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JSM Tropical Medicine and Research

Case Report

Detection of *Borrelia burgdorferi* Sensu Lato in Mato Grosso Do Sul, Brazil

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Submitted: 24 May 2016

Accepted: 01 August 2016

Published: 03 August 2016

Keywords
- *Borrelia burgdorferi*
- Lyme disease
- *FlgE*

Abstract

In this study, *Borrelia burgdorferi* sensu lato was molecularly detected in a human being and in *Rhipicephalus microplus* ticks of cattle from the same farm in the state of Mato Grosso do Sul, Brazil.

ABBREVIATIONS

BLD**:** Brazilian Lyme-like Disease; SBY: Síndrome de Baggio-Yoshinari; s.l.: Sensu Lato; s.s.: Sensu Stricto; PCR: Polymerase Chain Reaction; MS: Mato Grosso do Sul; UFMS: Federal University of Mato Grosso do Sul; UCDB: Dom Bosco Catholic University

INTRODUCTION

Baggio-Yoshinari Syndrome (BYS) or Brazilian Lyme-like disease (BLD), is an emerging multisystemic zoonotic disease, currently restricted to Brazil, caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l..) group with atypical morphology. The spirochete is transmitted by ticks, leading to the development of clinical manifestations similar to Lyme disease (LD), except for the high frequency of recurrent symptoms and immuno-allergic complications [1]. The heterogeneous clinical presentation of LD en linked to the genetic diversity of the *B. burgdorferi* s.l. complex in general and *B. burgdorferi* s.s. in particular. Only *B. burgdorferi* s.s. causes LD both in the USA and in Europe, with a wide spectrum of clinical conditions ranging from minor cutaneous erythema migrans (EM) to severe arthritis or neurological manifestations [2].

The BYS or BLD progresses in stages, with diagnosis based on the parameters of the National Reference Laboratory (LIM-17 HCFMUSP). Erythema migrans is indicative of the syndrome in the acute phase. During spread of the microorganisms, nonspecific signs and symptoms may appear, such as onset of fever and other flu-like symptoms, as well as new skin lesions. The lag phase or recurrence arises in the absence of an acute phase diagnosis or when conventional antibiotic treatment is inadequate or ineffective, causing cutaneous, neurological, articular and or cardiac secondary complications [3,4].

Laboratory diagnosis is based on detection of anti-*B. burgdorferi* antibodies, but the immunological response is weak and, although relevant for diagnosis, circulation of these antibodies is low and fluctuating, rapidly disappearing in the blood. Patients with BYS develop positive serology (Enzyme-linked Immunosorbent Assay/ELISA or Western blotting/WB) to *Borrelia burgdorferi* in approximately 65% of cases [5].

Thus, isolation and consequent characterization of the etiological agent responsible for BYS or BLD is still a major challenge in Brazil. Spirochetes have been detected in ticks, animals and humans in Brazil [1,5,6]. *In vitro* culturing of *Borrelia* spp. could be an alternative means of clarifying and confirming which spirochete species circulate in vertebrate and invertebrate BYS or BLD hosts in Brazil since. Currently, we do not know if the etiological agent belongs to the subtypes of *B. burgdorferi* s.l. complex, and/or subtypes of *B. burgdorferi* s.s., or whether they are spirochetes from a group related to the recurrent fever agent. Thus, this study investigates positive serology for *B. burgdorferi* DNA in patient samples and cell cultures of the tick, *Rhipicephalus microplus*, collected from the rural municipality of Campo Grande, Mato Grosso do Sul (MS), Brazil.

CASE PRESENTATION

We describe the case of a 49-year-old female patient presenting with chronic fatigue, anemia and arthralgia. The patient had a

history of frequent bites by ticks because of her work on a rural farm. Evaluated by the Rheumatology Department of University Hospital Maria Aparecida Pedrossian (HUMAP/UFMS–Ebserh), serology for BYS or BLD was requested (Opinion of Research Ethics Committee No. 1,065,681). Serology testing (ELISA and WB) of the patient's samples was conducted by the Clinical Immunology Laboratory of the UFMS, and returned positive results for *Borrelia burgdorferi* s.l. *Rhipicephalus microplus* ticks were also collected from the workplace of the patient and, through embryonic tissue culture, mobile spirochetes were detected (Figure 1). PCR was performed on the patient's blood serum and on tick tissue cultures in the laboratory BIOTECH Sinova of UCDB, MS. The amplified product was sequenced and exhibited 100% homology with the *Borrelia burgdorferi* targeted *flgE* gene.

DISCUSSION

Our results provide the first evidence of *Borrelia burgdorferi* DNA in human and *Rhipicephalus microplus* tick cells in the State of Mato Grosso do Sul, Brazil, amplified for the *flgE* gene. The *flgE* gene encoding the flagellin protein (41 kDa) [7] comprises 1119 nucleotides and is responsible for synthesis of the flagellar hook structure. Its sequence diversity is valuable for distinguishing species of *Borrelia* since the hook structure is highly conserved. In a study performed by Madureira [6], the *16S* rRNA of *B. burgdorferi* was amplified and sequenced from a human blood sample and [1,5] amplified the *flgE* gene from patients presenting symptoms compatible with BYS or BLD and later showed that the sequences had 99% homology with *flgE* sequences from *B. burgdorferi*.

Clinical manifestations vary according to the species of the *B. burgdorferi* s.l. complex found in a particular geographic location. Etiologic and antigenic diversity explains the varying organotropism and the appearance of different clinical and laboratory symptoms in different regions [8]. Cultivation of *B. burgdorferi* in BSK medium from clinical samples has thus far not been possible, but structures suggestive of spirochetes in human blood samples of patients with a compatible clinical profile have been described [1,5].

The presence of anti-*Borrelia* antibodies, as evidenced by ELISA and WB, has previously been demonstrated in symptomatic and asymptomatic patients, with positive epidemiology in terms of contact with ticks in different regions of Brazil [9].

This study shows that *Borrelia spp*. can infect humans and ticks on the same farm. We suggest that further molecular and epidemiological investigations are warranted, including attempts to cultivate and isolate *Borrelia spp*. from human, arthropod and other animal samples within this region of Brazil. Because this is a cerrado region that includes the Pantanal, characterized by high faunal and floral diversity and a favored route of migratory birds it presents ideal conditions for live and reproduce and possible spread of pathogens.

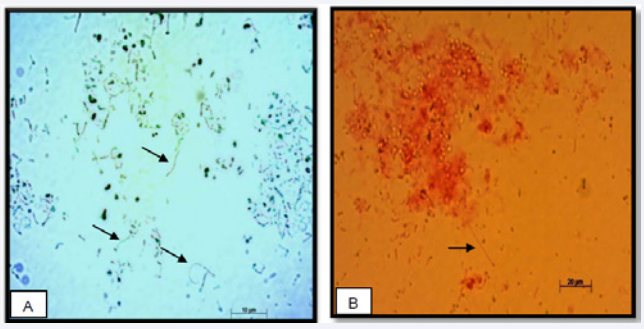

Figure 1 *In vitro* cultivation of spirochetes naturally infecting embryonic *Rhipicephalus microplus* cells. **A**- Unstained spirochetes (arrow), objective 100x, scale bar=10µm.
B- Gram-negative spirochete bacterium (arrow) under Gram staining, objective 100x, scale bar=20µm.

ACKNOWLEDGEMENTS

This study was supported by the National Council for Scientific and Technological Development (CNPq) and the Foundation to Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT). The authors thank the Laboratory of Research in Rheumatology HC-FMUSP (LIM-17) for cooperation during this study and Ana Rita Coimbra Motta de Castro (UFMS) and Cristiano Marcelo Espinola Carvalho (UCDB) for their invaluable collaboration in this study.

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Cite this article

de Rezende J, Lopes FA, de Cássia Gonçalves Alves F, Bruno AR, Moreno SE, et al. (2016) Detection of Borrelia burgdorferi Sensu Lato in Mato Grosso Do Sul, Brazil. JSM Trop Med Res 1(1): 1003.