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

CYTOLOGICAL AND MOLECULAR EVIDENCE OF TRIPLE COINFECTION BY HEPATOZOON, MYCOPLASMA, AND TRYPANOSOMA IN A WILD RODENT (OXYMYCTERUS QUAESTOR) COLLECTED IN NOVA FRIBURGO, RIO DE JANEIRO

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The investigation of hemoparasites in small wild mammals is essential for understanding pathogen ecology, host–parasite interactions, and the potential impacts of these infections on native fauna. Among sigmodontine rodents, species of the genus Oxymycterus play an important role in maintaining parasitic cycles, although they remain understudied from a hemoparasitological

perspective. In this context, the present study aimed to report a cytological finding, confirmed by molecular diagnosis, of triple coinfection by Hepatozoon, Mycoplasma, and Trypanosoma in a specimen of Oxymycterus quaestor collected in an Atlantic Forest area in the municipality of Nova Friburgo, Rio de Janeiro, Brazil. The analyzed samples were obtained during a field expedition conducted in July 2024 by the Laboratory of Biology and Parasitology of Wild Reservoir Mammals (LABPMR/IOC/Fiocruz) in the Atlantic Forest region of Nova Friburgo (RJ). Taxonomic identification of the rodent was performed through integrative taxonomy, including morphological, cytogenetic, and molecular analyses. All collection procedures followed protocols approved by CEUA/IOC (L-036/2018). A fresh blood sample was used to prepare blood smear slides, which were fixed in methanol and sent to the Laboratory of Hemoparasites and Vectors (LHV/UFRRJ), where they were stained with Giemsa (1:10) and examined under light microscopy using an oil immersion objective (1000×). To molecularly confirm the cytological diagnosis, DNA was extracted from 25 µg of hepatic parenchyma and 10 µg of splenic parenchyma using a genomic extraction kit (Invitrogen). Samples were subjected to two cPCR protocols: one targeting the amplification of an ~600 bp fragment of the 18S rDNA of Hepatozoon spp. using primers Hep300F and Hep900R and another using primers Myco322s and Myco938as to amplify an ~620 bp fragment of the 16S rDNA of Mycoplasmataceae bacteria. Trypanosomatid detection was performed via nested PCR using primers TRYP927F/TRYP927R in the first reaction and SSU561F/SSU561R in the second, aiming to amplify a 927 bp final fragment of the V7–V8 region of the 18S rDNA. Amplified products were visualized on 1.5% agarose gels stained with ethidium bromide. Cytological analysis revealed the concurrent presence of three hemoparasites. Intraleukocytic gamonts compatible with Hepatozoon were observed, occupying most of the leukocyte cytoplasm, with an elongated to oval shape, light-blue staining, and a granular nucleus located centrally or shifted toward one extremity. Basophilic coccoid organisms arranged in chains, compatible with hemoplasmas of the genus Mycoplasma, were identified adhered to erythrocyte membranes. Additionally, typical trypomastigote forms of Trypanosoma were observed, characterized by a free flagellum, prominent terminal kinetoplast, centralized nucleus, and a well-developed undulating membrane, morphology similar to that described for Trypanosoma cruzi and Trypanosoma lewisi, previously reported in sigmodontine rodents of the genera Oxymycterus, Akodon, and Oligoryzomys. Molecular analysis showed that both DNA samples tested positive in cPCR assays for Hepatozoon spp. and Mycoplasma spp., as

well as in the nested PCR for trypanosomatids. This record contributes to expanding the knowledge of hemoparasite diversity in Brazilian small mammals and reinforces the value of blood smear analysis as a rapid and efficient tool for initial detection of hematozoa. Further studies, including phylogenetic analyses, will be conducted to refine species-level identification of the agents involved and elucidate their potential ecological and One Health implications.

Palavras-chave: blood smear; rodentia; hemoparasites; atlantic forest; pcr.