MOLECULAR ANALYSIS OF THE rRNA GENES OF BABESIA SPP AND EHRLICHIA CANIS DETECTED IN DOGS FROM RIBEIRÃO PRETO, BRAZIL

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ABSTRACT

The partial DNA sequences of the 18S rRNA gene of Babesia canis and the 16S rRNA gene of Ehrlichia canis detected in dogs from Ribeirão Preto, Brazil, were compared to sequences from other strains deposited in GenBank. The E. canis strain circulating in Ribeirão Preto is identical to other strains previously detected in the region, whereas the subspecies Babesia canis vogeli is the main Babesia strain circulating in dogs from Ribeirão Preto.

Key words: Babesia canis vogeli, Ehrlichia canis, ticks, rRNA gene, PCR.

Canine monocytic ehrlichiosis (CME) and canine babesiosis (CB) are endemic diseases of great veterinary importance in Brazil. CME is caused by the Gram-negative endobacterium E. canis which multiplies inside monocytes of dogs, whereas CB is mainly caused by the intraerythrocytic protozoa B. canis. In Brazil, both parasites are transmitted to dogs by the brown tick Rhipicephalus sanguineus, a fact probably facilitating co-infection (1, 5). Thrombocytopenia is a common finding in E. canis-infected dogs and many clinicians tend to use it as an indication for antibiotic treatment. However, thrombocytopenia can also be a manifestation of other diseases, as well as of infections with other parasites such as B. canis itself (6). Therefore, many clinicians tend to use combinations of antibiotics for treatment of the two parasites. Indeed, in the region of Ribeirão Preto we found thrombocytopenic dogs co-infected with E. canis and Babesia sp or Anaplasma platys, a Gram-negative bacterium which multiplies inside dog platelets and causes canine infectious cyclic thrombocytopenia (4, 11). This fact emphasizes the need for more precise exams to permit a correct diagnosis by veterinary clinicians. In this respect, PCR-based methods can be designed to specifically detect and identify each of these parasites with high accuracy (7). DNA sequences obtained by PCR can also be used for the characterization and comparison of Brazilian strains to strains from other regions around the world.

This approach helps complement studies to determine the occurrence of strains with specific regional genetic traits, such as susceptibility to antibiotics. In this report we present such analysis using partial DNA sequences from the 16S and 18S ribosomal RNA genes of E. canis and B. canis detected in dogs from Ribeirão Preto, Brazil.

The rRNA gene sequences were generated by sequencing PCR products obtained during a previous study on the prevalence of blood parasites in dogs from Ribeirão Preto, Brazil (11). Briefly, nested PCR was used to detect the 16S rRNA gene of E. canis using the following set of primers: Apla-sense 5’-CTCAGAACGAACGCTGGCGGCAAGC-3’ and ECB 5’-CGTATTA CCGCGGCTGCTGGC-3’ were used in the first reaction; in the second reaction, 1 µL of the first reaction was used with primers ECA 5’-CAATTATTTATAGCCTCTGGCTA TAGGAA-3’ and HE-3 5’-TATAGGTACCGTCATTATCTT CCTAT-3’, which generated a 389-bp fragment, encompassing position 49 to 437 of the GenBank reference sequence (AF162860). A single PCR was used to detect B. canis with primers BabgenF 5’-GAAACTGCGAATGGCTCATTA-3’ and Babesiarev1 5’-CCATGCTGAAGTATTC AAGAC-3’, which target the 18S rRNA gene of a wide range of Babesia species and generate a 642-bp fragment, encompassing position 81 to 722 of the GenBank reference sequence (AY072926). The
amplified PCR products were purified and sequenced in an automated DNA sequencer (model 377, Applied Biosystems). Sequence analysis was performed using the ClustalX (12) and BLAST programs (2).

The *E. canis* nested PCR nucleotide sequences of 10 out of 86 positive dogs were analyzed and showed 100% similarity. No polymorphisms to sequences from other Brazilian strains deposited in GenBank were observed. Considering the 347-bp fragment analyzed, 100% similarity to the 16S rRNA gene of the reference sequence was observed. Moreover, the sequences showed 100% similarity to strains from Venezuela, where human cases of infection with *E. canis* have been reported (10). In Brazil, despite the high incidence of the bacterium, no human cases have so far been reported. A more complete characterization is necessary to determine the molecular basis of these differences in pathogenicity, which might be related to host-parasite interactions influenced by geographical factors (3,8,11).

*B. canis* and *B. gibsoni* are the two species associated with CB in Brazil (5). The PCR nucleotide sequences of 12 out of 18 dogs positive for the presence of *Babesia* spp were analyzed and were identical, indicating that the strains detected in Ribeirão Preto are classified as the subspecies *B. canis vogeli*. The sequences showed high similarity to sequences of *B. canis vogeli* previously detected in a study on dogs from the states of Minas Gerais and São Paulo, Brazil (9). In addition, these sequences also showed high similarity to strains from other parts of the world. A few polymorphisms were found among these sequences and are summarized in Table 1 for a fragment of 600 bp.

The tick *R. sanguineus* is assumed to be the common vector of the two parasites described in this study. The occurrence of other species of tick-transmitted pathogens might be too low to be detected in the tick or in the vertebrate host in which clinical signs may not manifest. One example is the detection of *A. platys* DNA in dogs (4). This parasite is also believed to be transmitted by *R. sanguineus*, although there are no molecular data to confirm it. Since tick borne diseases affecting humans and animals are considered to be emerging diseases, the present molecular data are important for the surveillance of strains with different degrees of pathogenicity or even of the emergence of new species. In addition, the data contribute to the development of methods permitting sensitive detection and precise identification of these strains.

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**Table 1.** Nucleotide sequence identities of the 18S rRNA sequences of *B. canis vogeli* with available *B. canis* sequences.

| Strain            | Nucleotide position<sup>a</sup> |
|-------------------|----------------------------------|
|                   | 55    | 71    | 72    | 100   | 348   | 410   | 480   | 521   | 554   | 571   | 574   | 586   | 588   | 589   | 591   | 598   | 599   | 600   |
| Brazil (AY371194) | C      | T      | A      | C      | A      | A      | C      | T      | T      | T      | G      | T      | C      | T      | T      | A      | T      | T      |
| RP1 (EF052623)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP2 (EF052624)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP3 (EF052625)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP4 (EF052626)    | N      | N      | N      | N      |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP5 (EF052627)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP6 (EF052629)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP7 (EF052628)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP8 (EF052629)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP9 (EF052630)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP10 (EF052631)   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP11 (EF052632)   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RP12 (EF052633)   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Japan (AB083374)  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Australia (AY102163) |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| USA (AY371198)    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| France (AY072925) |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Egypt1 (AY371197) |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Spain (AY150061)  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Egypt2 (AJ009796) |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |

<sup>a</sup>*B. canis vogeli* Brazil, accession number AY371194, was used as a reference sequence for nucleotide positions. (——) deletion; (°) identical base to reference strain; (N) not determined.
Oliveira, L.P. et al.

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RESUMO

Análise dos genes rRNA de Babesia spp e Ehrlichia canis detectados em cães de Ribeirão Preto, Brasil

As sequências parciais dos genes RNAr 18S de Babesia canis e RNAr 16S e Ehrlichia canis detectados em cães de Ribeirão Preto, Brasil, foram comparadas à sequências de outras linhagens depositadas no GeneBank. A linhagem de E. canis circulando em Ribeirão Preto é idêntica a outras detectadas previamente na região, enquanto a sub-espécie B. canis vogeli é a principal linhagem de Babesia circulando em cães de Ribeirão Preto.

Palavras-chave: Babesia canis vogeli, Ehrlichia canis, carrapatos, gene RNAr, PCR

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