Conformational dependence of an immunodominant epitope within the *Babesia equi* erythrocyte-stage surface protein EMA-1

C. W. Cunha [1,2*], L. S. Kappmeyer[3], T. C. McGuire[1], O. A. Dellagostin[2], D. P. Knowles [1,3]

[1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [2]Center for Biotechnology, Federal University of Pelotas, Brazil; [3]USDA/ARS, Animal Disease Research Unit, Pullman, WA

Equi merozoite antigen 1 (EMA-1) is a *Babesia equi* erythrocyte-stage protein that contains an immunodominant epitope which is defined by monoclonal antibody (mAb) 36/133.97. A competitive enzyme-linked immunosorbent assay (cELISA), based on inhibition of mAb 36/133.97 binding to recombinant EMA-1 (rEMA-1) by equine anti-*B. equi* antibodies, has been developed. Using this assay, persistently infected horses from 19 countries tested have antibodies that inhibit mAb 36/133.97 binding. The objectives of this work were to define the epitope recognized by mAb 36/133.97 and to determine the sequence conservation of this EMA-1 epitope. Full-length and truncated EMA-1 recombinant proteins were expressed and tested for binding to mAb 36/133.97. Binding required the presence of amino acids on both N- and C-terminal regions of the truncated protein EMA-1.2, containing amino acids 1-98. This result indicated the epitope defined by mAb 36/133.97 was conformational. Sera from persistently infected horses and those immunized with rEMA-1 bound full length and truncated EMA-1 proteins in Western blot and ELISA. However, sera from immunized horses inhibited binding more effectively, suggesting that infected and rEMA-1 immunized horses recognize different epitopes within EMA-1 and EMA-1.2 molecules. A comparison of the deduced amino acid sequence of full-length EMA-1 (Florida strain) and other published sequences from geographically distinct isolates, had 82.8-99.6% (median: 98.5%) identity and 90.5-99.6% (median: 98.9%) similarity. When the first 98 amino acids (truncated EMA-1.2 protein) containing the epitope defined by mAb 36/133.97 were compared, the sequences had 85.7-100.0% (median: 99.0%) identity with similarities of 94.9-100.0% (median: 100.0%). These results showed the conformational dependence of the epitope bound by mAb 36.133/97 and quantified the sequence conservation of the region containing this epitope among *B. equi* isolates.