

Title: PREVALENCE AND MOLECULAR CHARACTERIZATION *Anaplasma platys* DETECTED IN DOGS FROM THE METROPOLITAN REGION FROM BELÉM - PARÁ, BRAZIL

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Abstract:

The obligate intracellular gram-negative bacterium *Anaplasma platys* is the etiologic agent of canine cyclic thrombocytopenia, a disease that has been recorded in various parts of the world. Despite the range of studies on the occurrence of *A. platys*, researches of this nature have not yet been performed in dogs in the state of Pará, Brazil. Therefore, this study aimed to evaluate the prevalence and characterize the bacteria *A. platys* in dogs in the metropolitan area from Belém (Pará, Brazil), using molecular tools. The genomic DNA of 126 blood samples of dogs was extracted and evaluated its quality in agarose gel. For the specific detection of *A. platys*, it was used a nested PCR amplification of the partial 16S rDNA gene using the primer pairs ECC/ECB and ApysF/ApysR. The molecular characterization for further phylogenetic analysis was carried out using the pair of primer 8f/1492r. Samples positive by PCR were purified and subsequently cloned in *Escherichia coli*. Sequencing was performed by the dideoxy (Sanger) method and terminal electrophoresis using a 3500xL sequencer Genetic Analyzers. Phylogenetic analyses were performed in MEGA program and the phylogenetic tree was constructed using the neighbor-joining method and the Kimura-2-parameter model. Of the 126 blood samples of dogs, 40 individuals (31.74%) were positive for *A. platys*. The phylogenetic groupings showed that the sequence of 16S rDNA of *A. platys* found in dogs from Belém was 100% similar to the sequence recorded in the pathogen found in Lara region (Venezuela). The sequence obtained in this study had 1478 nucleotides in length and nucleotide polymorphisms were detected in twelve positions, when comparing to 16S rDNA gene of *A. platys* from other locations. The prevalence of *A. platys* in dogs from Belém was similar to those recorded in Okinawa (Japan) and North Carolina (USA). With regard to studies carried out in different states of Brazil, prevalence rates observed vary. The analyses performed in this study showed that was not observed high genetic diversity of this pathogen in Belém and other parts of the world, according to the literature. This work was the first to molecularly characterize *A. platys* in the state of Pará (Brazil) revealing its prevalence in dogs. So clearly, there is a lack of studies of this nature which are necessary for taking more effective measures in the fight against canine diseases of zoonotic potential.

Keywords: 16S rDNA, *Anaplasma platys*, Belém, dogs, prevalence.

Funding: CAPES and CNPq.