INHIBITION PROFILE OF A *Rhipicephalus appendiculatus* CYSTATIN

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**INTRODUCTION AND OBJECTIVES:** Tick cystatins are cysteine peptidase inhibitors, that regulate the activity of cathepsins involved in host immunologic system as well as parasite blood feeding process. The characterization of cystatins from different tick species is important to understand the physiological role of these inhibitors and to help the development of new tick control strategies. *Rhipicephalus appendiculatus* is a tick responsible for major drawback in livestock in African countries. Nevertheless, the role of its cystatins is unknown. With the goal to improve the understanding of cystatins role in the physiology of this tick specie, we analyzed the amino acid sequence of the *R. appendiculatus* cystatin QnRacys2a, expressed and purified the recombinant form (rQnRacys2a), and characterized its inhibitory profile.

**MATERIALS AND METHODS:** The QnRacys2a protein sequence was aligned with cystatin sequences from various tick species. The expression of rQnRacys2a was performed using *Escherichia coli* BL21(DE3)pLysS strain. The protein was purified by affinity chromatography and rQnRacys2a inhibitory profile was characterized using commercial bovine cathepsin C.

**RESULTS AND CONCLUSIONS:** The three conserved motifs of cystatins, (QXVXG, PW and a glycine in the N terminal region), were identified in QnRacys2a sequence. QnRacys2a amino acid sequence presented identity levels between 30% and 91% with tick cystatins, suggesting the potential use of rQnRacys2a as vaccine antigen against other tick species. rQnRacys2a was able to inhibit cathepsin C with a Ki of 90.9nM +/- 15.7nM. Cathepsin C inhibition suggests that QnRacys2a is involved in the final steps of hemoglobin digestion. Inhibitions assays with cathepsins B, L and G are in progress. In addition, conditions for large-scale purification of rQnRacys2a are being established to obtain protein for immunogenicity characterizations and for vaccination trials.

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