

THE SPREADING PROCESS OF *Ehrlichia canis* IN MACROPHAGES IS DEPENDENT ON ACTIN CYTOSKELETON AND IRON AND CALCIUM INFLUX

(*O PROCESSO DE PROPAGAÇÃO DE Ehrlichia canis EM MACRÓFAGOS É DEPENDENTE DO CITOESQUELETO DE ACTINA E DO INFLUXO DE CÁLCIO E FERRO*)

M. A. LEVENHAGEN^{1*}, R. N. ALVES¹, M. M. M. D. LEVENHAGEN¹, S. E. RIECK², M. B. LABRUNA³, M. E. BELETTI¹

Erliquioses are diseases of medical and veterinary importance, caused by a Gram-negative α -proteobacteria that belongs to the order Rickettsiales. In Brazil, the main causative agent of canine monocytic ehrlichiosis (CME) is *Ehrlichia canis*, obligate intracellular pathogen with tropism for monocytes and macrophages. This worldwide distributed microorganism is most commonly found in tropical and subtropical regions due to the geographical distribution of its main vector, the tick *Rhipicephalus sanguineus*. The invasion process already described for some bacteria of this genus, such as *E. muris* and *E. chaffeensis*, comprises four stages: adhesion, internalization, intracellular proliferation and intercellular propagation. However, little is known about the cellular mechanisms involved in the invasion process of the target cells by *Ehrlichia canis*. The objective of this study was to analyze the role of actin cytoskeleton, calcium and iron on the spreading process of *E. Canis*, São Paulo strain, in canine macrophage (DH82), in vitro. For each of these cellular components, different inhibitory drugs were used: cytochalasin D (inhibits the polymerisation of actin filaments), verapamil (a calcium channel blocker), both incubated for 3h, and deferoxamine (chelating iron), incubated for 24 hours. After the incubation time of each drug, the infected cells were washed and maintained in culture for four days in 5% CO₂ incubator at 37°C. After this time, semi-quantitative analyses already described, based on the size of morulae were performed to determine the rate of infectivity of treated infected cells, compared to untreated. The results were considered significant if p<0.05 (unpaired 2-tailed Student's t –test). The results showed a significant decrease in the total number of bacteria in infected cells treated with all drugs compared to control, suggesting that these components are essential elements for cell propagation of *Ehrlichia canis* in macrophages in vitro. The elucidation of these important mechanisms in cell propagation of the bacteria under study is the basis for the development of new therapeutic strategies.