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Babesia vogeli in dogs from Rio Branco, South-west Amazonia, Brazil

Babesia vogeli em cães de Rio Branco, sudoeste da Amazônia, Brasil

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Highlights

First Record of molecular diagnosis of *Babesia vogeli* in South-west Amazonia.
Frequency of 2.1% of *B. vogeli* in symptomatic dogs in Rio Branco.
Co-infection of *B. vogeli* with *Ehrlichia* spp. appeared in blood smears.

Abstract

This is the first report of *Babesia vogeli* molecular detection in dogs from the state of Acre, northern Brazil. This study aimed to perform the molecular detection of *Babesia vogeli* in dogs in the municipality of Rio Branco, Acre. Blood samples were collected from 47 dogs presenting with clinical signs comparable to hemoparasitosis. These were dogs which were attended in veterinary clinics from Rio Branco municipality, Acre. Physical examinations, packed cell volume (PCV) determination, platelet number estimation, hemoparasite investigation in the blood (collected from the pinna and peripheral blood), and polymerase chain reaction (PCR) for piroplasm based on the 18S rRNA gene, were performed. One dog (1/47, 2.1%; CI 95%: 0.1-11.3%) tested positive to *Babesia vogeli* in the polymerase chain reaction (PCR) assay for piroplasms and the resulting sequence showed 100% identity with *Babesia vogeli* isolates deposited in GenBank®. Co-infection with *Ehrlichia* spp. was also observed by direct examination (via blood smear). The

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clinical and hematological alterations observed in the positive animal were anorexia, dehydration, white mucous membranes, anemia and thrombocytopenia.
Key words: Diseases transmitted by ticks. Hemoparasitosis. Piroplasmosis.

Resumo

Este é o primeiro relato de detecção molecular de *Babesia vogeli* em cães do estado do Acre, norte do Brasil. Este estudo teve como objetivo realizar a detecção molecular de *B. vogeli* em amostras de sangue de 47 cães com sinais clínicos compatíveis com hemoparasitoses no município de Rio Branco, Acre. Tratavam-se de animais atendidos em clínicas veterinárias do município, sendo realizados exames físicos, determinação do volume globular (VG), estimativa do número de plaquetas, investigação de hemoparasitos no sangue (coletado da ponta da orelha e sangue periférico). Além disso, amostras de sangue foram submetidas a extração de DNA e reação em cadeia da polimerase (PCR) para amplificação de fragmentos do gene 18S rRNA de piroplasmas. Um cão (1/47, 2,1%; IC 95%: 0,1-11,3%) apresentou resultado positivo para *B. vogeli* na PCR para piroplasmas e a sequência resultante mostrou 100% de identidade com os isolados de *B. vogeli* depositados no GenBank®. Co-infecção com *Ehrlichia* spp. também foi observado por exame direto (esfregaço de sangue). As alterações clínicas e hematológicas observadas no animal positivo foram anorexia, desidratação, mucosas pálidas, anemia e trombocitopenia.
Palavras-chave: Doenças transmitidas por carrapatos. Hemoparasitose. Piroplasmose.

Introduction

Babesiosis is one of the most common infections caused by intraerythrocytic piroplasms in domestic animals worldwide, in addition to being an emerging zoonosis (Irwin, 2016). The transmission of *Babesia vogeli* infection in dogs has been observed in Brazil, considering the wide distribution of its tick vector, *Rhipicephalus sanguineus* sensu lato (A. P. Costa et al., 2015).

In Acre, the equatorial climate provides heat and humidity that is favorable to the survival and proliferation of ectoparasites. In Rio Branco city, the capital of the state of Acre, most dogs' infestations by ticks are associated to *R. sanguineus* species (Fernandes, Medeiros, Carvalho, Ribeiro, & Souza, 2018).

No studies on the detection of *Babesia* spp. in dogs from Acre have been reported. Thus, this study aimed to screen domiciled dogs for the presence of piroplasms in the Rio Branco municipality, Acre state, by direct examination and by polymerase chain reaction (PCR).

This study was conducted after the approval of the Ethics Committee on the Use of Animals from Universidade Federal do Acre (protocol 40/2014), and conducted according to the ethical principles of animal experimentation, adopted by the Brazilian College of Animal Experimentation.

Blood samples from forty-seven dogs of different ages (varying from 3 months to 11 years) and both sexes (27 males and 20 females) attended at private veterinary clinics of Rio Branco municipality, Acre, northern Brazil, were sampled. Dogs were eligible for the study if they presented with: (i) clinical signs (lethargy, hyperthermia,

weight loss, epistaxis, lymph nodes' enlargement and/or splenomegaly) and/or (ii) hematological abnormalities (anemia and/or thrombocytopenia) consistent with tick-borne diseases (TBD); (iii) presence of ticks on their body surface at the time of sampling.

Dog blood samples were collected by cephalic/jugular venipuncture and stored in EDTA tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) for hematological and molecular procedures. Packed cell volume (PCV) was measured by routine centrifugation and platelet count, indirectly estimated on peripheral blood smears (Thrall, Weiser, Allison, & Campbell, 2015). Dogs were considered anemic when PCV <0.37 LL^{-1}, and thrombocytopenia was considered when the platelet counts < 200,000μL^{-1}. Additionally, blood smears of capillary origin were obtained after the introduction of a hypodermic stylet/needle into the pinna, after antisepsis. Hemoparasites were investigated in peripheral blood smears and blood extensions of capillary origin at 1000x magnification.

The DNA was extracted from 200µL blood using a commercial kit (DNeasy Blood & Tissue Kit®, Qiagen, Hilden, Germany), according to the manufacturer's instructions. Negative control purifications using ultra-pure water were performed in parallel, to monitor cross-contamination. DNA concentration and purity were evaluated by spectrophotometry (NanoDrop™, Thermo Scientific, Waltham, USA).

To ensure successful DNA extraction, PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Birkenheuer, Levy, & Breitschwerdt, 2003) was performed on all samples. Thereafter, samples were screened using a conventional PCR targeting a fragment (≈ 500 bp) of the piroplasms 18S rRNA gene, as previously described (J. F. Soares et al., 2011; Mongruel et al., 2017).

Amplicons (551 bp) obtained from piroplasm-positive samples were purified on an agarose gel (Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, EUA), evaluated by spectrophotometry for concentration and purity (NanodropTM 2000 Spectrophotometer, Thermo Fisher Scientific, Wilmington, MA, USA), and sequenced in both directions by the Sanger method. The assembled partial sequences of the 18S rRNA gene were subjected to BLASTn to determine their identity with sequences deposited in the GenBank® database. The nucleotide sequence of the *B. vogeli* amplified herein was submitted to the GenBank® database (accession number: MT386936).

Thirty-two out of 47 (68.1%; 95%; CI: 53-81%) dogs were anemic and 30/47 (63.8%; CI 95%: 48-77%) were thrombocytopenic. Thirteen out of 47 (28%) dogs were anemic and thrombocytopenic. Additionally, 2/47 (4.3%; CI 95%: 0.5-14%) and 1/47 (2,1%; 95% CI: 0,1-11,3%) dogs showed intraerythrocytic and monocytic inclusions compatible with piroplasms and *Ehrlichia* spp., respectively, during blood smear evaluations.

One out of 47 (2.1%; 95%; CI: 0.1-11.3%) dogs tested positive for piroplasm by PCR. Sequencing of the 18S rRNA fragment showed 100% (551/551 bp) identity with multiple *B. vogeli* sequences deposited in GenBank® (accession nos. MH100722, MG041384, KT323936). The dog was presenting anorexia, dehydration, white mucous membranes, anemia (PCV = 0.25LL^{-1}), thrombocytopenia (platelet count = 15,000μL^{-1}), and inclusions suggestive of *Ehrlichia* spp. during blood smear evaluation.

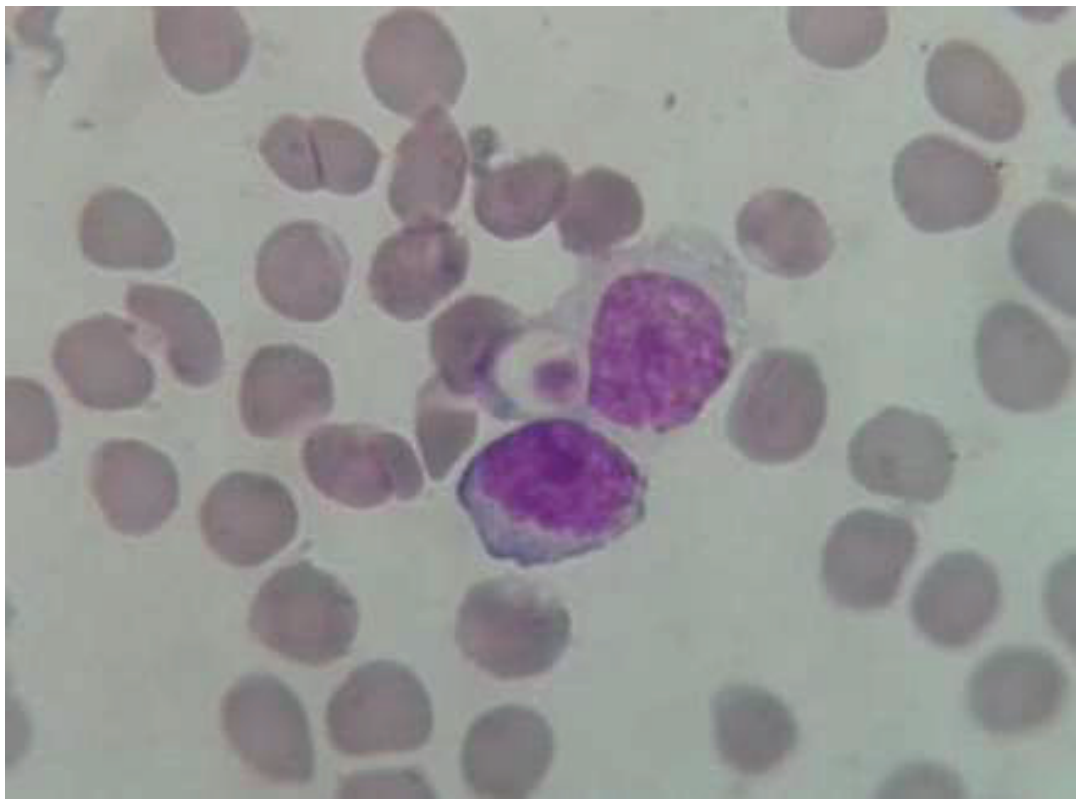

Figure 1. *Ehrlichia* spp. morulae in a monocyte. Blood smear, dog. 1000X magnification.

Unfortunately, ticks were not collected for taxonomic identification at the time of sampling.

Canine babesiosis cross-sectional molecular studies conducted in the Amazon biome are scarce. Galeno, Moreno and Alves (2018) observed 7.69% (5/65) of dogs infected by *B. vogeli* in Maranhão State. In the state of Pará, scientists obtained 5% (5/100) (Moraes et al., 2014) and 15.7% (27/172) (Moraes et al., 2015) of *B. vogeli* positive dogs, by targeting the 18S rDNA gene. Considering the biome and the studies that investigated the parasite in dogs showing clinical signs, Rio Branco obtained the lowest infection rate (2.1%).

The frequency of *B. vogeli* molecularly detected in symptomatic dogs from other regions in Brazil presents the following rates: 5% (15/300) in Goiás (H. X. Costa, 2011); 23.4% (66/282) in Paraná (Jojima et al., 2008), and 4.8% (7/146) in Pernambuco (Silva et al., 2016). Jojima et al. (2008) observed the highest detection rate (23.4%) of *B. vogeli* in a teaching hospital population.

Araujo et al. (2015) observed the presence of *Babesia* spp. in optical microscopy of 0.5% the evaluated animals from Pernambuco, a result lower than that found in this study. Castilho, Alves, Pereira and Coelho (2011) also found piroplasms in 3.9% of the evaluated animals, a result similar to that obtained in this research.

Jojima et al. (2008) observed hematological changes such as anemia and thrombocytopenia in parasitized dogs from Paraná, which were also observed in our study. Similarly, Vilela et al. (2013) verified anemia and thrombocytopenia in animals infected with *B. vogeli* in a study conducted in Rio de Janeiro. The authors observed clinical alterations such as apathy, fever, and white mucous membranes; signs that are nonspecific, as well as those found in the present study.

In a research conducted with dogs experimentally infected with *B. vogeli* Wang et al. (2018) observed fever, partial anorexia, malaise, regenerative anemia, thrombocytopenia and decreased white blood cell counts. These findings were similar to our study, corroborating its results.

Regarding *R. sanguineus,* the vector of several hemoparasites, the disease may be aggravated by *B. vogeli* co-infection with other organisms such as *Ehrlichia.* The interaction between *Ehrlichia* and *B. vogeli* was observed in a study conducted by H. X. Costa (2011) in Goiânia municipality. The author verified that among co-infections involving *Ehrlichia,* the most common was the mixed infection of *E. canis* and *B. canis vogeli.* Although most blood samples were collected from dogs with ticks, the vector species were not identified at the time of sampling. This may have compromised a possible relationship between the occurrence of the vector and the presence of hemoparasites, as described by H. S. Soares, Camargo, Gennari & Labruna (2014).

Babesia vogeli is present in the municipality of Rio Branco and studies conducted in the Amazon biome are scarce and have a heterogeneous frequency in dog population.

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