

RESUMO - PROTOZOÁRIOS E AGENTES TRANSMITIDOS POR VETORES
DE IMPORTÂNCIA EM SAÚDE

**ANALYSIS OF THE IMMUNOMODULATORY ROLE OF STOMOXYS
CALCITRANS SALIVA**

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Stomoxys calcitrans, known as stable flies, are hematophagous insects of the Diptera order. Their bites cause significant pain and blood loss in hosts, primarily cattle and horses, leading to substantial weight loss and reduced beef and dairy production when occurring in large numbers, resulting in significant economic impacts on livestock activities. During blood feeding, the insect can mechanically transmit various pathogens to the host, causing further losses. The saliva of hematophagous insects contains pharmacologically active molecules that facilitate feeding and affects pathogen infection through immunomodulatory effects on their hosts. This immunomodulatory potential can affect various immune cells, including macrophages, which are innate immunity cells capable of secreting large quantities of cytokines and pro-inflammatory mediators. The *Herpetomonas* genus is a monophyletic group with representatives on nearly all

continents. They infect exclusively insects of different classes, such as Diptera, Hemiptera, Hymenoptera, and Siphonaptera, with potential use as biological control agents for their hosts. Recently, the *Herpetomonas muscarum* was isolated from *S. calcitrans* for the first time by our group. Leishmaniasis comprises a group of diseases with four distinct clinical manifestations, depending on the parasite species (cutaneous tegumentary, diffuse cutaneous tegumentary, mucocutaneous tegumentary, and visceral). To this day, this group of diseases remains on the World Health Organization's (WHO) list of neglected tropical diseases. Brazil, where leishmaniasis has a high incidence, ranks among the top 10 countries with the highest number of cutaneous leishmaniasis cases and among the top 6 countries with the highest visceral leishmaniasis incidence worldwide. Visceral leishmaniasis, also known as kala-azar, among other regional names, is a systemic and chronic disease. In Brazil, it is caused by *Leishmania infantum* and transmitted by phlebotomine insect vectors. Visceral leishmaniasis is a zoonotic disease affecting various animal species, such as marsupials, rodents, edentates, and canines, with dogs being the primary reservoir and source of human infection in urban areas. The present study aims to analyze the effect of *S. calcitrans* saliva on *Leishmania infantum* and *Herpetomonas muscarum* promastigotes growth, as well as evaluate the cytotoxicity of the saliva on RAW 264.7 murine macrophage lineage, once macrophages are the host cells of *L. infantum*. One thousand pairs of *S. calcitrans* salivary glands were dissected, sonicated to obtain salivary glands homogenates (SGH), centrifuged and stored at -80°C. The protein concentration of the supernatant was calculated. For determining the effect on the protozoa, *L. infantum* and *H. muscaum* promastigotes at the logarithmic phase of growth were plated in 96-well plate and treated with different concentrations (20 – 100 µg/ well) of SGH for 48h at 27°C. The protozoa were then submitted to XTT assay that reveals the activity of a mitochondrial dehydrogenase and correlates with the amount of metabolic active cells. For macrophages, the same assay was performed, however these cells were incubated at 37°C with 5% CO₂. We also intend to elucidate the possible mechanisms of action of SGH on macrophages through nitric oxide and cytokines production and evaluate the parasitic load of *L. infantum*-infected macrophages in the presence and absence of fly saliva. Preliminary results have

shown that the *S. calcitrans* saliva has no cytotoxic potential against macrophages. When evaluating its effect directly on *L. infantum* and *H. muscarum* promastigotes *H. muscarum* proliferation was increased and *L. infantum* proliferation was reduced when compared to untreated controls. The infection of macrophages with *L. infantum* will soon be performed to evaluate the remaining parameters. The saliva of *S. calcitrans* plays an important role in *L. infantum* infectivity and possibly a crucial role in establishing *H. muscarum* in the fly.

Palavras-chave: *Stomoxys calcitrans*; immune system; macrophages; *Leishmania infantum*; *Herpetomonas muscarum*.