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Brief communication

Molecular evidence of *Borrelia burgdorferi sensu lato* in patients in Brazilian central-western region

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ABSTRACT

We aimed to detect DNA of *Borrelia burgdorferi* in whole blood and serum samples of patients with clinical symptoms and epidemiology compatible with Brazilian Lyme-like disease. Four patients with positive epidemiological histories were recruited for the study. Blood samples were collected, screened by serologic testing by ELISA and Western blotting and molecular identification of *B. burgdorferi* by amplifying a fragment of the conserved gene that synthesizes the hook flagellar flgE. The results showed positive serology and for the first time, the presence of *B. burgdorferi sensu lato* in humans in the Midwest region of Brazil. The resulting sequences were similar to GenBank corresponding sequences of *B. burgdorferi flgE* gene. By neighbor-joining the phylogenetic analysis, the flgE sequence of the Brazilian strain clustered in a monophyletic group with the sequence of *B. burgdorferi sensu lato* under 100% bootstrap support. This study opens up promising perspectives and reinforces the need for additional studies to determine the epidemiological characteristics of the disease, as well as the impact of the prevalence of Brazilian borreliosis in Mato Grosso do Sul State, Brazil.

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Evidência molecular de *Borrelia burgdorferi sensu lato* em pacientes no centro-oeste brasileiro

R E S U M O

Palavras-chave:

Borrelia burgdorferi

Doença de Lyme

flgE

Síndrome de Baggio-Yoshinari

Brasil

Este estudo promoveu a detecção de DNA de *Borrelia burgdorferi sensu lato* em amostras de sangue e soro de pacientes com manifestações clínicas e epidemiologia compatíveis com a doença de Lyme-símile brasileira ou síndrome de Baggio-Yoshinari. Para tanto, foi feita triagem sorológica pelos métodos de Elisa e *Western blotting* e a identificação molecular de *B. burgdorferi* por meio da amplificação de um fragmento do gene conservado que sintetiza o gancho flagelar (*flgE*). Os resultados demonstraram sorologia positiva e, pela primeira vez, a presença de DNA de *Borrelia burgdorferi sensu lato* em humanos na Região Centro-Oeste do Brasil. A análise genética das sequências dos isolados mostrou similaridade às sequências disponíveis no GenBank. Pela análise filogenética inferida pela sequência parcial do gene *flgE*, a cepa brasileira agrupou-se com a sequência de *B. burgdorferi sensu lato*. Este estudo abre perspectivas promissoras e reforça a necessidade de estudos adicionais a fim de determinar as características epidemiológicas da doença, bem como o impacto da prevalência da borreliose brasileira no Estado de Mato Grosso do Sul, Brasil.

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Introduction

Lyme disease (LD) is an emerging multisystemic zoonosis, caused by spirochetes of the *Borrelia burgdorferi sensu lato* group and transmitted by ticks of the *Ixodes ricinus* complex.¹

This is a disease with wide geographical distribution, and its clinical manifestations vary according to the species of *B. burgdorferi sensu lato* complex found in a certain geographical location.^{2,3} The etiological and antigenic diversity explains the organotropism and the appearance of different clinical and laboratory pictures in different regions, presenting an increasing challenge to the management of this emerging zoonosis.¹

In Brazil, the leading cases were described in the state of São Paulo in 1992,⁴ and since then other cases have been described with the use of serological and molecular techniques in several Brazilian states: Mato Grosso do Sul,⁵⁻⁷ Amazonas,⁸ Tocantins,⁹ and Paraná.¹⁰

Differences observed in epidemiological, clinical and laboratory characteristics in relation to LD described in the northern hemisphere allowed characterizing the Brazilian Lyme Disease-like Syndrome (BLDS) or Baggio-Yoshinari Syndrome^{11,12} which, despite the classic migratory erythema and the usual systemic complications found in LD, frequently occurs with a high frequency of recurrences and the production of autoantibodies over its lengthy clinical evolution.¹³

Studies using molecular methods from human samples with BLDS-compatible symptomatology have reinforced the existence of cases of borreliosis in Brazil^{10,14} Thus, this study aimed to investigate the presence of *B. burgdorferi* DNA in samples from patients with a clinical and serological diagnosis of borreliosis in Mato Grosso do Sul, Brazil.

Patients and methods

Four female patients with a mean age of 33.3 (\pm 11.9) years old and presenting clinical manifestations and BLDS-compatible epidemiology treated at the Rheumatology Department, Hospital Universitario Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul (HUMAP-UFMS), were selected. These patients had a history of tick bites and of visits to high-risk areas in southeastern and midwestern regions of Brazil; they met the Brazilian criteria for the diagnosis of borreliosis, as adopted by the Laboratório de Investigação em Reumatologia (Rheumatologic Research Laboratory), Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (LIM-17), a reference center in Brazil.^{12,15}

One of the patients was in an acute phase (diagnosed within three months of onset of disease) and the other three were in a late phase (diagnosed more than 3 months after the onset of the disease) of borreliosis. The patient who had early Lyme presented flu-like symptoms, including fever, headache, myalgia, and arthralgia. The other three patients, who had late Lyme disease, developed arthritis and also cognitive disorders, manifesting with nonspecific symptoms, including memory loss, sleep disturbances and mood changes, a depressive mood with social indifference and loss of appetite, and recurrence of flu-like symptoms and chronic fatigue. For all patients studied, serological tests for *B. burgdorferi* G39/40, of North American origin, were positive, using ELISA and WB¹⁶ methodologies according to the standardization and interpretation recommended by LIM-17, a national reference laboratory.^{12,15}

Forty individuals in good general condition, with no history of tick bite or of a recent trip to high-risk areas, provided blood samples and were included in the control group. Thirty of these were female (75.0%) and 10 were male (25.0%). The mean age was 34.6 (\pm 19.2) years old.

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