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Cloning, sequencing and antigenic characterization of rVirB9 of *Anaplasma marginale* isolated from Paraná State, Brazil

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ABSTRACT. *Anaplasma marginale*, a tick-borne bacterium, causes bovine anaplasmosis responsible for significant economic losses in tropical and subtropical regions worldwide. Various major outer membranes have been described, and VirB9, a type IV secretion system protein, has been recently indicated as a candidate in vaccine development against anaplasmosis. The *virB*9 gene of an *A. marginale* strain isolated in Paraná, Brazil, was cloned by polymerase chain reaction and sequenced; its cloning into the pETSUMO vector produced a *virB*9-SUMO-6X His fusion gene construct. This recombinant clone was over-expressed in *Escherichia coli* BL21 (DE3), and the expressed fusion protein was solubilized with urea and purified with an Ni-NTA column. This method produced a relatively high yield of rVirB9. The deduced amino acid sequence encoded by VirB9 showed 99% homology to *A. marginale* isolates from St. Maries. rVirB9 was recognized by serum from cattle immunized with PR1 strain and by bovine sera infected with heterologous strains, showing that rVirB9 has conserved epitopes, which suggests that rVirB9 could be useful for the development of a vaccine against anaplasmosis.

Key words: *Anaplasma marginale*; Bovine anaplasmosis; Sequence of VirB9

