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

ISOLATION, MAINTENANCE, AND CHARACTERIZATION OF EHRLICHIA CANIS IN ISE6 CELLS FROM IXODES SCAPULARIS

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The isolation of pathogens in tick cells, such as the ISE6 cell line of Ixodes scapularis, enables the reproduction of conditions that more closely resemble those found in the vector, allowing the study of essential stages of the biological cycle and pathogen–tick interactions. These cultures facilitate analyses of gene expression, transmission, and vector competence, providing information that cannot be obtained from mammalian cells. In addition, they allow the generation of stable and standardized isolates, which are useful for the development of

diagnostic tools, vaccines, and comparative studies. In the case of Ehrlichia canis, a Gram-negative, obligate intracellular bacterium, such approaches are particularly relevant, as previous studies have demonstrated different genotypes infecting dogs and high genetic variability of the agent in Rio de Janeiro State. Therefore, understanding this diversity is essential to elucidate regional epidemiological dynamics, clarify pathogen–tick interactions, and improve diagnostic and immunoprophylactic tools. The objective of this study was to describe the isolation, in vitro propagation, and characterization of Ehrlichia canis using ISE6 cells derived from I. scapularis. Three blood samples from dogs that showed morulae suggestive of E. canis in blood smears were used for the isolation procedure. In two of these animals, Histopaque® 1083 was used, with EDTA whole blood mixed with the reagent at a 1:1 ratio for mononuclear cell separation, following the manufacturer's instructions. In the third animal, the platelet-rich plasma (PRP) method was employed, based on two consecutive centrifugations to recover the leukocyte layer. The mononuclear cells and leukocytes obtained by both methods were seeded into 25 cm² culture flasks containing a monolayer of ISE6 cells in 5 mL of Leibovitz L-15B medium supplemented with 10% tryptose phosphate broth, 5% heat-inactivated fetal bovine serum, 0.1% bovine lipoprotein concentrate, 0.1% $NaHCO_3$, and 10 mM HEPES, without antibiotics. The cultures were maintained at 34 °C, with medium renewal every three days. Growth of the agent was monitored weekly using smears prepared by cytocentrifugation and stained with 10% Giemsa. Additionally, aliquots from each culture were subjected to DNA extraction using the Wizard® Genomic DNA Purification Kit, followed by PCR for the detection of Ehrlichia spp. through amplification of a dsb gene fragment (409 bp) and the E. canis-specific p28 gene (843 bp). The ISE6 cultures were successfully established as infected, and intracellular morulae were observed on average 13 days post-inoculation. After approximately 32 days of culture, more than 90% of the cells were infected, at which point the cultures were subcultured into new flasks containing uninfected ISE6 cells, and aliquots were cryopreserved in liquid nitrogen. DNA extracted from the cultures yielded amplification of both target genes. In BLASTn analysis, all isolates showed 100% identity in the dsb gene to E. canis sequences previously described in Rhipicephalus sanguineus in Rio de Janeiro (MT005830–MT005831). For the p28 gene, the isolates showed 99.40% (isolate 1) and 99.14% (isolate 2) identity to the Jake strain (CP000107), while isolate 3 showed 99.05% identity to the Jaboticabal strain (EF014897). The isolation of E. canis in ISE6 cells enabled the reproduction of vector-like conditions, resulting in stable cultures with high infection rates, which

are essential for future studies. The isolates showed complete identity in the dsb gene and variability in the p28 gene, reflecting the genetic diversity previously observed in Rio de Janeiro. These findings reinforce the relevance of the obtained isolates as biological models for research on pathogen–tick interactions and highlight the need to apply new molecular markers to discriminate the genotypes circulating in the state.

Palavras-chave: molecular characterization; tick cells; pathogen–vector interaction; canine ehrlichiosis.