

Molecular detection of *Borrelia burgdorferi* in free-living golden headed lion tamarins (*Leontopithecus chrysomelas*) in Rio de Janeiro, Brazil

Dear Editor;

Borreliosis is an infectious disease caused by *Borrelia* spirochetes, transmitted mainly by *Ixodes ricinus* ticks¹ to animals or humans². The known *Borrelia* species determine five distinct disease groups: human recurrent fever, avian borreliosis, bovine borreliosis, Lyme borreliosis and bovine scrapie³⁻⁶. Lyme disease (LD) is a multisystem disease with a wide geographical distribution and has *B. burgdorferi* as the main agent in several continents^{5,7}.

In Brazil, borreliosis is called Brazilian Lyme-like disease, Baggio-Yoshinari Syndrome or Brazilian Borreliosis (BB), is transmitted by ticks that are not from the *Ixodes ricinus* complex, and is caused by *B. burgdorferi* sensu lato⁸.

Borrelia species were described in non-human primates that currently serve as an experimental model for Lyme disease^{2,9-11}.

Borrelia species are transmitted primarily by ticks, but in rare cases or experimentally, they can be transmitted by tabanids, culicids and siphonaptera¹². Studies on borreliosis have been carried out to compare immunological, histopathological and clinical responses of animals and humans infected with *Borrelia burgdorferi*. In these studies, animals were inoculated or exposed to the *Ixodides dammini* tick^{1,9,11}, and findings such as conjunctivitis, rash, deep perivascular lymphocytic infiltration that are characteristic in humans¹, a large amount of spirochetes in nervous tissue of immunosuppressed animals⁹ and spirochetes in cardiac tissue¹⁰ were reported.

In order to identify arthropod-borne pathogens in an exotic invasive population of *Leontopithecus chrysomelas* (golden-headed lion tamarin), that inhabit Serra da Tiririca State Park (PESET), an urban Atlantic Rainforest area in Niteroi (Rio de Janeiro, Brazil), to translocate them to their native area (Bahia, Brazil), blood samples were analyzed. PESET harbor a large diversity of endemic fauna and flora and is considered an important ecotourism area. Inappropriate housing constructions, the increase of human and animal populations living inside the park or in its surroundings, hunting and inadequate garbage disposal have been recently causing damage to the park¹³.

This study was approved by the Ethics Commission on Animal Use (CEUA) from Universidade Federal Fluminense (process N° 367, issued in 10/10/2013) and was in full compliance with the federal authorizations issued by the Environment Ministry (SISBIO n 30939-4 issued in 18/05/2012). All procedures were also approved by the Committee on Ethics on Animal Research (CEUA) of Veterinary Medicine and Animal Science Faculty of the Universidade de Sao Paulo (Protocol N° 2662/2012 and 7085041215).

Blood samples of 200 *L. chrysomelas*, males and females, of different ages and independent of clinical alterations were subjected to DNA extraction using the Illustra blood genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, Sao Paulo, Brazil), according to the manufacturer instructions. Extracted DNA was then used as the template DNA in *B. burgdorferi* sensu lato (s.l.) amplifications. Nested Polymerase Chain Reaction (n-PCR) assay using primer 5Bor-out (5'-GTCAAACGGGATGTAGCAA TAC-3') and

¹Universidade Federal Fluminense, Faculdade de Veterinária, Niterói, Rio de Janeiro, Brazil

²Instituto Pri-Matas para a Conservação da Biodiversidade, Belo Horizonte, Minas Gerais, Brazil

³Fundação Oswaldo Cruz, Plataforma Institucional Biodiversidade e Saúde Silvestre-Presidência, Rio de Janeiro, Rio de Janeiro, Brazil

⁴Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, São Paulo, Brazil

⁵Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

⁶Centro de Primatologia do Rio de Janeiro, Guapimirim, Rio de Janeiro, Brazil

⁷Universidade Federal do Espírito Santo, Programa de Pós-Graduação em Biodiversidade Tropical, São Mateus, Espírito Santo, Brazil

Correspondence to: Aline Vieira Pinheiro dos Santos
Universidade Federal Fluminense,
Faculdade de Veterinária, Rua Vital Brasil Filho, 64 Vital Brasil, CEP 21230360, Niterói, RJ, Brazil
Tel: +55 21 26299522

E-mail: alineds@id.uff.br

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3Bor-out (5'-CACACTTAACACGTTAGCTTCG-3'), followed by a second reaction with primer 5Bor-in (5'-ATTCAGTGGCGAACGGGTG-3') and 3Bor-in (5'-AACAACGCTCGCCCCTTAC-3'), which amplifies a fragment of 811 bp/ 469 bp of the 16S rRNA gene of *B. burgdorferi* sensu lato, were performed.

A total of 16% (32/200) *L. chrysomelas* were positive to *B. burgdorferi* by n-PCR. Sequencing of five positive samples showed 99% of similarity with *B. burgdorferi* sequences available in GenBank. This was the first time that borreliosis is found in non-human primates in Brazil.

Borrelia infection in humans was first described in Brazil in 1992, and has an increasing number of suspect cases, differing from LD due to the recurrence rate after treatment and the intense immune response⁸. The presence of *Borrelia burgdorferi* in free-living golden-headed lion tamarins that inhabit Atlantic Forest biome of Rio de Janeiro, Brazil, confirms that this spirochete is circulating in this region and suggests that these small primates may play a role in transmission of this pathogen to other animals or human beings. Veterinarians and medical doctors should consider this zoonotic pathogen in their diagnostic routine.

Further studies are needed, including sequencing of all positive samples, in order to compare these small primates positive sequences with those of humans and other animals in study area.

Aline Vieira Pinheiro dos Santos¹
 Aline Moreira de Souza¹
 Marina Galvão Bueno^{2,3}
 José Luiz Catao-Dias⁴
 Helena Keiko Toma⁵
 Alcides Pissinati^{4,6}
 Camila Vieira Molina²
 Maria Cecília Martins Kierulff^{2,7}
 Danilo Gomes Freitas Silva^{2,4}
 Nádia Regina Pereira Almosny¹

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