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Molecular and serological detection of *Ehrlichia canis* and *Babesia vogeli* in dogs in Colombia

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ABSTRACT

Ehrlichiosis and babesiosis are tick-borne diseases, caused mainly by *Ehrlichia canis* and *Babesia canis*, respectively, with a worldwide occurrence in dogs, whose main vector is the brown-dog tick, *Rhipicephalus sanguineus*. The present work aimed to detect the presence of *E. canis* and *Babesia* sp. in 91 dog blood samples in Colombia, by molecular and serological techniques. We also performed sequence alignment to indicate the identity of the parasite species infecting these animals. The present work shows the first molecular detection of *E. canis* and *B. vogeli* in dogs from Colombia. Immunoglobulin-G (IgG) antibodies to *E. canis* and *Babesia vogeli* were found in 75 (82.4%) and 47 (51.6%) sampled dogs, respectively. Thirty-seven (40.6%) and 5 (5.5%) dogs were positive in PCR for *E. canis* and *Babesia* sp., respectively. After sequencing, amplicons showed 99% of identity with isolates of *E. canis* and *B. vogeli*. The phylogenetic trees based on 16S rRNA-Anaplasmataceae sequences and 18S rRNA-piroplasmid sequences supported the identity of the found *E. canis* and *B. vogeli* DNAs, respectively. The present work shows the first molecular detection of *E. canis* and *B. vogeli* in dogs in Colombia.

1. Introduction

Ehrlichiosis and babesiosis are tick-borne diseases, caused mainly by *Ehrlichia canis* and *Babesia* sp., respectively, with a worldwide occurrence in dogs, whose main vector is the brown-dog tick, *Rhipicephalus sanguineus* (Dantas-Torres, 2008).

E. canis is the primary etiologic agent of canine monocytic ehrlichiosis (CME), a cosmopolitan tick-borne disease of dogs (Stich et al., 2008). Other closely related bacterial pathogens of Anaplasmataceae family, namely *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Neorickettsia risticii* can cause atypical disease in dogs (Stich et al., 2008). The clinical signs caused by *E. canis* in the acute phase of the disease are characterized by fever, anorexia, emaciation, hepato/splenomegaly, lymphadenopathy, cardiac and respiratory disturbance, and ocular alterations (Breitschwerdt et al., 1998; Castro et al., 2004; Nakaghi et al., 2008). The chronic phase, which follows the subclinical phase (with no evidence of clinical signs but persistent hematological alterations), is characterized by pancytopenia, bone marrow hypoplasia, and bleeding tendencies (Stich et al., 2008).

Babesia species are tick-borne apicomplexan parasites that infect eythrocytes of a variety of domestic and wild animals, and human beings (Solano-Gallego and Baneth, 2011). Large (namely *Babesia vogeli*, *Babesia rossi*, and

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Babesia canis) and small (*Babesia gibsoni*, *Babesia conradae*, and *Babesia microti*-like piroplasm) piroplasms have been detected in dogs around the world by blood smears evaluation and molecular techniques (Irwin, 2009; Solano-Gallego and Baneth, 2011). The clinical manifestations or canine piroplasmosis are characterized by fever, lethargy, anorexia, jaundice, pale mucous membranes, splenomegaly, and weight loss. The diseases caused by different piroplasm species show a good prognosis except when caused by *B. rossi*, *B. gibsoni*, *B. conradae* and *Theileria annae* (Irwin, 2009; Solano-Gallego and Baneth, 2011).

In South America, Anaplasmataceae agents have been detected in dogs from Brazil (Dagnone et al., 2003, 2009), Venezuela (Unver et al., 2001), Chile (Abarca et al., 2007), and Peru (Vinasco et al., 2007). Also, *Babesia* sp. have been detected in dogs from Brazil (Passos et al., 2005), Venezuela (Criado-Fornelio et al., 2007), and Argentina (Eiras et al., 2008). Although *E. canis* and *B. canis* are cosmopolitan parasites, to the authors' knowledge there is no molecular confirmation of infections by these pathogens in dogs from Colombia, where only the presence of antibodies to *Ehrlichia* sp. (Parrado and Vargas, 2002; Gomez, 2002; Benavides and Ramirez, 2003; Contreras and Sanabria, 2006; Hidalgo et al., 2009) and the observation of piroplasm-like structures similar to *Babesia* spp. in dogs blood smears (Batista, 1980) have been reported. The present work aimed to detect the presence of these both agents in dog blood samples in Colombia, by molecular and serological techniques. We also performed sequence alignment to indicate the identity of the parasite species infecting these animals.

Fig. 1. Map of the Republic of Colombia showing the three cities of central-west region. (A) Bogota city, (B) Villavicencio city, and (C) Bucaramanga city (Institute Geographical Agustin Codazzi – IGAC).

2. Materials and methods

In Colombia, blood was collected from the cephalic vein of 91 dogs and kept in EDTA-and plain tubes (Fig. 1): 21 dogs from Bogotá city, latitude 4°20′48″N/longitude 74°09′49″W (dogs were used for detection of explosives in several regions of the country), 31 dogs from Villavicencio city, latitude 4°15′48″N/longitude 73°65′49″W (31 dogs from an animal shelter), and 39 dogs from Bucaramanga city, latitude 7°07′48″N/longitude 73°11′49″W (19 dogs from a local veterinary clinic and 20 from an animal shelter). Blood smears were fixed with methanol and stained with Giemsa (Sigma–Aldrich, St. Louis, MO, USA). The inclusion criteria were the presence or history of tick exposure.

The presence of anti-*E. canis* and *B. vogeli* IgG antibodies in the sera of each animal was detected by Indirect Fluorescent Antibody Test (IFAT). *E. canis* antigen was obtained from culture of DH82 cells infected with *E. canis* (Jaboticabal strain) at the Immunoparasitology Laboratory, UNESP (Aguiar et al., 2007). The *B. vogeli* antigen was prepared by inoculation intravenously into a splenectomized three-month-old dog, negative for hemoparasites by PCR and serology (Furuta et al., 2009). Blood smears were performed twice a day to check for the presence of parasites in microscopic examination of Giemsa-stained. The parasitaemia peak occurred on the fifth day after inoculation; infected blood was collected with Alsever solution (113.7 mM glucose, 27.2 mM sodium citrate, 71.8 mM sodium cloride) (LabSynth, Diadema, São Paulo, Brazil). The slides containing air-dried fixed *B. vogeli* trophozoite-infected blood were used in IFAT (Indirect Fluorescent Antibody Test) as described previously (Furuta et al., 2009). Prior to its use, IFAT slides were stored frozen (at −20 °C). Antigen slides were removed from storage and allowed to thaw at room temperature for 30 min. Ten microliters of twofold dilutions of sera (cut-off of 1:20 for *E. canis* and 1:40 for *B. vogeli*) were placed in wells on antigen slides. Known positive canine sera (titre of 2560 for *E. canis* and 5120 for *B. canis*) were obtained from symptomatic dogs with ehrlichiosis and babesiosis, respectively, at Governador Laudo Natel Veterinary Hospital – UNESP, Jaboticabal, São Paulo, Brazil. Negative control serum samples were obtained from dogs (that had not been exposed to these agents, according to negative PCR and IFAT results) maintained in the kennel (a free-tick installation) of the Department of Veterinary Pathology, UNESP, Jaboticabal, São Paulo, Brazil. Slides were incubated at 37 °C in a moist chamber for 45 min, washed 3 times in PBS (pH 7.2) for 5 min, and air dried at room temperature. FITC-labeled anti-dog IgG conjugate (Sigma–Aldrich, St. Louis, MO, USA) was diluted according to the manufacturer (dilution of 1:80) and then added to each well. These slides were incubated again at 37 °C, washed 3 times in PBS, once more in distilled water, and air dried at room temperature. Next, slides were