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

DETECTION AND GENETIC DIVERSITY OF HEMOPLASMAS IN BATS FROM SOUTHERN AND SOUTHEASTERN BRAZIL

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The present study aimed to detect and analyze the genetic diversity of hemotropic mycoplasmas in bats captured in Magé, Rio de Janeiro, and Ponta Grossa, Paraná. In Magé, 16 samples were obtained from splenic fragments of bats, while in Ponta Grossa, 19 samples were collected from liver parenchyma. Molecular diagnosis of hemoplasmas was performed by extracting DNA from 25 mg of tissue using a commercial kit and PCR with primers Myco322s and Myco938as to amplify 620 base pairs (bp) of the 16S rRNA. For phylogenetic

analysis, primers Myco41s–Myco938as and Myco322s–Myco1420as were used to amplify a 1,600 bp fragment of the 16S rRNA. The phylogenetic analysis included seven sequences generated in this study and 69 hemoplasma sequences retrieved from GenBank, using Mycoplasma pneumoniae as the outgroup, totaling a dataset of 76 sequences. Alignment was performed using the ClustalW method, and the dataset was then trimmed, resulting in a final alignment of 716 bp. Phylogeny was reconstructed using the maximum likelihood method with 1,000 bootstrap replicates, applying the best-fit nucleotide substitution model (GTR+G+I) in MEGA 10 software. Intergroup, intragroup, and overall genetic distances were also calculated. For haplotype analysis, sequences from the “Haemofelis group” and those obtained in this study were evaluated in DnaSP v.6.12.03, determining the number of polymorphic sites (S), haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), and frequencies. A median-joining (MJ) haplotype network was constructed in PopART v.1.7 to visualize haplotypes and intermediate mutations. Of the 35 bats captured, 42.85% (n=15) were positive for hemoplasma DNA by PCR. In Magé, 37.5% (n=6/16) were positive, exclusively in Molossus molossus (50%, n=6/12), while the other tested species were negative. In Ponta Grossa, 47.36% (n=9/19) of individuals were positive, with detection in Myotis riparius (100%, n=2/2), Mimon bennettii (60%, n=3/5), Desmodus rotundus (42.85%, n=3/7), and Sturnira lilium (33.33%, n=1/3). The remaining analyzed species did not show amplification. Phylogenetic analysis positioned the seven sequenced samples within the larger “Haemofelis group” with an overall mean distance of 12%, intragroup distances of 7% (Haemofelis) and 8% (Suis), and intergroup distance (Haemofelis × Suis) of 21%. Two sequences from M. riparius from Paraná clustered with hemoplasmas from Molossus rufus (Belize), Myotis chiloensis (Chile), and Myotis lucifugus (USA). Two sequences from M. molossus from Rio de Janeiro clustered with Ca. M. haematomolossi from M. rufus and Mycoplasma sp. from Molossus alvarezi (Belize). The D. rotundus sequence from Paraná grouped with hemoplasmas from D. rotundus, Glossophaga soricina, and Carollia spp. from Brazil and Belize. Finally, two sequences from M. bennettii from Paraná clustered with hemoplasmas from different Artibeus species in Brazil. Analysis of nucleotide alignment polymorphism identified 48 haplotypes, Hd = 0.992 ± 0.005, π = 0.06729, and S = 169. Among this dataset, the seven samples obtained in this study were distributed across five distinct haplotypes. The haplotype network showed that the M. molossus sample shared one haplotype; the two M. riparius samples occupied unique haplotypes; the M. bennettii sample formed a shared

haplotype; and the D. rotundus sample grouped with a previously recorded sequence from the database. The results indicate high genetic diversity among hemoplasmas of bats within the larger “Haemofelis group.” The distribution of samples across different haplotypes suggests that distinct bat species may harbor specific haplotypes, or that cross-species transmission occurs. These findings provide important information for future investigations on the diversity and evolution of these microorganisms.

Palavras-chave: hemotropic mycoplasmas; epicellular bacteria; small mammals; phylogeny; haplotype.