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Short communication

Babesia gibsoni genotype Asia in dogs from Brazil☆

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Abstract

Babesia gibsoni was first recognized in India in 1910. Analysis of 18S rRNA has shown that there are at least three distinct isolates that are morphologically identical. Although organisms similar to *B. gibsoni* have been identified in peripheral blood smears from one dog in Brazil, this isolate has not been molecularly characterized. Accordingly, we obtained blood samples from 16 dogs with intraerythrocytic inclusion bodies. DNA was extracted and amplified with primers that detect a segment of the 18S rRNA gene of the *Babesia* genus. Amplicons of the expected size for *B. gibsoni* were observed in 4 of the 16 dogs. The sequence of the 18S rRNA yield a 460 base pair segment that had a 99% homology with the *B. gibsoni* genotype Asia 1. Our findings suggest that the small piroplasm that naturally infects dogs in Brazil is *B. gibsoni* genotype Asia.

Keywords: *Babesia gibsoni*; Brazil; South America; Dogs

1. Introduction

Babesia gibsoni was first recognized in India in 1910 (Patton, 1910). Analysis of 18S rRNA has shown that there are at least three distinct isolates of *B. gibsoni* that are morphologically identical (Kjemtrup et al., 2000): one from Asia, one from California, and a third from Europe. The Asian isolate is the original organism found in India and is considered *Babesia gibsoni sensu stricto*. The California isolate is a different species with some characteristics of the genus *Theileria* (Kjemtrup et al., 2000). The name *B. conradae* has recently been proposed for this species (Kjemtrup et al., 2006). The isolate from Europe is a *Theileria* closely related to *B. microti* (Criado-Fornelio et al., 2003) that has been named *Theileria annae* (Camacho et al., 2001). Based on maximum-likelyhood analysis, it appears that

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B. conradae and *Cytauxzoon felis* are likely ancestral to the remaining *Theileria* and to the classic *B. gibsoni* (Criado-Fornelio et al., 2003). A small piroplasm morphologically similar to *B. gibsoni* was observed in peripheral blood smears from one Brazilian dog (Braccini et al., 1992), whereas antibodies against *B. gibsoni* have been detected in another dog after traveling through Brazil (Gothe and Wegerdt, 1991). Another small piroplasmid that causes hemorrhagic disease has been described in Brazil in the early 20th century (Carint, 1915). This organism tested positive by immunohistochemistry for *B. microti* but has an intraendothelial stage and likely does not belong to the genus *Babesia* (Loretti and Barros, 2005). Although a small piroplasm related to *B. gibsoni* is thought to occur in Brazil, this isolate has not been molecularly identified. Accordingly, we attempted to characterize the small canine piroplasm from South Brazil.

2. Material and methods

We obtained blood samples from 16 dogs with intraerythrocytic inclusion bodies. The inclusion bodies were morphologically identified as piroplams in two of the 16 dogs. All 16 dogs were seronegative for *B. canis* by indirect immunofluorescence test as previoulsy described (Trapp et al., in press). Blood samples were lysed and DNA was extracted using a commercially available kit (Gentra Systems, Minneapolis, MN). The primers PIRO-F (5′-AGTCATATGCTTGTCTCA-3′) and PIRO-R (5′-CCATCATTCCAATTACAA-3′) were developed based on the sequences available in the GeneBank to amplify a 460-, 483-, 480-bp fragment of the 18 S ribosomal RNA gene of the *B. gibsoni, B. conradae*, and *T. annae*, respectively. This set of primers would also amplify a 454 bp segment from the Brazilian strain of *B. vogeli*. In dogs that were positive for *B. gibsoni*, PCR was used to test for coinfection with *Ehrlichia canis* and *Mycoplasma haemocanis*. Primers (5′-GCCATTAGAAATGGTGGGTA-3′ and 5′-GCCACTGGTGTTCCTCCTAAT-3′) were developed based on the available GenBank sequences to amplify a 536-bp fragment of the 16S ribosomal DNA of *Ehrlichia* genus, whereas the 16S ribosomal DNA of *M. haemocanis* was amplified by PCR as previously described (Brinson and Messick, 2001). Standard amplification reactions were carried out in a thermal cycler (Brinson and Messick, 2001). The products were subsequently separated by electrophoresis in a 1% agarose gel containing 5 μg/ml of ethidium bromide, and photographed under UV light with an Alpha Imager 2200 imaging system. Gel-purified 18S ribosomal RNA products of *Babesia* genus and gel-purified 16S ribosomal RNA for the *Ehrlichia* genus and *M. haemocanis* were sequenced in the sense and antisense directions by use of a dideoxy terminator method.

3. Results and discussion

Amplicons of the expected size for the genus *Babesia* were observed in 4 of the 16 dogs with inclusion bodies. The sequence of the 18S ribosomal RNA yield a 460 base pair segment that had a 99% (458/460) homology with the *B. gibsoni* genotype Asia 1 (GenBank no. AF175300). However, both mutations are within the primer sequence. Homology with *B. gibsoni* genotype Asia 1 is 100% (424/424) if the primer sequences are removed. Amplicons of the expected size for *E. canis* were obtained in two dogs. The sequence of the 16S ribosomal RNA revealed a 563-bp fragment with 99% (533/536) homology with *E. canis* strain Jake (complete genome in GeneBank no. CP000107). In the two dogs that did not have ehrlichiosis, amplicons of the expected size for *M. haemocanis* were observed. Sequence of the 16S ribosomal RNA revealed a 393-bp fragment with 100% homology with *M. haemocanis* (GenBank no. AF197337) in one dog and 99% (391/393) in the other.

Intraerythrocytic inclusion bodies suggestive of haemoplasmosis were found in the peripheral blood smear of both dogs simultaneously infected with *B. gibsoni* and *M. haemocanis.* These two dogs were previously included in a case series of haemotropic mycoplasmosis in non-splenectomized dogs (de Morais et al., 2003). Coinfection with *E. canis* and *B. gibsoni* was found in two intact female dogs. Clinical findings, hematological abnormalities, therapy and outcome for all four dogs are summarized on Table 1. Those are the first confirmed cases of *B. gibsoni* infection in dogs in South America. *B. gibsoni* was likely an incidental finding in one dog. In the remaining animals, non-specific signs previously reported in experimental *B.*