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

MOLECULAR SURVEY AND PRELIMINARY DETECTION OF PIROPLASMIDA PARASITES IN FREE-LIVING DIDELPHIS ALBIVENTRIS IN GUAÍRA, PARANÁ, BRAZIL.

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Environmental changes resulting from human activities, such as deforestation and urbanization, have progressively brought wildlife closer to humans and domestic animals, altering the dynamics of vector transmission and favoring the emergence of infectious diseases. Although Brazil is home to the greatest diversity of mammals in the world, most research still focuses on humans or domestic animals. In this context, monitoring wild species is essential, as they

can act as reservoirs, contributing to the maintenance, adaptation, and evolution of infectious agents. Piroplasmid (Phylum Apicomplexa, Order Piroplasmida) are protozoa of great relevance to public health, transmitted by ticks and distributed worldwide in the Babesiidae and Theileriidae families, with Babesia, Theileria, and Cytauxzoon being the main recognized genera. Given this scenario, this study aims to detect these agents in marsupials through molecular diagnosis. To achieve this goal, samples obtained in April 2024 during a field expedition conducted by the Laboratory of Biology and Parasitology of Wild Mammal Reservoirs (LABPMR/IOC-Fiocruz) were used in the municipality of Guaíra, Paraná, an area where the Atlantic Forest and Pantanal biomes meet, on the banks of the Paraná River, on the border with Paraguay. The individuals collected belonged to the species Didelphis albiventris, whose taxonomic identification was performed using integrative taxonomy, including morphological, cytogenetic, and molecular analyses. All capture and handling procedures followed the protocols approved by CEUA/IOC (L-036/2018). After capture, the specimens were sent to a field laboratory structured according to level 3 biosafety standards, where they were anesthetized and subsequently euthanized for the collection of biological samples. A total of 13 animals were captured, 54% of which were male (n=7) and 46% female (n=6). For molecular analysis, the samples were sent to the Hemoparasites and Vectors Laboratory at UFRRJ, where DNA was extracted from 25 µg of liver parenchyma and 10 µg of spleen parenchyma using a commercial genomic extraction kit (Invitrogen). The samples were subjected to conventional PCR targeting the 18S rRNA gene of parasites of the order Piroplasmida, using the primers BAB2143-167F and BAB2694-667R, amplifying fragments of approximately 500 bp. The amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide. Regarding males, 71% (n=5/7) were positive in both tissues analyzed (liver and spleen), and 29% (n=2/7) were negative. Among females, 50% (n=3/6) were positive in both tissues, 17% (n=1/6) were positive only in the spleen and negative in the liver, while 33% (n=2/6) were negative in both tissue samples. The results obtained to date refer to the initial stage of molecular detection, characterizing this as a preliminary study. The next phases include the purification of positive products using ExoSAP-IT (Amersham Biosciences, Piscataway, New Jersey, USA) and sequencing by the Sanger method (SANGER et al., 1977), to be performed with the BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, California, USA), following the manufacturer's recommendations and using the same primers employed in PCR. The detection of protozoa of the order

Piroplasmida in marsupials highlights their potential ecological role in the transmission dynamics of these parasites to other animals and even humans, which may influence both the health of wildlife and the risk of spreading zoonotic diseases. Thus, this study contributes to the initial understanding of these agents and, in the future, aims to integrate phylogenetic data encompassing variables linked to the animals and to their surroundings.

Palavras-chave: molecular diagnosis; marsupials; hemoparasites;emerging zoonoses.