
Title: Molecular characterization and antigenic properties of a novel Babesia gibsoni glutamic acid-rich protein (BgGARP).

Abstract: Identification and molecular characterization of Babesia gibsoni proteins with potential antigenic properties are crucial for the development and validation of the serodiagnostic method. In this study, we isolated a cDNA clone encoding a novel B. gibsoni 76-kDa protein by immunoscreening of the parasite cDNA library. Computer analysis revealed that the protein presents a glutamic acid-rich region in the C-terminal. Therefore, the protein was designated as B. gibsoni glutamic acid-rich protein (BgGARP). A Blastp analysis of a translated BgGARP polypeptide demonstrated that the peptide shared a significant homology with a 200-kDa protein of Babesia bigemina and Babesia bovis. A truncated BgGARP cDNA (BgGARPt) encoding a predicted 13-kDa peptide was expressed in Escherichia coli (E. coli), and mouse antisera against the recombinant protein were used to characterize a corresponding native protein. The antiserum against recombinant BgGARPt (rBgGARPt) recognized a 140-kDa protein in the lysate of infected erythrocytes, which was detectable in the cytoplasm of the parasites by confocal microscopic observation. In addition, the specificity and sensitivity of enzyme-linked immunosorbent assay (ELISA) with rBgGARPt were evaluated using B. gibsoni-infected dog sera and specific pathogen-free (SPF) dog sera. Moreover, 107 serum samples from dogs clinically diagnosed with babesiosis were examined using ELISA with rBgGARPt. The results showed that 86 (80.4%) samples were positive by rBgGARPt-ELISA, which was comparable to IFAT and PCR as reference test. Taken together, these results demonstrate that BgGARP is a suitable serodiagnostic antigen for detecting antibodies against B. gibsoni in dogs.