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Evidence of *Borrelia* in wild and domestic mammals from the state of Minas Gerais, Brazil

Evidência de *Borrelia* em mamíferos silvestres e domésticos no Estado de Minas Gerais, Brasil

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Abstract

The main of the study was to evaluate the presence of *Borrelia burgdorferi* infection in domestic and wild vertebrates and ectoparasites in endemic areas from the state of Minas Gerais, Brazil. A total of 445 serum samples were examined by ELISA, which used the *Borrelia burgdorferi* strain G39/40 U.S. source and 3,821 tick samples were tested by polymerase chain reaction (PCR). *B. burgdorferi* antibodies were found in 30 serum samples (6.74%); three in marsupials (7.69%), three in rodents (2.80%), nine in dogs (6.25%), and 15 in horses (9.68%). Nested-PCR performed in DNA samples obtained from collected ticks demonstrated negative results. Although attempts to amplify *B. burgdorferi* DNA from ticks had been not successful, the presence of seroreactive vertebrates suggests the possibility the *Borrelia* species circulating in these regions. Further research is required to provide information on the presence of *Borrelia* in Brazilian territory and its association with Baggio-Yoshinari syndrome.

Keywords: Lyme-disease, Baggio-Yoshinari Syndrome, tick-borne diseases, borreliosis.

Resumo

O principal objetivo do estudo foi avaliar a presença de infecção por *Borrelia burgdorferi* em vertebrados domésticos e silvestres e ectoparasitas em áreas endêmicas do estado de Minas Gerais, Brasil. Um total de 445 amostras de soro foram examinadas por ELISA, onde usou-se a cepa americana G39/40 de *Borrelia burgdorferi* e 3.821 amostras de carrapatos foram testados pela reação em cadeia da polimerase (PCR). Anticorpos anti -*B. burgdorferi* foram encontrados em 30 amostras de soro (6,74%); três marsupiais (7,69%), três em roedores (2,80%), em nove cães (6,25%) e 15 em cavalos (9,68%). Nested-PCR realizada em amostras de DNA obtidas a partir de carrapatos coletados demonstraram resultados negativos. Apesar das tentativas para amplificar o DNA de *B. burgdorferi* a partir de carrapatos não tenha sido bem sucedido, a presença de soroatividade em vertebrados sugere a possibilidade de espécies de *Borrelia* circulando nestas regiões. Mais pesquisas são necessárias para fornecer informações sobre a presença de *Borrelia* em território brasileiro e sua associação com a Síndrome de Baggio-Yoshinari.

Palavras-chave: Doença de Lyme, Síndrome Baggio-Yoshinari, doenças transmitidas por carrapatos, borreliose.

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Microorganisms of genus *Borrelia* are Gram-negative multi-flagellated bacteria, belonging to family Spirochetaceae (KRUPKA et al., 2007). Lyme disease (LD) is considered the most important emerging zoonosis in the United States of America and Europe (KRUPKA et al., 2007), causing skin, joint, neurological, cardiac and ocular symptoms. LD is caused by *Borrelia burgdorferi sensu lato* bacterium complex, which comprises a group of 12 distinct species; it is transmitted by ticks of *Ixodes ricinus* complex (JOPPERT, 1995; RANKA et al., 2004).

In Brazil, a similar disease, referred as Brazilian Lyme disease-*simile* illness or Baggio-Yoshinari Syndrome (BYS) has been described, since last ending century (YOSHINARI et al., 2010; GOUVEIA et al., 2010). Mantovani et al. (2007) were unable to confirm whether this syndrome is caused by new *Borrelia* species or it is related to genetically modified *Borrelia burgdorferi sensu lato* microorganisms or other infectious agent.

Give that BYS is considered a zoonosis as LD, it is very important to verify the presence of the causing agents in wild and domestic animals and theirs ectoparasites. In this scenario, the goal of this research was to investigate the occurrence of antibodies against *B. burgdorferi* in domestic and wild animals and the presence of *Borrelia* DNA in ticks.

This study was realized in the municipalities of Santa Cruz do Escalvado (20°14'09" S 42°48'50" W), Pingo D'Água (19°43'37" S 42°24'28" W), and Caratinga (19°47'24" S 42°08'20" W). These municipalities are located in the Rio Doce Hydrographic Basin in the State of Minas Gerais, Brazil, being classified as endemic areas for tick-borne zoonosis, and characterized by intense anthropogenic destructive actions against the ecological system. In the municipalities of Santa Cruz do Escalvado and Pingo D'Água, blood samples from 445 animals were collected from the tail vein of rodents and marsupials, cephalic vein of dogs, and jugular vein of horses, as previously approved by Brazilian Institute of Environment and Natural Renewable Resources (IBAMA) and the Ethics Committee of the Federal University of Vicosa. These samples were stored at –20 °C until the time of use. After blood collection, the wild animals captured were morphologically identified (ROSSI et al., 2006; OLIVEIRA; BONVICINO, 2006) and returned to their natural habitat.

A total number of 3,821 ticks were collected from wild and domestic animals in the parasitic stage and from the ground in the municipalities of Santa Cruz do Escalvado, Pingo D'Água, and Caratinga. The ticks were taxonomically identified by external morphological characteristics according to Aragão and Fonseca (1961) and Linardi and Guimarães (2000); they were sorted into pools according to each mammalian parasitized, been maintained at 37 °C under biochemical oxygen demand (BOD) conditions for 48 hours. After that, the ectoparasites were separated in pools according to taxonomic identification and animal origin, followed by storage at –20 °C until DNA extraction.

Of 445 animals, 332 were from the municipality of Santa Cruz Escalvado - 38 *Didelphis aurita,* 64 rodents, 119 dogs and 11 horses -, and 113 were of Pingo D'Água - one *Didelphis aurita*, 43 rodents, 25 dogs, and 44 horses. The rodents were identified as *Nectomys squamipes*, *Rattus rattus*, *Oryzomys subflavus,* and *Akodon* sp. The serological test using immunoenzymatic assay (ELISA) as previously described (GRODZICKI; STEERE, 1988; COSTA, 1998) showed reactivity in 30 (6.74%) serum samples of 445 animals tested; 7.89% in marsupials (n= 3), 2.80% in rodents (n= 3), 6.25% in dogs (n= 9), and 9.68% in horses (n= 15) (Tables 1 and 2).

In this study we found high anti-*Borrelia* titers - 1:800 to 1:3,200 - among serum samples from dogs and horses in the municipality of Santa Cruz do Escalvado, where only three marsupials and two (3.13%) rodents presented antibodies against *B. burgdorferi*, with titers of 1:100 and 1:400. In Pingo D'Água municipality, one (2.33%) of 43 rodent, one (4.00%) of 25 dog and six (13.64%) of 44 horse serum samples were positive with titers from 1:100 to 1:800 titers (Table 2).

To roll out possible cross reaction, but not covering all of them, serology for leptospirosis pre-exposure against 30 different serovars was performed in all serum samples using microscopic agglutination test (MAT), as previously described (POSTIC et al., 2000). In the analyzed samples, 20 (4.49%) showed reactivity against *B. burgdorferi* and *Leptospira* spp. antigens, being 211 animals (47.42%) *Leptospira* spp. reactive (Table 3).

Taxonomic identification of ectoparasites collected from marsupials, small rodents, dogs, and horses in the municipalities of Santa Cruz do Escalvado, Pingo D'Água, and Caratinga demonstrated the occurrence of the following genus/species of ticks in the studied animals species: *Amblyomma cajennense* (n= 1,926), *Amblyomma brasiliensis* (n= 1), *Amblyomma dubitatum* (n= 1),

Table 1. Frequencies of antibodies against *Borrelia burgdorferi* (ELISA - IgG) in wild and domestic animals from the municipalities of Santa Cruz do Escalvado and Pingo D'Água, Minas Gerais State, Brazil.

Animals		Municipalities				Total Positive Samples (%)
		Santa Cruz do Escalvado		Pingo D'Água		
		n	Positive Samples (%)	n	Positive Samples (%)	
Wild	Marsupials	38	3 (7.89)	1	0 (0.00)	3 (7.69)
	Rodents	64	2 (3.13)	43	1 (2.33)	3 (2.80)
	Subtotal	**102**	**5 (4.90)**	**44**	**1 (2.27)**	**6 (4.11)**
Domestic	Dogs	119	8 (6.72)	25	1 (4.00)	9 (6.25)
	Horses	111	9 (8.11)	44	6 (13.64)	15 (9.68)
	Subtotal	**230**	**17 (3.48)**	**69**	**7 (2.90)**	**24 (8.03)**
	Total	**332**	**22 (6.63)**	**113**	**8 (7.08)**	**30 (6.74)**

N.S.: n - number; % - percentage.

Table 2. Anti-*Borrelia* antibody titers in serum samples from animals captured in the municipalities of Santa Cruz do Escalvado and Pingo D'Água, state of Minas Gerais, Brazil.

Municipality	Animals	n	Antibodies titers						
			1:100	1:200	1:400	1:800	1:1,600	1:3,200	Total
Santa Cruz do Escalvado	Marsupials	38	1	1	1	0	0	0	3
	Rodents	64	1	0	1	0	0	0	2
	Dogs	119	0	0	4	3	0	1	8
	Horses	111	0	0	3	3	2	1	9
Pingo D'Água	Marsupials	01	0	0	0	0	0	0	0
	Rodents	43	1	0	0	0	0	0	1
	Dogs	25	1	0	0	0	0	0	1
	Horses	44	0	0	4	2	0	0	6
	Total	445	04	01	13	08	02	02	30

Note: Serology against *Borrelia* using *Borrelia burgdorferi* of North American origin strain G39/40 performed by ELISA. n - number.

Table 3. Serological response against *Leptospir*a spp. and *Borrelia burgdorferi* in wild and domestic animals (n= 445) from the municipalities of Santa Cruz do Escalvado and Pingo D'Água, Minas Gerais State, Brazil.

Organism	Frequencies of antibodies n (%)	
	Positive	Negative
Borrelia burgdorferi (only)	10 (2.25)	435 (97.75)
Leptospira spp. (only)	211 (47.42)	234 (53.58)
Borrelia burgdorferi + *Leptospira* spp.	20 (4.49)	425 (95.51)

Note: Serology against *Leptospira* spp. (30 serovars) performed by microscopic agglutination test; Serology against *Borrelia* using *Borrelia burgdorferi* of North American origin strain G39/40 performed by ELISA. n - number; % - percentage.

Amblyomma spp. (n= 74), *Rhipicephalus* (*Boophilus*) *microplus* (n= 198), *Rhipicephalus sanguineus* (n= 659), and *Dermacentor nitens* (n= 133). The following flea species were also identified: *Ctenocephalides canis* (n= 691), *Ctenocephalides felis* (n= 80), *Xenopsylla cheopis* (n= 19), *Pulex irritans* (n= 1), and *Rhopalopsyllus* sp. (n= 38).

The 3,821 ectoparasites collected were organized into 1,395 pools to DNA extraction, according host and evolutionary stage. The molecular analysis using the primers *flgE* 470 FW (5'-CGCCTATTCTAACTTGACCCGAAT-3') and *flgE* 470 Rev (5'-TTAGTGTTCTTGAGCTTAGAGTTG-3') was performed as previously described (SAL et al., 2008; BILLINGS et al., 1998; MANTOVANI, 2010). Nested-PCR assays presented negative results for the 3,821 DNA samples tested.

The results of our work support the evidence of *Borrelia* spp. infection in wild and domestic animals; it was lowest in rodents (2.80%) and highest in horses (9.68%). In the municipality of Santa Cruz do Escalvado the highest antibody titers were found, especially in serum samples collected from dogs and rodents. Similar results were observed by Alves et al. (2004) in dogs from the metropolitan region of Rio de Janeiro, Brazil, with titles ranging from 1:400 to 1:6,000.

Serological evidence of *B. burgdorferi* infection in dogs was observed in 6.25% animals; this frequency was similar to date previously reported in areas of risks to Lyme disease. In a study with 237 dogs from the risk area of Cotia, State of São Paulo, Joppert (1995) demonstrated by ELISA 33 animals (9.70%) showing IgG antibodies against *B. burgdorferi*.

In horses tested, 9.68% presented antibodies against *B. burgdorferi*, indicating the potential relevance of horses in the epidemiology of *Borrelia* spp. or non-identified BYS agent transmission. This result is similar to the results found in the municipalities of Três Rios and Vassouras in the State of Rio de Janeiro, Brazil (MADUREIRA et al., 2007).

In relation to wild animals, three of the 39 samples examined of marsupials (7.69%). showed antibodies against *B. burgdorferi*.

Barros-Battesti (1998) captured 62 marsupials and 72 rodents in a residential condominium located in the Atlantic Rain forest in the municipality of Itapevi, State of São Paulo, where the first cases of BYS in brothers were described in Brazil. This author observed under dark field microscopy the presence of *Borrelia*-like spirochetes in blood of 13.00% marsupials and 36.40% wild rodents. Despite of low frequency of antibodies against *B. burgdorferi* verified in rodents (2.80%), these animals are considered major reservoirs and carriers of some *Borrelia* species (PAVLOVSKY, 1965; BARBOUR; HAYES, 1986).

By nested-PCR, using *flgE* primers, the DNA samples from 3,821 ticks collected in three municipalities showed negative results. Similar results were obtained by Ataliba (2006), who used nested-PCR to verify the presence of *Borrelia* DNA in a total of 349 *A. cajennense* adult ticks collected from BYS suspected areas. We propose some explanations to justify these PCR negative results: (1) The etiological agent of BYS is quite different from the *B. burgdorferi* described in LD, since it is probably found at its atypical morphology cystic presentation (MANTOVANI et al., 2007, 2012; MANTOVANI, 2010); (2) The BYS is caused by a new *Borrelia* species, or by a genetically modified *B. burgdorferi* strain, or other spirochete that was not yet described, that do not can be correctly identified when using *B. burgdorferi* Northern hemisphere primers (MANTOVANI et al., 2007, 2012; YOSHINARI et al., 2010); (3) As a rule, enormous amounts of DNA are necessary to obtain positive nested-PCR with the use of *flgE* primer in Brazil (MANTOVANI, 2010; MANTOVANI et al., 2012).

In conclusion, although the obtained results require further studies aiming the characterization of the etiologic agent in both samples of wild and domestic animals, the research demonstrated

by the first time in the State of Minas Gerais, Brazil, serological evidence of circulation of borrelia or the other related agent in wild and domestic animals in the municipalities of Santa Cruz do Escalvado and Pingo D'Água. Further research is required to provide information on the presence of *Borrelia* in Brazilian territory and its association with Baggio-Yoshinari syndrome.

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