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Short communication

First molecular detection of *Babesia vogeli* in dogs from Brazil

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Abstract

The present work describes the detection and first molecular characterization of *Babesia vogeli* in dogs, naturally infected in Brazil and even in South America. Microscopic examination of Giemsa-stained peripheral blood smears collected from dogs originating from four different locations in Brazil revealed the presence of large *Babesia* merozoites and trophozoites (>2.5 μm). DNA was extracted from infected blood samples and PCR amplifications of the 18S rDNA were carried out. As a reference, DNA from an isolate of *B. vogeli* originated from Egypt was used. PCR products were purified and sequenced. The DNA sequences demonstrated 100% identity among the Brazilian isolates. Comparisons with the 18S rDNA sequence of the *B. vogeli* isolate from Egypt and with other *B. vogeli* sequences from Spain, France, Japan, Australia and South Africa confirmed the affiliation of all Brazilian isolates to the species *B. vogeli*.

Keywords: *Babesia vogeli*; *Babesia canis*; PCR; 18S rDNA; Dog; Brazil

1. Introduction

Canine Babesiosis is a tick-borne disease of domestic and wild canidae characterized by fever, depression and anaemia. In dogs, large and small babesias were described, which generally were attributed to two species, *Babesia canis* and *Babesia gibsoni*, respectively. Usually, the diagnosis is made upon size and morphological appearance of the intra-erythrocytic forms in peripheral blood smears. However, taxonomy of canine *Babesia* species is under great discussion and the question arises whether

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morphological features are sufficient for differentiation of these pathogens. Previous studies have suggested that large Babesias should be divided into three subspecies: *Babesia canis canis*, *Babesia canis rossi*, and *Babesia canis vogeli*, depending on vector specificity, pathogenicity and antigenic properties (Uilenberg et al., 1989; Hauschild et al., 1995). The existence of three taxonomic entities was confirmed recently with molecular biological methods (Zahler et al., 1998; Carret et al., 1999; Cacciò et al., 2002). Since, at least for *B. canis canis* and *B. canis rossi*, differences in main pathogenicity mechanisms have also been detected (Schetters et al., 1997), the differentiation of species is not only of mere academic interest but has also implications for vaccination and therapy. However, the concept of three subspecies based on the characterization of isolates from distinct geographic regions is still unclear. Besides, molecular biological techniques and comparisons of the 18S rDNA of isolates from different areas of the world have shown that small Babesia, generally attributed to *B. gibsoni*, separated in genetically distantly related species (Zahler et al., 2000a; Kjemtrup et al., 2000). Furthermore, a small *Babesia microti*-like piroplasm has been described most recently in north-west Spain (Zahler et al., 2000b; Camacho et al., 2001), which has initiated new considerations and discussions about distribution and epidemiology of Babesioses in dogs. Since the discussion about classification of canine *Babesia* species is not the aim of the present paper, the classical nomenclature (*B. canis*, *Babesia vogeli* and *Babesia rossi*) will be used as suggested by Schetters et al. (1997), Zahler et al. (1998), Carret et al. (1999) and Depoix et al. (2002).

In Brazil, canine Babesiosis is commonly distributed as reported in a previous study when anti-*B. canis* (sensu lato)-antibodies were detected in 66.9% of dogs examined at a veterinary hospital in Minas Gerais state (Ribeiro et al., 1990). *Rhipicephalus sanguineus* Latreille, 1806 ticks have been considered the vector of canine Babesiosis. Because *R. sanguineus* is a three-host tick, very well adapted to urban areas, this would support the fact that the disease assumes an endemic character.

Although the importance of canine Babesiosis has increased over the last years in the state of Minas Gerais (Bastos et al., 2004), few epidemiological studies have been carried out in the country and little is known about its epidemiological aspects in Brazil. Characterization of *Babesia* isolates on a molecular basis is completely lacking. Therefore, the aim of the present study was to characterize Brazilian isolates of canine *Babesia* species with molecular biological methods in order to fit them into a taxonomic system, together with *Babesia* isolates from other geographic regions.

2. Materials and methods

Five canine samples were obtained in four separate geographic locations in Brazil: four dogs from the state of Minas Gerais (two in Belo Horizonte, one in Lavras and one in Uberlândia) and finally one dog from the state of São Paulo. Peripheral blood smears were made from dogs naturally infested with *R. sanguineus* showing clinical manifestations suspicious of Babesiosis. Giemsa-stained smears were examined under the light microscope for direct detection of intra-erythrocytic stages. Blood samples from infected dogs were collected in EDTA tubes and the DNA was extracted using a commercial kit (Wizard Genomic DNA Purification, Promega, Madison, USA).

For comparison, blood from a dog naturally infected with a large *Babesia* in the United States of America was included as well as an isolate of *B. vogeli* originated from Egypt (Egypt 1), which had served as a reference isolate for the description of the species (Uilenberg et al., 1989). From these two samples DNA was extracted from 200-μl aliquots of EDTA blood using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. PCR amplifications of the 18S rDNA were carried out according to Zahler et al. (2000a), with the PCR primers RIB-19 (5′-CGG GAT CCA ACC TGG TTG ATC CTG C-3′) and RIB-20 (5′-CCG AAT TCC TTG TTA CGA CTT CTC-3′), which are located at the 5′ and 3′ end of the small subunit rRNA gene, respectively. Using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany), PCR products of a size of 1.6 kb were purified and sequenced with the primers RIB-19, RIB-20, RIB-21 (5′-TTT CCC CGT GTT GAG TC-3′) and RIB-24 (5′-GAC GGT AGG GTA TTG GC-3′), (Zahler et al., 2000a). The sequences are available in GenBank