Detection of *Hepatozoon* spp in Naturally Infected Brazilian Dogs by Polymerase Chain Reaction

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ABSTRACT

Hepatozoon canis was molecularly identified in Rio de Janeiro State, Brazil. Twelve dogs from urban areas were studied by blood smear examination and the polymerase chain reaction (PCR) assay. From these dogs, only 1 was positive in both blood smears and PCR.

INTRODUCTION

Canine hepatozoonosis is a tick-borne disease caused by the protozoan *Hepatozoon*. There are 2 described species: *Hepatozoon canis*, transmitted by the dog tick *Rhipicephalus sanguineus*, and *Hepatozoon americanum*, transmitted by *Amblyomma maculatum*.[1,2]

In Brazil, the canine *Hepatozoon* infection has been diagnosed during laboratory examinations[3-5] and during epidemiological studies in urban and rural areas.[6-9] Previous studies observed high prevalence (39.2%) in dogs from rural areas from Rio de Janeiro State[7] and low prevalence (5.9%) in stray dogs from São Paulo State, Brazil.[8] *Rhipicephalus sanguineus* is more common in urban than in rural areas, whereas in rural areas *Amblyomma* spp is more prevalent on dogs.[10] A positive correlation between *Amblyomma* spp and *H canis* infection were observed.[7] Recently in Brazil, the canine *Hepatozoon* species was molecularly identified and characterized for the first time.[11] The authors demonstrated that the Brazilian species was closely related to the Japanese isolate, which has 99% nucleotide identity with *H canis*, but showed the presence of one polymorphic site with a transversion

(T ↔ G). Other authors also observed close similarity between canine *Hepatozoon* from Brazil and the species described in Japan.[12] In 2005, *Hepatozoon* spp were diagnosed in *A ovale* and achieved transmission to dogs,[13] confirming the hypothesis that the *Amblyomma* spp could be associated with *H canis* infection in dogs from rural areas.[7] The higher frequency of *H canis* in rural than urban areas and the possibility that *Amblyomma* spp may be the most important vector of this protozoa, demonstrate the need to perform more epidemiological, transmission, and pathological studies about canine hepatozoonosis in Brazil.

The objective of this study was to report the first molecular identification of *H canis* in dogs from urban areas of Rio de Janeiro State, Brazil.

MATERIALS AND METHODS

Blood Samples

Twelve samples of venous blood were collected from dogs during the "Anti-rabies Vaccination Campaign" on October 23, 2004, in Rio de Janeiro, Brazil. The direct microscopic examination of Giemsa-stained blood smears were performed in all dogs. About 200 µL of blood was aliquoted into 1.5-mL eppendorf tubes and stored at –20°C until DNA was extracted.

DNA Extraction

DNA was isolated from 100 µL of EDTA blood with GFX™ Genomic Blood Purification Kit (Amersham Biosciences, Piscataway, New Jersey, USA) according to manufacturer instructions. DNA samples were eluted in 100 µL of UltraPure™ DNase/RNase-Free Distilled Water (Gibco/Invitrogen, Carlsbad, California, USA).

PCR Assay

Polymerase chain reaction (PCR) was conducted with set primers that amplified a partial 18S rRNA gene sequence of *Hepatozoon* spp. The forward primer HepF (5′ ATA-CAT-GAG-CAA-AAT-CTC-AAC 3′) and the reverse prime HepR (5′ CTT-ATT-CCA-TGC-TGC-AG 3′) were previous described.[14]

The following conditions were used: an initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 90 sec, followed by a final extension at 72°C for 5 min. PCR amplification was performed using a programmable thermal cycler (PTC-100, MJ Research, Inc., Waltham, Massachusetts, USA)

The reaction mixture (50 µL) contained 10 µL of extracted DNA, 1.5 U of Taq DNA polymerase (Amersham Biosciences, Piscataway, New Jersey, USA), 0.2 mM of each deoxynucleoside triphosphate, 0.25 µM of each primer, 1.5 mM of $MgCl_2$, 50 mM of KCl, and 10 mM of Tris-HCl pH 9.0 (Amersham Biosciences, Piscataway, New Jersey, USA)

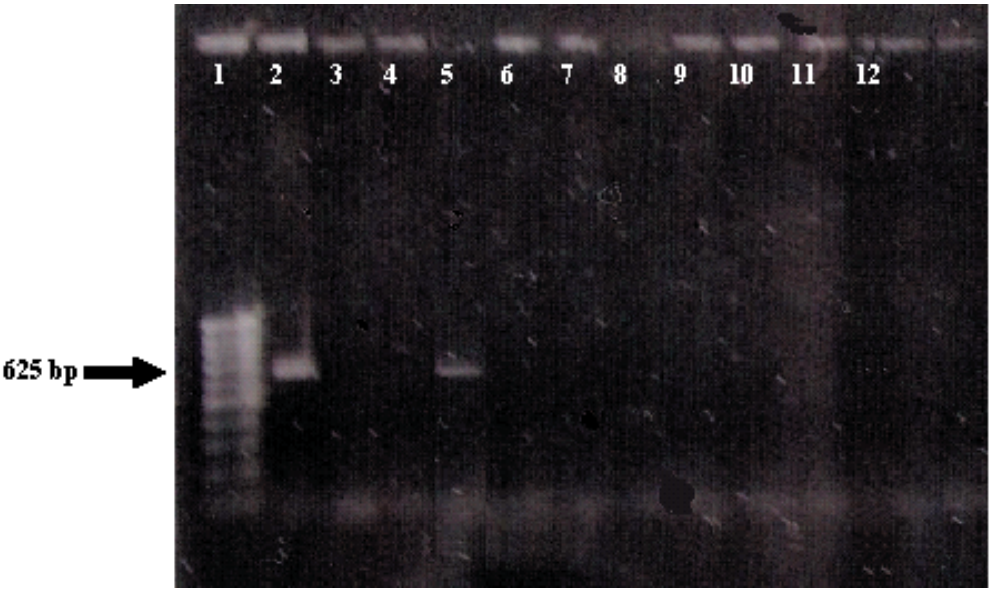
Results were visualized on 1% agarose gel electrophoresis and stained with ethidium bromide.

Positive and negative samples for H canis were used as control. To avoid DNA contamination, the extraction, amplification, and electrophoresis of the amplified products were done in different locales.

RESULTS AND DISCUSSION

Microscopic evaluation revealed *Hepatozoon* spp. gametocytes within neutrophils in stained peripheral blood smears from 1 of the 12 dogs (8.3%). The same result was achieved by PCR assay (Figure 1). Results of microscopic evaluation of blood smears and the PCR assay were negative for all other dogs. Although the number of examined dogs was low, the prevalence of the infection was less than 10% (like the results obtained in urban dogs from São Paulo State [5.9%]), and significantly lower than the results from rural areas (39.2%).[8] In this study, the results of the PCR assay were identical to the blood smear examination. Thirty-one dogs were examined from rural areas; 7 (22.6%) were identified as positive by blood smear examination and 21 (67.7%) positive by PCR, demonstrating the elevated occurrence of *H canis* infection in dogs from rural regions.[11] Our results showed the importance of ample molecular studies of this agent to better understand the epidemiology of *H canis* in Brazil.

Figure 1. PCR amplification of *Hepatozoon* rDNA from Brazilian dog samples. The products sizes were approximately 625 bp. (1) Molecular weight marker – 100 bp; (2) *Hepatozoon canis*-positive control; (3) Negative control; (4) Reaction control; (5) Positive sample; (6-12) Negative samples.



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