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SHORT COMMUNICATIONS

Borrelia theileri in Bovine in the northern and southeastern regions of Brazil

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Abstract

The present study aimed to describe the occurrence of *Borrelia* spp. in cattle in the states of Minas Gerais and Pará in southeastern and northern Brazil, respectively. Bovine whole blood samples were examined by blood smear and polymerase chain reaction (PCR) to detect the flagellin B (*flaB*) gene of *Borrelia* spp. Frequencies of positive animals for *Borrelia* spp. were 1.52% (2/132) in the municipality of Unaí, Minas Gerais, and 14.2% (2/7) in the municipality of Marabá, Pará. Subsequent genetic sequencing confirmed that the detected spirochetes close to the species *B. theileri*. In both locations, the animals positive for *B. theileri* were also highly infested by *Rhipicephalus microplus* ticks. Despite the low frequency of *Borrelia* spp., the occurrence of this spirochete indicates that further studies are needed to determine the consequences in cattle herds.

Keywords Borreliosis · Cattle · Tick

Introduction

Borrelia theileri is a cosmopolitan microorganism identified in cattle, goats, sheep, and solipeds (Callow 1967). This species belongs to the relapsing fever group Borreliae (RFGB) from the Spirochaetaceae family, which includes gram-negative spirochetes (McCoy et al. 2014; Cordeiro et al. 2018). Species in this group differ from others in the order Spirochaetales in that they have a greater number of periplasmic flagella, few turns, and are microaerophiles (Barbour and Hayes 1986).

Borreliosis, also known as spirochetosis, caused by *B. theileri* is still poorly understood regarding its pathogenicity in ruminants but is initially associated with fever (Koch et al. 1990) and anemia (Abanda et al. 2019). Furthermore, co-infection by *B. theileri* and other pathogens, such as piroplasmid protozoa (Koch et al. 1990; Sharma et al. 2000), may worsen clinical signs and, therefore, require an integrated treatment.

Biological transmission by either transovarial or transstadial perpetuation of *B. theileri* was described for the first time in Brazil in *Rhipicephalus* (*Boophilus*) *australis* ticks (probably *Rhipicephalus* (*Boophilus*) *microplus*) by Brumpt (1919), who also reported the first finding of *B. theileri* in a bovine in the country. Transmission has also been identified between populations of *Rhipicephalus decoloratus* and *Rhipicephalus evertsi* ticks in South Africa (McCoy et al. 2014). Experimental transmission of *B. theileri* by *R. microplus* in splenectomized calves was reported by Smith et al. (1985), who confirmed the presence of the spirochete after 15 to 17 days of tick infestation. The only change observed in the animals in the experiment was an increase in rectal temperature, reaching 40.2 °C.

Recently, Morel et al. (2019) characterized a new bovine isolate in Argentina. In Brazil, characterization has been

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performed only in *R. microplus* ticks (Cordeiro et al. 2018), and subclinical infection in a cow (Paula et al. 2022). No other studies have been conducted to determine the molecular frequency of *B. theileri* in herd of cattle in Latin America. To address some of these data gaps, the objective of this study was to describe the occurrence of *Borrelia* sp. in blood samples from cattle in two different Brazilian regions.

Materials and methods

A cross-sectional study was carried out from 2019 to 2020 on nine dairy farms in the cities of Unaí, Cabeceira Grande, and Arinos, located in the micro-region of Unaí in the northwestern region of the state of Minas Gerais (MG), Brazil (16°21′6″S, 46°54′43″W). This area is located in Cerrado biome. Its climate, according to Köppen (1948), is type Aw, which corresponds to a tropical rainy climate, a savannah climate, with a predominance of dry winters. Average annual temperatures range between 31 °C, maximum, and 15 °C, minimum. The average annual rainfall is between 1400 and 1500 mm (Naime et al. 2014). Additionally, an incidental finding of infection by spirochete on calf in the city of Marabá, in southeastern Pará, Brazil (5°33′44.5″S and 49°06′01.1″W) was sampled. This area is located in the Amazon biome. Its climate, according to Köppen (1948), is type Aw, which corresponds to a tropical, hot and rainy climate, with predominant rains in the summer. Average annual temperatures vary between 35 °C, maximum, and 22 °C, minimum. The average annual precipitation is 2200 mm (Junior et al. 2017).

All animals in the study showed nonspecific signs, such as a drop in milk production in cows and reduced food consumption.

Blood was collected from Holstein cows between 3 and 9 years of age in the state of Minas Gerais and from a crossbred calf (Holstein x Gyr) in the state of Pará by puncture of the jugular vein using Vacutainer® tubes containing ethylenediaminetetraacetic acid (EDTA), which were kept at a temperature of 2–8 °C. In the laboratory, blood smear slides were made, and the hematocrit centrifugation technique for diagnosis of African trypanosomiasis (Woo's test) was used to detect hemoparasites with motility (primarily *Trypanosoma vivax*). The Woo's test was performed by centrifuging whole blood with anticoagulant EDTA in microhematocrit tubes and identifying motility by direct observation of the capillary under a microscope at×40 magnification (Woo 1970).

The slides were fixed in methyl alcohol for 3 min, stained with a 10% Giemsa stain for 30 min, and viewed under the 100×objective of an optical microscope.

A 300-µl aliquot of blood from each animal was used for total DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The extracted DNA was amplified by polymerase chain reaction (PCR) to detect the flagellin B (*flaB*) gene of *Borrelia* spp., Samples from animals positive for *Borrelia* spp. were also tested for the *16SrRNA* genes of bacteria of the Anaplasmataceae family, a genomic region that encodes the *8 kDa* antigen of the protozoan *T. vivax*, the *sbp* gene of the protozoan *Babesia bovis*, and the rap gene that characterizes *Babesia bigemina*. The primers used are shown in Table 1. All reactions and thermocycling protocols were performed as described in the original articles. The products of the PCR reactions were analyzed using an electrophoresis gel. For each reaction, a positive control of *Borrelia anserina* strain AL (culture), *Anaplasma marginale* strain AmRio 2 (culture), *B. bovis*, *B. bigemina* and *T. vivax* (cattle positives) and two negative controls (water) were included.

The amplification products from samples of positive animals were sent for sequencing, having been previously treated with Exo-Sap-IT (GE Healthcare®) following manufacturer's guidelines, and were sequenced through the capillary-type Sanger platform in an ABI 3730 DNA Analyzer (Applied Biosystems, Life Technologies®). The generated sequences were compared with published data using the NCBI nucleotide BLAST program. Phylogenetic analysis was performed using MEGA version 11.0 software.

Results and discussion

Among a total of 132 samples taken from dairy cows from Minas Gerais, one blood smear sample was positive for *Borrelia* sp. (Fig. 2), reflecting a positivity rate of 0.75% (1/132) by this technique. Additionally, a spirochete was detected in a blood smear from the calf from Pará.

Molecular analysis revealed two positive animals from Minas Gerais (2/132, 1.52%) and confirmed the diagnosis in the calf from Pará. The positivity of the three animals was confirmed by the presence of spirochetes on the blood smear slides (Fig. 1) and/or by molecular analysis (Table 2).

Blood smear and molecular analyses for detecting bacteria of the Anaplasmataceae family and the protozoa *Babesia* spp. and *T. vivax* in animals positive for *Borrelia* spp. found bacteria of the Anaplasmataceae family in the calf and only one of the cows, as shown in Table 2.

Sequencing of the amplified flagellin B fragments revealed a shared identity of over 99% with *B. theileri* isolate C5 (MG601737). Analysis of the phylogenetic relationship based on the *flaB* gene sequences obtained in this study showed that they formed a clade highly related to the *B. theileri* strains deposited in GenBank. This clade includes the *B. theileri* strain C5 detected in *R. microplus* in Brazil (Fig. 2). The three spirochetes detected in this study are herein referred to as *B. theileri* isolate MG7, *B.*

Table 1 Sequences of the primers used, together with the respective target genes and the size of the amplified fragment

Gene/*primers*	Specificity	Sequence of *primers* (5′-3′)	Amplified fragment	Reference
16SrRNA	Family Anaplasmataceae			
EHR16SD		GGTACCYACAGAAGAAGTCC	345 bp	Inokuma et al. (2000)
EHR16SR		TGCACTCATCGTTTACAG		
*fla*B	*Borrelia* spp.			
BORFLAF1		TACATCAGCTATTAATGCTTCAAGAA	760 bp	Blanco et al. (2017)
BORFLAR1		GCAATCATWGCCATTGCRGATTG		
BORFLAF2		CTGATGATGCTGCTGGWATGG		
BORFLAR2		TCATCTGTCATTRTWGCATCTT		
sbp gene	*Babesia bovis*			
BbSBP-4F1		AGTTGTTGGAGGAGGCTAAT	907 bp	Terkawi et al. (2011)
BbSBP-4R1		TCCTTCTCGGCGTCCTTTTC		
BbSBP-4F2		GAAATCCCTGTTCCAGAG	503 bp	
BbSBP-4R2		TCGTTGATAACACTGCAA		
rap gene	*Babesia bigemina*			
BbigRAP-1aF1		GAGTCTGCCAAATCCTTAC	879 bp	Terkawi et al. (2011)
BbigRAP-1aR1		TCCTCTACAGCTGCTTCG		
BbigRAP-1aF2		AGCTTGCTTTCACAACTCGCC	412 bp	
BbigRAP-1aR2		TTGGTGCTTTGACCGACGACAT		
8KDa	*Trypanosoma vivax*			
ILO1264		CAGCTCGCCGAACACTTGGCTGGG	400 bp	Masake et al. (1997)
ILO1265		TCGCTACCATCGCAATCGTCGTCTCAAGG		

theileri isolate MG8, and *B. theileri* isolate Pará. The novel sequences were deposited in the GenBank with the following accession numbers OQ344268 (*B. theileri* isolate MG7), OQ344269 (*B. theileri* isolate MG8), and OQ344270 (*B. theileri* isolate Pará).

Since it was first described in 1902, this spirochete has been identified in Africa, Australia, and both North and South America (McCoy et al. 2014). Although *B. theileri* is a tick-borne agent of cosmopolitan occurrence, its molecular description in South America has been reported infrequently. Ours is the first study showing the molecular frequency of *Borrelia* sp. in bovine in South America and the only study based on such a large number of animals.

The first molecular description of *B. theileri* was performed on homogenized samples of *R. microplus* by Yparraguirre et al. (2007) in the southeastern region of Brazil. Subsequently, Cordeiro et al. (2018) described the morphological and molecular characteristics of this spirochete in the hemolymph of this same tick species collected from a calf in the state of Rio de Janeiro, Brazil. Recently, *B. theileri* was

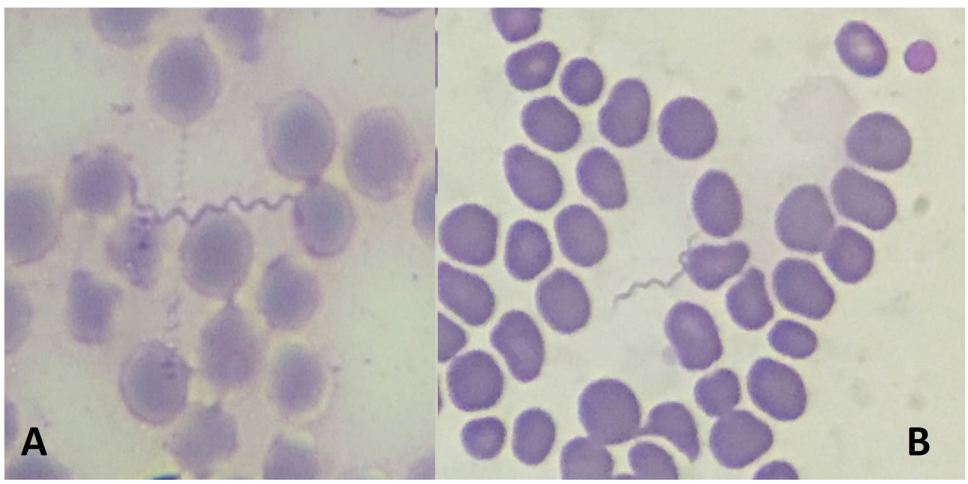

Fig. 1 Photomicrograph of two specimens of *Borrelia theileri* observed in blood smear from a lactating cow (**A**) and Calf from Pará (**B**). Giemsa, ×1000 magnification

Table 2 List of tests performed on animals positive for *Borrelia theileri* in northern and southeastern Brazil

Test	Cow MG7	Cow MG8	Calf PA
Polymerase chain reaction			
Borrelia spp.	Positive	Positive	Positive
Anaplasmataceae	Positive	Negative	Positive
Trypanosoma spp.	Negative	Negative	NT
Babesia spp.	Negative	Negative	NT
Blood smear			
Spirochetes	Positive	Negative	Positive
Anaplasmataceae	Negative	Negative	Positive
Trypanosoma spp.	Negative	Negative	Negative
Woo's test			
Motility	Positive	Negative	NT

NT not tested

incidentally detected in one of the cows during the examination of stained blood smears of 10 cows from Goiás State, Brazil (Paula et al. 2022). Morel et al. (2019) detected the presence of *B. theileri* in a bovine in Argentina, indicating that the agent is present in much of South America.

It is noteworthy that positive animals showed infestation with ticks of the species *R. microplus*. This suggests that infestation with *R. microplus* may be a determining factor for the presence of *Borrelia* sp. in cattle. However, other tick species, including *R. decoloratus* and *R. evertsi* in South Africa, have also been reported to transmit this spirochete (McCoy et al. 2014).

Two important features of RFGB are a high concentration of bacteria in the blood of competent hosts and the presence of transovarial transmission in ticks (Dworkin et al. 1998). The high concentration of the *B. theileri* bacteria has

Fig. 2 Sequences from the study and GenBank aligned using MAFFT in JALVIEW 2.11 software. The best-fit evolutionary model determined with Bayesian Information Criterion. Phylogenetic relationships estimated using Maximum Likelihood (ML) phylogenetic inference with PhyML and Bayesian Markov Chain Monte Carlo (MCMC) method with MrBayes v.3.2.6. MCMC settings included two independent runs with 4 chains each, run for 10 million generations with a sampling frequency of 100th generation, resulting in 100,000 trees. After removing 25% of the samples as burn-in, a consensus tree was built. Statistical support for clades was measured using a heuristic search with 1000 bootstrap replicates and Bayesian posterior probabilities. Numbers ($\geq 70/\geq 0.7$) above branches indicate node bootstrap or probabilities values (ML/ MrBayes). Asterisks indicate values below 70/0.7

been observed in the hemolymph of *R. microplus*, as well as transovarial transmission (Cordeiro et al. 2018). However, parasitemia appears to be low in cattle. Thus, the spirochete is rarely found circulating in the blood and almost always with the presence of a single specimen in the blood smear (Fig. 1).

Although little studied, infection by *B. theileri* alone is known to be of low pathogenicity (Abanda et al. 2019). However, infection with other arthropod-borne agents may potentiate symptoms generated by these agents in co-infected animals.

Despite a negative test for *T. vivax* in the blood smear and in the molecular analysis, occasionally, Woo's test was positive in one of the dairy cows, demonstrating that this technique, possible can be used to detect the presence of *Borrelia* spp. Woo's technique, described more than 50 years ago, is widely used in the diagnosis of bovine trypanosomiasis and has a high sensitivity compared to other techniques (Woo 1970). However, although further studies are needed, the results of the present study suggest that both *Borrelia* spp. and trypanosomiasis should be included in the differential diagnosis for positive cattle using Woo's technique.

The frequency of 1.52% for *B. theileri*-related relapsing fever group spirochete in cattle from northwestern Minas Gerais suggests a low prevalence of this agent in this region. Among the few previous studies on *B. theileri* as a disease agent, none address the actual molecular prevalence of this microorganism or elucidate the host-parasite relationship. Abanda et al. (2019) detected a frequency of 17.9% (255/1260) but confirmed 100% shared sequence identity with *B. theileri* in only 42 samples (3.33%). Serological diagnoses of *Borrelia* spp. in bovids have been carried out in several regions of Brazil (Guedes-Junior et al. 2008; Silva et al. 2013). Using *Borrelia burgdorferi* crude antigens, these studies obtained frequencies ranging between 30 and 90%. As these were crude antigens, the possibility of cross-reaction cannot be ruled out; nonetheless, we hypothesize that the serological prevalence of *B. theileri* is within this range. This hypothesis can only be tested, however, by obtaining an isolate of the agent in pure culture to develop a standardized serological technique taking into account cross reactions with other pathogens like *Leptospira* and *Treponema*. Thus, it can be used in future seroepidemiological studies.

Therefore, our results present novel data for *B. theileri*-related relapsing fever group spirochete in bovine from two Brazilian regions, contributing to the expansion of knowledge about this agent in the country.

Author contribution Cordeiro MD and Figueiroa T designed the project and experiments. Figueiroa T, AH Fonseca, BA Baêta, JB Silva, Lima DHS, and Silva MM carried out the experimental procedures. Guterrez contributed with phylogenetic analyses. Cordeiro MD and Figueiroa T have written the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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Data availability The datasets generated and analyzed during this study are available upon request from the corresponding author.

Declarations

Ethical approval This study was approved by the Council of Ethics in the Use of Animals (CEUA) of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM) protocol No. 060/2016.

Conflict of interest The authors declare no competing interests.

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