RESUMO - HELMINTOLOGIA

PARTIAL WOLBACHIA MULTILOCUS SEQUENCING PROFILE BASED ON FBPA, FTSZ AND GATB LOCI AND DETECTION OF NOVEL BACTERIAL HAPLOTYPES IN DIROFILARIA IMMITIS ADULTS FROM RIO DE JANEIRO, BRAZIL

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Wolbachia is a genus of Gram-negative intracellular symbiotic bacteria that infect a wide range of arthropods and nematodes, including Dirofilaria immitis. The present study aimed to molecularly detect and characterize Wolbachia haplotypes in adult filariids of D. immitis obtained from dogs through surgical procedures or necropsy performed at a private veterinary clinic and at the two Federal Universities of the state, respectively. DNA was extracted from 81 adult worms (78 females and 3 males) and subjected to PCR targeting the endogenous filarial cox-1 gene. All samples tested positive (81/81; 100%) and were subsequently subjected to PCR assays targeting five MLST loci (gatB, coxA, hcpA, ftsZ, and fbpA) and additional molecular markers (16S rRNA, groEL, gyrA, and dnaA). Allelic comparisons against the Wolbachia MLST database demonstrated that the obtained sequences were most closely related to gatB allele 192 and fbpA allele 247, both previously described from Wolbachia supergroup C associated with Onchocerca cervipedis in Canada, as well as to ftsZ allele 152, previously reported for the Wolbachia MP3 strain infecting D. immitis in the United States (USA). Complete sequence type (ST) assignment was not possible because amplification of the coxA and hcpA loci failed. In addition to the MLST loci, positive amplification and high-quality sequences were also obtained for the 16S rRNA and groEL genic regions. Phylogenetic analyses (based on five genic regions: 16S rRNA, fbpA, ftsZ, gatB, and groEL) clustered the sequences obtained in the present study within supergroup D, a clade comprising Wolbachia spp. associated with nematodes. Genotypic analyses revealed novel haplotypes for the 16S rRNA, fbpA, and gatB loci. This study represents the first report of Wolbachia haplotypes associated with D. immitis in Brazil. Three 16S rRNA haplotypes were identified, including two not yet described Brazilian haplotypes and one shared with isolates from Italy, Myanmar, Portugal, Puerto Rico, Russia, South Korea, and USA. Six fbpA haplotypes were detected: five have been described only from Brazilian samples and one was shared with isolates from France and USA. Two gatB haplotypes were identified, including one unique Brazilian haplotype and another shared with sequences from France and USA. The genotypic analyses based on the ftsZ and groEL loci revealed a single haplotype shared between Brazilian sequences and sequences detected in Cuba, France, Italy, Thailand and USA. Further studies should investigate the genetic diversity and distribution of

Wolbachia haplotypes in Brazil and their role in the pathogenesis of canine dirofilariasis.

Palavras-chave: haplotypes; nematodes; endosymbiont.