

## **A novel 57-kDa merozoite protein of *Babesia gibsoni* is a prospective antigen for diagnosis and serosurvey of canine babesiosis by enzyme-linked immunosorbent assay**

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### **Abstract**

We isolated a novel single copy gene encoding a 57-kDa merozoite protein of *Babesia gibsoni* (BgP57). The nucleotide sequence of the cDNA was 2387 bp with an open reading frame (ORF) of 1644 bp encoding a 57-kDa predicted polypeptide having 547 amino acid residues. The recombinant BgP57 (rBgP57) without a predicted signal peptide was expressed in *Escherichia coli* as a soluble glutathione S-transferase (GST) fusion protein. Western blotting showed that the corresponding native protein was 57-kDa, consistent with molecular weight of predicted mature polypeptide. An indirect enzyme-linked immunosorbent assay (ELISA) using the rBgP57 detected specific antibodies in the sequential sera from a dog experimentally infected with *B. gibsoni*. Moreover, the antigen did not cross-react with antibodies to *B. canis* sub-species and closely related apicomplexan parasites indicating that the rBgP57 was a specific antigen for *B. gibsoni* antibodies. The diagnostic performance of ELISA based on rBgP57 using 107 sera from *B. gibsoni*-naturally infected dogs was the same as the previously identified rBgP32 but performed better than the previously studied rBgP50. Although, seminested peR detected higher proportions (82%) of positive samples than the ELISAs, the McNemar's chi-square test showed that there was no significant difference in relative effectiveness of rBgP57-ELISA and seminested peR ( $\chi^2 = 2.70$ ;  $P = 0.1003$ ) in identifying positive samples. The rBgP57-ELISA when used in combination with rBgP32-ELISA and rBgP50-ELISA appeared to improve sensitivity of the rBgP57-ELISA for detection of *B. gibsoni* antibodies. Overall, the rBgP57-ELISA and seminested peR when used in combination, could improve epidemiological surveys and clinical diagnosis of *B. gibsoni* infection.