Borrelia burgdorferi sensu lato in humans in a rural area of Paraná State, Brazil

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Abstract

This study describes the detection of Borrelia garinii and Borrelia burgdorferi sensu stricto (s.s.) in Brazilian individuals using PCR and DNA sequencing. Our results suggest that these species are emerging pathogens in this country, and additional studies are necessary to determine the epidemiological characteristics of this disease in Brazil.

Key words: Brazil, human, lyme borreliosis, PCR, sequencing.

Lyme borreliosis (LB) is a tick-borne disease caused by genospecies of the Borrelia burgdorferi sensu lato (s.l.) complex (Steere, 1997). The genospecies causing LB vary according to the geographic region: B. andersonii, is mainly found in North America, B. afzelii and B. garinii in Europe, B. japonica in Japan, and B. burgdorferi sensu stricto (s.s.) has been detected on several continents (Qiu et al., 2008; Rudenko et al., 2011). Migratory birds cause the dissemination of Borrelia spp. between continents, and the establishment and maintenance of these spirochetes in a new environment depends on the presence of their reservoir hosts (tick species) and host-vector interactions (Hasle, 2013; Norte et al., 2013).

In Europe and North America, B. burgdorferi genospecies causing LB are mainly transmitted by the tick Ixodes ricinus (Steere, 1997; Qiu et al., 2008; Rudenko et al., 2011). In contrast, in Brazil, some studies have indicated the presence of these spirochetes in ticks from the Amblyomma, Rhipicephalus and Dermacentor genera, demonstrating the need for further studies to determine the vectors able to transmit LB in this country (Yoshinari et al., 2010; Gonçalves et al., 2013; Montovani et al., 2013).

In Brazil, this disease, which is known as Brazilian lyme-like disease or Baggio-Yoshinari syndrome, has been poorly studied (Yoshinari et al., 2010). Therefore, its epidemiology and most prevalent genospecies are not well defined (Dantas-Torres et al., 2008; Steps et al., 2009). Cases of this disease have been detected in humans and animals by serologic methods and/or by clinical symptoms in the northern (Amazonas and Tocantins States) (Abel et al., 2000; Carranza-Tamayo et al., 2012), midwestern (Mato Grosso do Sul State) (Costa et al., 2002; Naka et al., 2008), southeastern (Espírito Santo, Rio de Janeiro and São Paulo States) (Azulay et al., 1991; Passos et al., 2009; Yoshinari et al., 2003, 2010) and southern (Paraná State) (Gonçalves et al., 2013a, 2013b) regions of Brazil. Most of the cases affecting humans have been detected in inhabitants of rural areas, where the incidence of this zoonosis is high due to the close proximity of humans to the animal population, which are often parasitized by ticks.

Despite these findings, studies have reported negative serology in most of the individuals showing clinical signs of this disease and have failed to define its etiologic agent (Yoshinari et al., 2010). Studies involving this pathogen in Brazil have mainly assessed serology; thus, the aim of this
Of the 207 human serum samples analyzed, two (0.96%) showed positive nested-PCR results for the B. burgdorferi s.l. complex with amplicon sizes of ~230 bp. The BLAST analysis showed high sequence similarity (100%) with two different Borrelia genospecies. The nucleotide sequences obtained have been submitted to the GenBank database under the accession numbers KF790698 and KF790699. One strain (J-70) was identified as B. garinii, and the other strain (J-96) was identified as Borrelia burgdorferi s.s. (Figure 1A and 1B). Both samples were identified in males (15 and 72 years old, respectively) who worked with different animal species and performed various functions, such as assisting with births and slaughtering and castrating cattle. The analysis of the variables associated with the presence of Borrelia burgdorferi s.l. DNA is shown in Table 1.

The two nested PCR-positive serum samples for B. burgdorferi s.l. in this study have also been detected by indirect immunofluorescence assay (IFA) and western blot (WB) in a previously published study (Gonçalves et al., 2013a).

Brazilian lyme-Like disease, or Baggio-Yoshinari syndrome, was first reported in Brazil in 1992, but the causative agent of Borrelia infection has not been isolated or identified to date (Yoshinari et al., 2003, 2010). Many aspects of the disease, such as the symptoms and frequency of recurrence after treatment, appear to differ in Brazilian individuals compared with those inhabiting the northern hemisphere (Yoshinari et al., 2010). Moreover, Amblyomma cajennense and Rhipicephalus microplus ticks are believed to be involved in the transmission cycle of B. burgdorferi s.l. (Barros-Battesti et al., 2000; Yoshinari et al., 2003; Yparraguire et al., 2007).

Researchers from different countries have detected B. burgdorferi s.l. DNA in ticks of the Dermacentor (Gonçalves et al., 2013b; Lledó et al., 2014), Ixodes (Leyedet et al., 2014; Morshed et al., 2006; Hjgaard et al., 2014; Dingler et al., 2014; Prusinski et al., 2014; Masuzawa et al., 2014; Barbieri et al., 2013) and Rhipicephalus (Maia et al., 2014; Niu et al., 2014) genera, which parasitize humans and different animal species. These studies have contributed to the understanding of borreliosis epidemiology, as they have indicated the main vectors involved in the transmission of this disease according to the region studied.

The presence of Borrelia burgdorferi s.l. was detected in Brazilian individuals by serological and molecular tests. Different researchers have demonstrated the presence of antibodies against B. burgdorferi s.s. and B. garinii by WB and/or ELISA tests in symptomatic and asymptomatic humans with histories of contact with ticks in Brazil (Costa et al., 2002; Naka et al., 2008; Gonçalves et al., 2013a).

A recent study in Brazil detected the flgE gene from B. burgdorferi by PCR and DNA sequencing in three peripheral blood samples collected from humans with clinical symptoms of borreliosis and histories of tick exposure.
Figure 1 - BLAST sequence analysis. (A) Alignment comparison of sequences generated from serum sample J-96 with the 5S(rrf)-23S (rrl) intergenic spacer region of *Borrelia burgdorferi* B31 strain (AE000783.1); (B) Alignment comparison of sequences generated from serum sample J-70 with the 5S (rrf)-23S (rrl) intergenic spacer region of *Borrelia garinii* (Pbi strain) (CP000013.1).

Table 1 - Variables associated with the presence of DNA *Borrelia burgdorferi* s.l. in serum samples from 207 residents of the rural area of Jataizinho (PR), 2007.

| Disease variables       | Positive DNA total (%) | p value  | OR CI (95%) |
|-------------------------|------------------------|----------|-------------|
| Lyme Borreliosis        |                        |          |             |
| Ticks attached to the body |                      |          |             |
| Yes                     | 02/16 (12.50)          | 0.0056*  |             |
| No                      | 00/191 (0.00)          |          |             |
| Presence of ticks inside of house |            |          |             |
| Yes                     | 02/27 (7.40)           | 0.0164*  |             |
| No                      | 00/180 (0.00)          |          |             |

p = probability; * Fisher’s exact test; OR = Odds ratio; CI = Confidence interval (Gonçalves *et al.*, 2013).
(Mantovani et al., 2012). Gonçalves et al. (2013b) also detected the presence of these bacteria in Brazil using PCR and DNA sequencing, indicating that the detected DNA sequences in two ticks of the Dermacentor nitens species shared 99.99% homology with the B. burgdorferi sensu stricto (s.s.) strain B31. Despite these findings, further studies are necessary to delineate the presence of this pathogen in Brazil.

In the present study, B. garinii and B. burgdorferi s.s. were detected by molecular methods for the first time in residents of rural areas, who were directly or indirectly exposed to wild and/or domestic animals and ticks in the northern region of Parana State, confirming the presence of these genospecies in Brazil. The variables studied, such as the presence of ticks inside homes (p = 0.0164) and the presence of ticks attached to the body (p = 0.0056), were significant when associated with the B. burgdorferi s.l. DNA findings. These data are in accordance with other studies, which have also associated tick exposure with illness in humans by serological techniques (Yoshinari et al., 2003, 2007; Mantovani et al., 2012; Gonçalves et al., 2013a).

However, the low frequency of Borrelia genospecies observed can be justified if these species are emerging pathogens in the country due to the dissemination of B. burgdorferi s.l. by migratory birds, and this hypothesis should not be discarded (Yoshinari et al., 2010; Hasle, 2013).

Studies of the Brazilian lyme-Like disease, or Baggio-Yoshinari syndrome, have revealed differences in epidemiological, clinical and laboratory characteristics compared with those reported in affected individuals in the northern hemisphere, suggesting the existence of differing etiological agents in the two locations (Yoshinari et al., 2010). In Brazil, despite the wide geographical distribution of both invertebrate and vertebrate hosts for Borrelia spp., there are few descriptions of these spirochetes. Thus, further serological and molecular studies are needed in humans, different species of domestic and wild animals, and ticks, in particular, to better understand the epidemiology of Borrelia spp.

Ethics Committee

This research was approved by the Committee of Ethics in Research involving Humans (CEP) from the State University of Londrina (UEL) (No. 319/06).

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