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

GENETIC PROFILE OF EHRLICHIA CANIS ISOLATES FROM THE STATE OF RIO DE JANEIRO BASED ON P28 GENE ANALYSIS

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Canine ehrlichiosis, caused by the bacterium Ehrlichia canis, remains one of the most important and prevalent hemoparasitic diseases that affect domestic dogs in tropical and subtropical regions worldwide. E. canis is an obligate intracellular organism belonging to the family Anaplasmataceae, and it is recognized for its strong tropism for monocytes and its capacity to modulate the immune response of the host during infection. Due to its clinical and epidemiological relevance, studies involving the isolation, cultivation, and molecular

characterization of circulating strains of this pathogen are essential for understanding the dynamics of infection, parasite–vector interactions, and the extent of genetic variability among isolates from different geographical regions. The objective of the present study was to conduct the molecular characterization of E. canis isolates using the p28 gene as the molecular target. All experiments were carried out at the Laboratory for the Study of Parasite–Host Interaction (LEIPH) at UFRRJ. The isolates named “E.canisPretinha” and “E.canisScooby” were previously obtained from naturally infected dogs in the state of Rio de Janeiro, Brazil, and maintained in cryopreservation in SPG medium at −196 °C. After thawing the samples in a 37 °C water bath, the isolates were inoculated into tick cells (ISE6 lineage) maintained in 25 cm² culture flasks containing L15B medium supplemented with Hepes and without antibiotics. The cultures were incubated at 34 °C, and the medium was replaced twice a week. Infection progression was monitored by inverted light microscopy and by cytological examination of infected cells processed by cytocentrifugation and stained with Giemsa. Throughout the infection, distinct developmental phases of the pathogen were observed inside host cells, including structures compatible with morulae and reticulate forms, consistent with the intracellular cycle previously described for E. canis isolates maintained in ISE6 cells. For initial diagnosis and screening, DNA extraction was performed using the Wizard® kit (Promega), and conventional PCR targeting fragments of the 16S rDNA and gp19 genes was used as the primary detection method. The amplification produced positive bands compatible with the genus Ehrlichia and suggestive of E. canis, confirming infection in the cultures. Subsequently, species confirmation and molecular characterization were performed using PCR directed at the p28 gene, which encodes an outer membrane protein known for its sequence variability among isolates. The obtained amplicons were purified and sequenced, allowing for detailed phylogenetic analysis. Phylogenetic reconstruction using the Maximum Likelihood method under the Tamura–Nei model, with 1000 bootstrap replicates, revealed genetic variability between the analyzed isolates. The E.canisPretinha strain showed evolutionary proximity to sequences originating from India and North America, forming clusters with international isolates and suggesting shared ancestry or possible introduction of non-native variants into the local population. Conversely, the E.canisScooby strain positioned basally in the cladogram and clustered with isolates previously described in Rio de Janeiro, indicating that it represents a more ancestral lineage circulating locally, with a distinct evolutionary history. Differences in infection behavior were also observed in vitro. The E.canisPretinha isolate

showed rapid growth in ISE6 cells, quickly invading and occupying the cellular monolayer and reaching high infection levels. In contrast, the E.canisScooby isolate showed slower replication and a more gradual infection pattern. These differences suggest variations in virulence, adaptability, or replicative fitness consistent with the divergence identified by p28 analysis. Such results reinforce the importance of maintaining regional isolates in culture to evaluate biological behavior, genetic diversity, and parasite–host interactions and contribute to advancing the molecular epidemiology of canine ehrlichiosis.

Palavras-chave: ehrlichiosis; phylogeny; genetic variability.