Brazilian Journal of Microbiology 46, 2, 571-575 (2015)
ISSN 1678-4405
DOI: http://dx.doi.org/10.1590/S1517-838246220140097

Short Communication

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

Daniela Dib Gonçalves[1,2], Rodrigo Assunção Moura[2], Mônica Nunes[3], Teresa Carreira[3], Odilon Vidotto[4], Julio Cesar Freitas[4], Maria Luísa Vieira[3]

[1]Programa de Pós-gradução em Ciência Animal, Universidade Estadual de Londrina, Londrina, PR, Brazil.
[2]Medicina Veterinária Preventiva, Universidade Paranaense, Umuarama, PR, Brazil.
[3]Leptospirosis and Lyme Borreliosis Group, Medical Microbiology Unit, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Lisbon, Portugal.
[4]Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina, Londrina, PR, Brazil.

Submitted: February 2, 2014; Approved: August 7, 2014.

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. afzelli* 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, 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

Send correspondence to D.D. Gonçalves. Programa de Pós-Gradução em Ciência Animal, Universidade Estadual de Londrina, Londrina, PR, Brazil / Medicina Veterinária Preventiva, Universidade Paranaense, Umuarama, PR, Brazil. E-mail: danieladib@unipar.br.

study was to use molecular methods to determine the particular species of the *B. burgdorferi* s.l. complex that are present in humans in a small rural area in the northern region of Parana State, Brazil.

From February to November 2007, blood samples were collected voluntarily from 207 asymptomatic humans between 15 and 72 years of age living on 63 small rural properties in the northern region of Parana State. These residents also worked on family farms with animals.

After collection, the blood samples were forwarded to the Leptospirosis Laboratory of Preventive Veterinary Medicine Department at Universidade Estadual de Londrina (UEL) to obtain serum samples. Each sample was kept in a sterile container and stored at -20 °C until its use in the molecular tests, which were performed at the Laboratory of Leptospirosis and Lyme Borreliosis, Medical Microbiology Unit, Institute of Hygiene and Tropical Medicine (IHMT), Universidade Nova de Lisboa (UNL), Portugal.

DNA from the serum samples and the *B. garinii* culture (strain PBi), which contained approximately 2 x 10^7 cells/mL (used as a positive control), was extracted using the Puregene™ Gentra Cell & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturers protocol. The extracted DNA was stored at -20 °C until further use.

The detection of the *B. burgdorferi* s.l. complex genospecies was performed by nested PCR targeting the 5S(*rrf*)-23S(*rrl)* intergenic spacer region, as previously described (Schwartz *et al.*, 1992; Postic *et al.*, 1994; Chao *et al.*, 2011). Amplicons of ~226-266 bp, depending on the strain of *Borrelia* spp., were purified, and both strands were directly sequenced by the Macrogen Sequencing Service, Inc. (Seoul, Korea). The primers used for DNA amplification were also used for sequencing. The results obtained were compared with existing Borrelia genospecies sequences in the GenBank database using the BLAST sequence analysis tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

An epidemiological survey was also conducted using a structured questionnaire, in which the participants answered questions regarding risk factors related to the disease, including education level, the presence of domestic animals (dogs, cats and others), wild animals and rodents at the rural property, the presence of ticks attached to the body and the observation of ticks inside homes (Gonçalves *et al.*, 2013).

The data obtained from the epidemiological survey were statistically analyzed using Yates or Fisher's exact test with a chi-square (χ^2) correction. The tabulations of the epidemiological data and analyses were performed using the EpiInfo statistical program version 6.04 (CDC) at a 5% significance level. As an association measure, odds ratios (ORs) were calculated with confidence intervals of 95% (Dean *et al.*, 1994).

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; Yparraguirre *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; Prusinki *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

```
>gb|AE000783.1| Borrelia burgdorferi B31, complete genome                    A
Length=910724

 Score =   407 bits (450),  Expect = 3e-110
 Identities = 225/225 (100%), Gaps = 0/225 (0%)
 Strand=Plus/Plus

Query  4       CATAGACTCTTATTACTTTGACCATATTTTTATCTTCCATCTCTATTTTGCCAATTTATT  63
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438241  CATAGACTCTTATTACTTTGACCATATTTTTATCTTCCATCTCTATTTTGCCAATTTATT  438300

Query  64      TATACAACATAAAATAATATATATCTTTGTTTAATACATGTCAATATATATTTTATTTTT  123
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438301  TATACAACATAAAATAATATATATCTTTGTTTAATACATGTCAATATATATTTTATTTTT  438360

Query  124     TATGTTATTTAAACAACACATTCAAAAACACCAATATTTAAAAAACATAAAAATAAAATC  183
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438361  TATGTTATTTAAACAACACATTCAAAAACACCAATATTTAAAAAACATAAAAATAAAATC  438420

Query  184     AAAGTTTAAAGTATAAAAATAAAAACCCTGGCAATAACCTACTCT  228
               |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438421  AAAGTTTAAAGTATAAAAATAAAAACCCTGGCAATAACCTACTCT  438465


>gb|CP000013.1| Borrelia garinii PBi, complete genome                         B
Length=904246

 Score =   387 bits (428),  Expect = 2e-104
 Identities = 214/214 (100%), Gaps = 0/214 (0%)
 Strand=Plus/Plus

Query  1       CATAGACTCTTATTACTTTGACCATATTTTTATCTTCCATCTCTATTTTGCCAATTTGTT  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438886  CATAGACTCTTATTACTTTGACCATATTTTTATCTTCCATCTCTATTTTGCCAATTTGTT  438945

Query  61      TATGCAACATAGAATAATATATATCTTTGTTTAATCCATGTCAATATATATTTTATTTTT  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438946  TATGCAACATAGAATAATATATATCTTTGTTTAATCCATGTCAATATATATTTTATTTTT  439005

Query  121     TATATTATTTGAATAAAACATTCAAAAAAATGAACATCTAAAAACATAAAAAATAAAATC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439006  TATATTATTTGAATAAAACATTCAAAAAAATGAACATCTAAAAACATAAAAAATAAAATC  439065

Query  181     AATGTTTAAAATATAAAATAAAAACCCTGGCAAT  214
               |||||||||||||||||||||||||||||||||
Sbjct  439066  AATGTTTAAAATATAAAATAAAAACCCTGGCAAT  439099
```

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 laboratorial 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).

Acknowledgments

We would like to acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), the technical support of the Laboratory of Leptospirosis and Lyme Borreliosis, Medical Microbiology Unit, Institute of Hygiene and Tropical Medicine (IHMT) from the Universidade Nova de Lisboa (UNL), Portugal, and Universidade Paranaense (UNIPAR) for the financial support.

References

Abel IS, Marzagao G, Yoshinari NH *et al.* (2000) Borrelia-like spirochetes recovered from ticks and small mammals collected in the Atlantic Forest Reserve, Cotia county, State of São Paulo, Brazil. Mem Inst Oswaldo Cruz 95:621-624.

Azulay RD, Azulay-Abulafia L, Sodre CT *et al.* (1991) Lyme disease in Rio de Janeiro, Brazil. Int J Dermatol 30:569-571.

Barbieri AM, Venzal JM, Marcili A *et al.* (2013) *Borrelia burgdorferi* sensu lato infecting ticks of the *Ixodes ricinus* complex in Uruguay: first report for the Southern Hemisphere. Vector Borne Zoonotic Dis 13:147-53.

Barros-Battesti DM, Yoshinari NH, Bonoldi VL *et al.* (2000) Parasitism by *Ixodes didelphidis* and *I. loricatus* (Acari: *Ixodidae*) on small wild mammals from an Atlatic Forest in the State of Sao Paulo, Brazil. J Med Entomol 37:820-827.

Carranza-Tamayo CO, Costa JN, Bastos WM (2012) Lyme disease in the state of Tocantins, Brazil: report of the first cases. Braz. J Infect Dis 16:586-589.

Chao LL, Chen YJ, Shih CM (2011) First isolation and molecular identification of Borrelia burgdorferi sensu stricto and Borrelia afzelii from skin biopsies of patients in Taiwan. Int J Infect Dis 15:182-187.

Costa IP, Bonoldi VL, Yoshinari NH (2002) Search for Borrelia sp. in ticks collected from potential reservoirs in an urban forest reserve in the State of Mato Grosso do Sul, Brazil: a short report. Mem Inst Oswaldo Cruz 97:631-635.

Dantas-Torres F (2008) Canine vector-borne diseases in Brazil. Parasites & Vectors 1:25.

Dean A, Dean J, Coulombier D *et al.* (1994) Epi info. A word processing database and statistics program for epidemiology on microcomputers [computer program]. Version 6.

Dingler RJ, Wright SA, Donohue AM *et al.* (2014) Surveillance for *Ixodes pacificus* and the tick-borne pathogens *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in birds from California's Inner Coast Range. Ticks Tick Borne Dis. pii: S1877-959X(14)00047-8. doi: 10.1016/j.ttbdis.2014.02.002. [Epub ahead of print].

Gonçalves DD, Benitez A, Lopes-Mori FM *et al.* (2013a) Zoonoses in humans from small rural properties in Jataizinho, Parana, Brazil. Braz J Microbiol 44:125-131.

Gonçalves DD, Carreira T, Nunes M *et al.* (2013b) First record of Borrelia burgdorferi B31 strain in Dermacentor nitens ticks in the northern region of Parana (Brazil). Braz J Microbiol 44:883-887.

Hasle G (2013) Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front Cell Infect Microbiol 3:48.

Hojgaard A, Lukacik G, Piesman J (2014) Detection of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti*, with two different multiplex PCR assays. Ticks Tick Borne Dis 5:349-351.

Leydet BF Jr, Liang FT (2014) Detection of Lyme Borrelia in questing *Ixodes scapularis* (Acari: Ixodidae) and small mammals in Louisiana. J Med Entomol 51:278-282.

Lledó L, Gegúndez MI, Giménez-Pardo C *et al.* (2014) A seventeen-year epidemiological surveillance study of *Borrelia burgdorferi* infections in two provinces of northern Spain. Int J Environ Res Public Health 11:1661-1672.

Maia C, Ferreira A, Nunes M *et al.* (2014) Molecular detection of bacterial and parasitic pathogens in hard ticks from Portugal. Ticks Tick Borne Dis pii: S1877-959X(14)00043-0. doi: 10.1016/j.ttbdis.2014.01.009 [Epub ahead of print].

Mantovani E, Marangoni RG, Gauditano G *et al.* (2012) Amplification of the *flgE* gene provides evidence for the existence of a Brazilian borreliosis. Rev Inst Med Trop S Paulo 54:153-158.

Masuzawa T, Masuda S, Fukui T *et al.* (2014) PCR detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in *Ixodes persulcatus* ticks in Mongolia. Jpn J Infect Dis 67:47-49.

Morshed MG, Scott JD, Fernando K *et al.* (2006) Distribution and characterization of *Borrelia burgdorferi* isolates from *Ixodes scapularis* and presence in mammalian hosts in Ontario, Canada. J Med Entomol 43:762-773.

Naka EN, Costa IP, Arão CAB *et al.* (2008) Detection of anti-Borrelia and anti-Babesia antibodies in the serum of children with clinical manifestations and compatible epidemiology with Lyme-Like disease in the State of Mato Grosso do Sul. Rev Bras Reumat 48:74-85.

Niu Q, Guan G, Yang J *et al.* (2011) Detection and differentiation of *Borrelia burgdorferi* sensu lato in ticks collected from sheep and cattle in China. BMC Vet Res 7:17.

Norte AC, Lobato DN, Braga EM *et al.* (2013) Do ticks and Borrelia burgdorferi s.l. constitute a burden to birds? Parasitol Res 112:1903-1912.

Passos SD, Gazeta RE, Latorre MR *et al.* (2009) Epidemiological characteristics of Lyme-like disease in children. Rev Assoc Med Bras 55:139-144.

Postic D, Assous MV, Grimont PA *et al.* (1994) Diversity of Borrelia burgdorferi sensu lato evidenced by restriction fragment length polymorphism of *rrf(5S)-rrl(23S)* intergenic spacer amplions. Int J Syst Bacteriol 44:743-752.

Prusinski MA, Kokas JE, Hukey KT *et al.* (2014) Prevalence of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) collected from recreational lands in the Hudson Valley Region, New York State. J Med Entomol 51:226-236.

Qiu WG, Bruno JF, McCaig WD *et al.* (2008) Wide distribution of a high-virulence Borrelia burgdorferi clone in Europe and North America. Emerg Infect Dis 14:1097-1104.

Rudenko N, Golovchenko M, Grubhoffer L *et al.* (2011) Updates on Borrelia burgdorferi sensu lato complex with respect to public health. Ticks Tick Borne Dis 2:123-128.

Schwartz JJ, Gazumyan A, Schwartz I (1992) rRNA gene organization in the Lyme disease spirochete, Borrelia burgdorferi. J Bacteriol 174:3757-3765.

Steere AC (1997) Diagnosis and treatment of Lyme arthritis. Med Clin North America 81:179-194.

Yoshinari N, Spolidorio M, Bonoldi VL *et al.* (2007) Lyme disease like syndrome associated lymphocytoma: first case report in Brazil. Clinics 62:525-526.

Yoshinari NH, Abrao MG, Bonoldi VL *et al.* (2003) Coexistence of antibodies to tick-borne agents of babesiosis and Lyme borreliosis in patients from Cotia county, State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz 98:311-318.

Yoshinari NH, Mantovani E, Bonoldi VLN *et al.* (2010) Brazilian lyme-like disease or Baggio-Yoshinari syndrome: exotic and emerging Brazilian tick-borne zoonosis. Rev Assoc Med Bras 56:363-369.

Yparraguirre LA, Machado-Ferreira E, Ullmann AJ *et al.* (2007) A hard tick relapsing fever group spirochete in a Brazilian Rhipicephalus (Boophilus) microplus. Vector Borne Zoonotic Dis 7:717-721.

Associate Editor: Agnes Marie Sá Figueiredo