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

INTERACTION OF ANAPLASMA PLATYS-LIKE IN A CELL LINE OF RHIPICEPHALUS (BOOPHILUS) MICROPLUS

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Anaplasma platys-like is a bacterium from the Anaplasmataceae family, characterized by hosting species of obligate intracellular parasites. Although it has already been described in ruminants in several countries, its biology and pathogenicity are still poorly understood. In Brazil, it was isolated during a study approved by the Ethics Committee on the Use of Animals (CEUA/UFRRJ), No. 2134171215, from the blood of an 11-month-old calf (Bos taurus) belonging to the Laboratory of Parasitic Diseases of UFRRJ, in Seropedica, Rio de Janeiro,

and has since been cultivated in vitro in different tick strains. This study aimed to analyze, in vitro, the interaction of the bacterium A. platys-like with the BME2 cell line, seeking to understand its behavior and growth rate. The experiment was conducted at the Laboratory for the Study of Parasite-Host Interaction (LEIPH), at the Federal Rural University of Rio de Janeiro (UFRRJ). Initially, the agent was cultivated in the ISE6 tick strain (Ixodes scapularis), widely used for the propagation of hemoparasites, and subsequently subjected to semi-purification and transferred to the Rhipicephalus (Boophilus) microplus (BME2) cell line. For bacterial separation, 2 mL of sample containing cells in suspension were transferred to a Potter-Elvehjem type tissue homogenizer to promote cell lysis and release the microorganisms. The samples were applied to slides and subjected to a Cytospin cytocentrifuge (Thermo Fisher Scientific), operating at 1,500 rpm for 5 minutes, fixed in methanol for 3 minutes, and stained with Giemsa (1:10 dilution) for 20 minutes and examined by optical microscopy (1000x magnification), revealing ruptured cells and bacterial structures. After this, 0.5 mL of the solution were inoculated onto monolayers of the BME2 strain cultivated in the laboratory in a 25 cm² culture flask in Leibovitz's L15 medium at 28ºC. In order to ensure that the purification process was successful, a molecular diagnosis was performed using a fragment of the mitochondrial 16S rDNA gene of approximately 460 bp. Subsequently, sequencing was carried out, confirming that it corresponded to the R. (B.) microplus lineage. For cultivation after inoculation, the medium for maintaining the lineage was replaced with L15B supplemented with Hepes (N-(2-hydroxy-3-methylaminopropyl) piperidine-3-propanesulfonic acid), free of antibiotics, and incubated at 34ºC. The medium was changed twice a week by removing and discarding 3 mL of supernatant and replacing it with an equivalent volume of fresh medium. Optical microscopy examinations were conducted to monitor the infection. The analysis revealed intense formation of intracellular morulae within the first few days of culture. After 20 days, the first subculture was performed: 0.5 mL of suspended cells were transferred to a new flask containing BME2, and then supplemented with 4.5 mL of fresh medium. Seven days after this procedure, a large number of free cells were observed, with no signs of contamination, which led to the performance of a new subculture. Currently, the isolate is at passage 17. During this period, dense intracellular morulae have been identified, often associated with significant destruction of host cells. In some areas, bacteria were observed leaking into the extracellular medium, indicating cell rupture and an advanced stage of infection. The morphological findings indicate that the in vitro infection progressed significantly, with a high replication capacity of the agent in the cell

line used, contributing to studies on its interaction with vectors and hosts. Although there are still gaps regarding the transmission routes of this agent, laboratory results, which indicate a high multiplication rate, suggest that this tick species may play a significant role in its transmission cycle. These data reinforce the need for further studies to clarify its involvement in the epidemiology of the infection.

Palavras-chave: anaplasmataceae; in vitro culture; vector.