Heparin-Binding Proteins (HBPs) of Infestant Larvae from the Cattle Tick
*Rhipicephalus (Boophilus) microplus*

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**INTRODUCTION:** *Rhipicephalus (Boophilus) microplus* is an obligate hematophagous parasite and vector of several infectious agents to cattle. Heparin is a sulfated glycosaminoglycan produced by mast cells and has role in blood clothing, cytokine release, proteolysis control and angiogenesis. All heparin functions rely on its binding to specific proteins. This work aims to identify heparin-binding proteins (HBPs) in this parasite.

**MATERIAL AND METHODS:** 10-days old larvae homogenates were prepared and the HBPs were purified by affinity (HiTrap Heparin) using a step-wise elution gradient of NaCl (0 – 2 M). Eluted proteins were monitored at 280 nm, the chromatogram was recorded and each fraction analyzed by SDS-PAGE. Proteins were excised from the gel, reduced with DTT, alkylated with iodoacetamide, digested by trypsin and the peptides were analyzed by LC-MS/MS (ESI-Q-TOF). Spectra generated were searched against the RmINCT-EM database using Mascot server (fragment mass tolerance 0.6 Da, ion charged +2, cleavage set for trypsin, carbamidomethylated cysteine and oxidized methionine selected as peptide modification). Functional annotation of each identified protein was based on GeneOntology (GO) classifications.

**RESULTS AND DISCUSSION:** LC-MS/MS analysis identified about 100 HBPs, including: (i) endopeptidases as cathepsin D-like, cathepsin L-like, tick-heme aspartic protease (THAP), legumain-like and serine-carboxipeptidase; (ii) endopeptidase inhibitors belonging to the serpin and Kunitz-type family; (iii) other proteins like heat shock proteins (HSPs), lipocalin, tumoral necrosis factor (TNF) and mucins. The GO annotation reveals that the most of the identified proteins have binding (42%) and catalytic (34%) activities.

**CONCLUSION:** This approach allowed the identification of HBPs in tick larvae. As in mammals, the inhibitory activity of these inhibitors is regulated by heparin it is feasible that these tick endopeptidase inhibitors also are regulated by heparin. The function of most of the identified proteins still remains elusive. The biochemical characterization of this interaction will allow new insights in heparin-protein relationship.

**Key-words:** heparin-binding proteins, *R. microplus*, LC-MS/MS

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