## THE MAINTENANCE OF *Ehrlichia canis* IN MACROPHAGES IS DEPENDENT ON LYSOSOMAL EVASION)

(A MANUTENÇÃO DE Ehrlichia canis EM MACRÓFAGOS É DEPENDENTE DA EVASÃO LISOSSOMAL)

## R. N. ALVES<sup>1</sup>\*, M. A. LEVENHAGEN<sup>1</sup>, M. M. M. D. LEVENHAGEN<sup>1</sup>, S. E. RIECK<sup>2</sup>, M. B. LABRUNA<sup>3</sup>, M. E. BELETTI<sup>1</sup>

Ehrlichia canis is the etiologic agent of canine monocytic ehrlichiosis (CME), one of the most important infectious diseases of dogs in Brazil. Obligate intracellular bacteria of monocytes and macrophages like E. canis develop various cellular mechanisms to avoid host defenses, which involve different adaptations for survival in intracellular compartments. However, studies about these strategies are scarce. Thus, the present study aimed to investigate whether *E. canis* has the ability to prevent the fusion of lysosomes to cytoplasmic inclusions, which would favor its multiplication. The fusion of lysosomes with endosomes containing E. canis was investigated using cell culture of canine histiocytes (DH82) infected with the São Paulo strain of E. canis when 50% of the cells were parasitized. To this end, infected cells were cytochemistrically labeled for acid phosphatase, an enzyme present in the fused lysosome compartments. After resin embedding process, the ultrathin sections were analyzed and photographically documented in a transmission electron microscope (TEM). We compared three control pairs and experimental groups in relation to the number of bacteria per cell, number lysosomes marked per cell and the number of labeled vacuoles containing E. canis per cell. Statistical analysis was performed using the Mann-Whitney test, at p<0.05 significance level. The cytochemical staining of the lysosomal enzyme was not observed in vacuoles containing intact E. canis indicating that these bacteria are able to inhibit lysosomal fusion to endosomes, as a mechanism of immune evasion. The evasion of lysosomal fusion by erlichia inclusions is critical to the survival and reproduction of the pathogen, despite being based on different strategies according to the species of Ehrlichia spp. Demonstration of lysosomal escape during the propagation of E. canis serves as a basis for the development and design of new therapeutic strategies.