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Characterization of the 48 kDa *Babesia caballi* Merozoite Antigen

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Equine babesiosis, caused by *Babesia equi* and *B. caballi*, is widely distributed in tropical and subtropical regions of the world and results in the great economic losses. Since aerological methods such as complement fixation test and enzyme-linked immunosorbent assay have problems in specificity and sensitivity, there, is need to improve these methods to detect antibodies in equine babesiosis. The aim of present study in to produce monoclonal antibodies (mAb) against *B. caballi* and to isolate species specific antigen using mAb, and to characterize the parasite gene encoding specific antigen for expression of *B. caballi* recombinant protein.

Nine mAbs were produced and showed the different fluorescent staining patterns determined by indirect immunofluorescent antibody test. mAbs, BC265C, BC253B, BC142B, BC202A and BC223B recognized antigens with molecular weight of 18-, 20-, 34-, 36- and 200-kDa, respectively. BC11D, BC21D, BC233D and BC255C reacted with antigen with molecular weight of 48 kDa and these four mAbs were used for immunoscreening of a cDNA expression library. Total RNA was prepared from *B. caballi*-infected horse erythrocytes cultured in vitro by acid guanidinium-phenol chloroform extraction and polyadenylated RNA purified by oligotex-dT 30. The cDNA was synthesized using Zap-cDNA synthesized kit and ligated to λ Zap II phage expression vector and packaged using Gigapack III packaging system. The cDNA library was screened with mAbs recognizing 48 kDa antigen. Recombinant phage (pBC48) was collected for pBluescript clone by in vivo excision. The sequence of insert contains 1,374 bp of open reading frame (ORF). This ORF encodes a protein consisting of 458 amino acid residues with a predicted molecular weight of 52-kDa. The expression of pBC48 gene by recombinant baculovirus in insect cells is now under investigation on potential candidates for diagnostic antigens of aerological tests to *B. caballi* infection.

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