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Cloning and sequencing of the rap-1α1 gene from Mexican isolates of *Babesia bigemina*

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The Babesia bigemina RAP-1 antigen is a 58 kDa protein located in the merozoite roptries of the protozoan parasite, which participates in the invasion to the bovine erythrocyte. rap-1 sequences of B. *bigemina* isolates from Mexico, Brazil, Argentina, Uruguay and Puerto Rico are known. However, the degree of conservation of rap-1 sequence in different geographic isolates of Mexico is not known. The aim of this study was to analyze rap-1α1 sequences in 8 different isolates of B. *bigemina* from Mexico. The methodology included the extraction of genomic DNA from B. *bigemina*-infected erythrocytes; PCR amplification of rap-1α1 using rap-1F and rap-1R as primers; Cloning the amplified fragment (1440 bp) into TOPO 10 vector and transforming competent E. coli cells; Selection of clones and purification of recombinant plasmids; Sequencing and analysis of rap-1α1 from the B. *bigemina* isolates, obtained from at least 3 recombinant clones per isolate, sequencing the inserts in both directions; and sequence alignments and assembling with the CLC application genomic workbench 4.8. The identity of the *rap-1α1* sequences was determined by a homology search with BLAST tools. The results showed amplification of rap-1α1 in all the B. *bigemina* isolates. The comparative analysis of nucleotide sequences revealed a high degree of conservation (98-99% identity) between the *rap-1α1* genes of the different Mexican isolates, compared to the reference *rap-1α1* sequence. BLASTX analysis revealed identities up to 99% in the deduced amino acid sequences of RAP-1 compared to the sequence of the reference isolate. The high degree of conservation in rap-1α1 sequences among geographically distant Mexican isolates suggests that there is no strong bovine immune pressure that translates into genetic variation of this particular gene. The RAP-1 antigen is a viable candidate for inclusion in a diagnostic test for bovine babesiosis caused by *B. bigemina* in Mexico.

Biography

Julio V Figueroa is currently working as a researcher and head of the National Research Center for Veterinary Parasitology, INIFAP, in Jiutepec, Morelos, Mexico. He obtained his Veterinary Medicine Degree from the State of Mexico Autonomous University in Toluca, Mexico, and the MSc and PhD degrees in Veterinary Pathology and Microbiology at the University of Columbia-Missouri, in Columbia, MO, USA. He has conducted research on tick borne diseases of cattle during the past 30 years and has published over 70 research papers in peer reviewed international journals.

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