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Case Report

Negative results for *Borrelia burdgorferi* in cows and ticks from a border region between Brazil and Paraguay: a case report

Resultados negativos para *Borrelia burgdorferi* em vacas e carrapatos de região fronteiriça entre Brasil e Paraguai: relato de Caso

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A B S T R A C T

Lyme borreliosis is an infectious disease caused by bacteria of the genus *Borrelia*. In ruminants, most infections are asymptomatic, but the animals can present myalgia, lameness, laminitis, arthritis, synovitis, neurological symptoms, and also decreased production and abortion. The objective was to investigate *Borrelia burgdorferi* DNA in cows and cattle ticks on a small dairy farm in a border region. Blood samples and ticks were collected from Holstein cows with a history of decreased milk production and abortions. *Borrelia burgdorferi* DNA was extracted from blood samples using a commercial extraction kit, and from ticks using an alkaline hydrolysis solution for subsequent nested-PCR. Serum and tick samples did not present *Borrelia burgdorferi* DNA, and 100% of the ticks were identified as *Rhipicephalus (Boophilus) microplus*. Although this study shows negative results it contributes to understanding the epidemiology of this etiological agent in Paraná and in Brazil, since there are few studies on bovine species. The negative results of this work demonstrate that the animals and ticks researched were not exposed to *Borrelia burgdorferi*, however, as it is a property located in a border region, the sanitary monitoring of the herd must be performed constantly since this is a region. vulnerable to the entry of potential threats to human, animal and environmental health from vectors and pathogenic microorganisms, given the large extension of the land border with the neighboring country and which also has different health status.

Palavras-chave:
Borreliose de Lyme
Borrelia spp.
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R E S U M O

A borreliose de Lyme é uma doença infecciosa causada por bactérias do gênero Borrelia. Nos ruminantes, a infecção é assintomática, mas os animais podem apresentar mialgia, claudicação, laminite, artrite, sinovite, sintomas neurológicos e também diminuição da produção e do aborto. O objetivo foi investigar o DNA de *Borrelia burgdorferi* em vacas e carrapatos de uma pequena propriedade rural de gado leiteiro em uma região de fronteira. Amostras de sangue e carrapatos foram coletados de vacas da raça Holandesa com histórico de diminuição da produção de leite e abortos. O DNA de *Borrelia burgdorferi* foi extraído de amostras de sangue usando um kit de extração comercial e de carrapatos usando uma solução de hidrólise alcalina para subsequente nested-PCR. Amostras de soro e carrapato não apresentaram DNA de *Borrelia burgdorferi*, e 100% dos carrapatos foram identificados como *Rhipicephalus (Boophilus) microplus*. Embora este estudo apresente resultados negativos o mesmo contribui para o entendimento da epidemiologia desse agente etiológico no Paraná e no Brasil, uma vez que existem poucos estudos sobre espécies bovinas. Os resultados negativos deste trabalho demonstram que os animais e

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carrapatos pesquisados não foram expostos a Borrelia burgdorferi, no entanto, por se tratar de uma propriedade localizada em uma região de fronteira, o monitoramento sanitário do rebanho deve ser realizado constantemente, pois é uma região vulnerável à entrada de ameaças potenciais à saúde humana, animal e ambiental por vetores e microrganismos patogênicos, dada a grande extensão da fronteira terrestre com o país vizinho e que também apresenta diferentes condições de saúde.

INTRODUCTION

Lyme borreliosis is an infectious, multisystemic and cosmopolitan disease caused by spirochete bacteria of the genus *Borrelia*, which is a gram-negative, motile, microaerophilic and helical-shaped microorganism (MONTANDON *et al.*, 2014).

This disease can affect domestic and wild animals, and it can accidentally infect humans (MASSARD, 2004). It is transmitted through the bite of its vectors (i.e., ticks, mainly of the genus *Ixodes*) that inoculate saliva infected with the bacteria while they are sucking blood (CORRÊA, 2007; GALO *et al.*, 2009; LOPES, 2013).

Different *Borrelia* strains can infect cattle; a study by Johnson *et al.* (1984) reported that *Ixodes* ticks are the most important vectors of *Borrelia burgdorferi*. However, Mather; Fish; Coughlin (1994) attributed the transmission of *Borrelia burgdorferi* in cattle through *Dermacentor variabilis* or *Amblyomma americanum*.

Some studies in Brazil have detected antibodies against *Borrelia burgdorferi* in bovine species, as demonstrated by Ishikcnva *et al.* (1999) in Rio de Janeiro, São Paulo (SP), and Espírito Santo (ES); and Guedes-Junior *et al.* (2008) in Pará (PA), who detected 13.12% and 54.90% seropositive samples using the ELISA test, respectively. In Rio Grande do Sul (RS), Martins *et al.* (1996) identified spirochetes of the species *Borrelia theileri* in the hemolymph of *Boophilus microplus* ticks. In a more recent study in Paraná, Gonçalves *et al.* (2013) detected a specimen of *Dermacentor nitens,* using molecular biology, with 99.9% similarity to *Borrelia burgdorferi* s.s. strain B31.

Wells *et al.* (1993) reported that *Borrelia burgdorferi* infection in ruminants is usually asymptomatic, even in animals from high seroprevalence regions. However, some animals can present weight loss, myalgia, sporadic lameness, fever, laminitis, arthritis, synovitis, joint augmentation, neurological symptoms such as depression, and behavioral changes, as well as decreased reproduction and abortion (FONSECA *et al.*, 1996; ISHIKAWA, 1996; SOARES *et al.,* 2000).

Border regions are localities with high geographic heterogeneities and intense population flow and, consequently, favorable conditions for the transmission of different pathogens (PEITER *et al.*, 2008) and due to the scarcity of data concerning this disease in the bovine species and in ticks from border regions the objective of this work was to investigate *Borrelia burdgorferi* DNA in cows and their respective ticks from a small dairy farm in a border region between Brazil and Paraguay.

CASE REPORT

This project was approved by the Animal Experimentation Ethics Committee (CEPEEA) of the Paranaense University (UNIPAR) under protocol number 31974/2017.

Blood samples and ticks were collected from Holstein cows, aged one to fifteen years, in a small rural property in the municipality of Umuarama, in the northwest region of the state of Paraná, Brazil, in November 2017. These animals presented decreased milk production and abortions. This municipality is considered a border region, since it is located approximately 100 km from Paraguay.

Collections were conducted by veterinarians and veterinary students from the Veterinary School of of Paraná University (UNIPAR), Brazil. Blood was collected from a puncture in the jugular vein. Approximately 10 mL of blood was collected in sterile tubes without anticoagulant and stored in isothermal boxes. The ticks were manually collected using anatomical tweezers.

Blood and tick samples were sent to the Molecular Biology Laboratory of the Graduate Program in Animal Science and Bioactive Products at UNIPAR. In the laboratory, the blood samples were centrifuged to obtain serum. This was divided into three aliquots of equal volume in microtubes (Eppenddorf™), packed in sterile vials, and kept at -20 °C for molecular diagnosis. The ticks were stored in microtubes (Eppenddorf™) and kept at -20 °C for identification using the taxonomic key of the Brazilian Ixodidae fauna (ARAGÃO; FONSECA, 1961; BARROS-BATTESTI; ARZUA; BECHARA, 2006).

DNA extraction from the blood clot samples of the cows was performed using the PureLinkGenomic DNA mini extraction kit (Invitrogen - USA). DNA was extracted from the ticks using alkaline hydrolysis solution, according to the methodology described by Guy; Stanek (1991) and De Michaelis *et al.* (2000).

DNA amplification of *Borrelia burgdorferi* s.l. was performed using the nested-PCR reaction by using the oligonucleotide sequences previously described by (RIJPKEMA *et al.*, 1995; KURTENBACH *et al.*, 1998; DE MICHELIS *et al.*, 2000; COUCEIRO *et al.*, 2003) in a Thermocycler-PX2 Thermal Cycler (MyCycler™, Bio-Rad, Hercules, CA, USA).

The positive control contained 380-bp and 230-bp fragments obtained during the first and second amplifications, respectively, which were kindly provided

by the Lyme Borreliosis Group of the Institute of Hygiene and Tropical Medicine (IHMT) of the Nova Lisboa University (UNL), Portugal. To avoid false results, negative controls were incorporated into the PCR using ultrapure autoclave water.

The final n-PCR amplification product were subjected to 2% agarose gel electrophoresis containing ethidium bromide and was visualized in a transilluminator with ultraviolet light, using a molecular weight of 100 bp.

All ticks were identified as *Rhipicephalus (Boophilus) microplus*, and there was no DNA amplification in the 50 blood samples or in the 50 ticks tested using the n-PCR.

DISCUSSION

This study showed that it was not possible to detect *Borrelia burgdorferi* DNA in bovine blood samples from the city of Umuarama (PR), corroborating the study by Silva (2008) in Rio de Janeiro (RJ), which also did not detect anti-*Borrelia* spp. antibodies in calves using the ELISA method. However, a study by Ishikawa *et al.* (1999) in Rio de Janeiro (RJ), São Paulo (SP), and Espírito Santo (ES) detected 13.12% seropositive samples using ELISA, which do not corroborate our findings. A similar study by Guedes-Junior *et al.* (2008) in Pará (PA), detected 54.90% seropositive samples, both through the ELISA test, not corroborating the results of this study.

This difference in results may be due to the different diagnostic techniques performed, as the present study investigated the presence of DNA from the etiological agent (possibly a newly installed infection) and the other studies investigated antibodies (later infection) it was concluded in the others studies that the animals had already been exposed to the etiological agent showing already some immunological behavior of the disease.

Related to the ticks of the *Rhipicephalus* (*Boophilus*) *microplus* species of this study, no DNA amplification was detected either. This result does not corroborate with those of Gonçalves *et al.* (2013), who also in the state of Paraná (PR), more specifically in the northern region of the state, investigated *Borrelia burgdorferi* DNA using the same diagnostic technique and detected in a specimen *Dermacentor nitens* 99.9% similarity to *Borrelia burgdorferi* ss strain B31. In the northern region of the state of Paraná, studies to detect DNA and antibodies to this disease have already confirmed the presence of the etiological agent, as revealed by Gonçalves *et al.* (2013) and Gonçalves *et al.* (2015), however recently cases of clinical disease have also been confirmed and reported by the local press (ORIKASA, 2017) and this situation becomes alarming since only about 150km separate these two regions.

The results of this study show that neither animals nor ticks were infected with the respective etiological agent, however we cannot rule out the possibility of a future infection, since in the same region Gonçalves *et al.* (2015) detected a positivity of 83.07 % in stray dogs using the Western Blot technique which is considered the gold standard and definitive for this disease. The animals of this property had no contact with other domestic animals of neighboring properties and possibly for this reason the health of the herd is better preserved for infection by pathogenic microorganisms such as *Borrelia burgdorferi*.

CONCLUSIONS

The negative results of this work demonstrate that the animals and ticks researched were not exposed to Borrelia burgdorferi, however, as it is a property located in a border region, the sanitary monitoring of the herd must be performed constantly since this is a region. vulnerable to the entry of potential threats to human, animal and environmental health from vectors and pathogenic microorganisms, given the large extension of the land border with the neighboring country and which also has different health status.

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