 11 kDa on 1-DE were dominantly recognized by antisera from *B. canis*-infected dogs. In the immunoblot profiles on 2-DE, ten immunodominant proteins in CPAg were detected with antisera of infected dogs between pI 3.5-6.5 at approximate 35 to 10 KDa, without any nonspecific reaction with sera in non-infected dogs. Ten immunodominant proteins identified by MALDI-MS/MS were identified as superoxide dismutase, bacteroferritin, amino acid ABC transporter substrate-binding protein, extracellular solute-binding protein family3, transaldolase, 26kDa periplasmic immunogenic protein, Rhizopine-binding protein, enoyl-CoA hydratase, arginase and type1 glyceraldehyde-3-phosphate dehydrogenase. Most of these proteins were determined by their cytoplasmic or periplasmic localization with metabolism and transporter functions. Consequently, this study discovered and identified the prominent immunogenic proteins in *B. canis* CPAg, highlighting that those antigenic proteins may accomplish a specific serodiagnosis for canine brucellosis. Furthermore, we will evaluate those immunodominant proteins for applying to the advanced diagnostic methods with high specificity and accuracy.

Keywords : *Brucella canis*, Canine brucellosis, cytoplasmic antigen, immunogenic proteins

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