



Molecular identification of *Ehrlichia* sp. in a beef cattle from Brazilian Cerrado – case report

[Identificação molecular de *Ehrlichia* sp em um bovino do Cerrado brasileiro – relato de caso]

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ABSTRACT

Ehrlichia infections in cattle are frequent in Africa but have also been reported in Brazil and North America. This paper reports natural infection by *Ehrlichia* sp. associated with *Babesia bigemina* and *Anaplasma marginale* in a calf in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil, presenting polioencephalomalacia. The molecular evidence, based on a fragment of the *dsb* gene, indicates a species of *Ehrlichia* genetically related to *Ehrlichia canis* and other species of the genus found in the tick *Rhipicephalus (Boophilus) microplus* and a calf from Brazil (99 to 100% identity). It was not possible to associate the clinical signs with *Ehrlichia* infection due to co-infections and histological evidence of another disease. However, the circulation of the bacteria in bovines in Brazilian Cerrado was confirmed and more attention should be given to clinical suspicion of tick-borne pathogens in cattle to clarify the pathogenic potential of *Ehrlichia* sp.

Keywords: tick-borne pathogen, cattle, *Ehrlichia*, neurological signs

RESUMO

Infecções por *Ehrlichia* em bovinos são frequentes na África, mas também foram relatadas no Brasil e na América do Norte. Este artigo relata uma infecção natural por *Ehrlichia* sp. associado a *Babesia bigemina* e *Anaplasma marginale* em um bezerro, no município de Campo Grande, Mato Grosso do Sul, Brasil, o qual apresentava polioencefalomalácia. A evidência molecular, baseada em um fragmento do gene *dsb*, indica uma espécie de *Ehrlichia* geneticamente relacionada a *Ehrlichia canis* e outras espécies do gênero encontradas no carrapato *Rhipicephalus (Boophilus) microplus* e em um bezerro do Brasil (99 a 100% de identidade). Não foi possível associar os sinais clínicos à infecção por *Ehrlichia* devido a coinfeções e evidências histológicas de outra doença. No entanto, a circulação da bactéria em bovinos no Cerrado brasileiro foi confirmada, e mais atenção deve ser dada à suspeita clínica de patógenos transmitidos por carrapatos em bovinos para esclarecer o potencial patogênico de *Ehrlichia* sp.

Palavras-chave: patógenos transmitidos por carrapatos, gado, *Ehrlichia*, sinais neurológicos

INTRODUCTION

Anaplasmataceae (*Ehrlichia/Anaplasma*) infections in cattle are frequently associated with *Ehrlichia ruminantum*, *Anaplasma marginale*, *Anaplasma bovis* (formerly *Ehrlichia bovis*), and *Anaplasma phagocytophilum* (Aktas and

Özübek, 2015; Lorusso *et al.*, 2016). In recent years, however, a novel genotype of *Ehrlichia* (proposed name: *Ehrlichia minasensis*) (Cruz *et al.*, 2016) closely related to *E. canis* has been detected in cattle from North America (Gajadhar *et al.*, 2010) and Brazil (Aguiar *et al.*, 2014) as well as the cattle tick *Rhipicephalus (Boophilus) microplus* (Cruz *et al.*, 2012).

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The geographical distribution and clinical significance of *Ehrlichia/Anaplasma* infections in cattle are well documented for some species (*E. ruminantium*, *A. marginale*, and *A. phagocytophilum*) (Brito *et al.*, 2010; Aktas and Özübek, 2015). However, little information is available on other species (e.g., *E. minasensis*). In Brazil, only *A. marginale* (Brito *et al.*, 2010) and recently *Ehrlichia* sp. infections have been reported in cattle (Aguiar *et al.*, 2014). The clinical and epidemiological importance of these species of *Ehrlichia* to cattle remains unknown, although clinical and hematological abnormalities consistent with ehrlichiosis have been observed in experimentally infected calves (Aguiar *et al.*, 2014). In the present study, we report the natural infection of *Ehrlichia* sp. in a calf from the state of Mato Grosso do Sul, Brazil.

CASUISTRY

A 10-month-old Nelore female with a three-day history of apathy, anorexia, and lateral decubitus was sent for clinical care at the Veterinary Hospital of the *Universidade Federal de Mato Grosso do Sul* (UFMS), Campo Grande, Brazil, in September 2015. The calf belonged to a lot of 130 calves of the same age group allocated to 130 hectares of pasture (*Brachiaria* sp.) and supplemented with protein and mineral salt. The farm was located in the municipality of Campo Grande (20°26'34" S, 54°38'47" W). According to information from the owner, no other sick animals were found in the herd. Blood was collected for the hematological and molecular analyses in EDTA tubes (BD

Vacutainer®, USA). DNA extraction was performed with a blood sample of 350ul using the method in house. The integrity and quantity of the DNA sample was verified by electrophoresis in agarose gel (0.8%) and spectrophotometry (A260/A280nm) in a BioPhotometer Plus (Eppendorf®,USA), respectively. PCR reactions were performed according to Buling *et al.* (2007) for *Babesia bovis* and *B. bigemina*, Silva *et al.* (2006) for *Anaplasma marginale*, and Doyle *et al.* (2005) for *Ehrlichia* sp. The primers used are shown in Table 1.

To identify the *Ehrlichia* species involved, the amplicon obtained from the PCR reaction to *Ehrlichia* sp. was purified using the QIAEX II kit (Qiagen®, Germany) and sequenced in both directions using ABI 3130 automated capillary DNA sequencing. The chromatograms were analyzed, and a consensus sequence was obtained using the BioEdit program. Phylogenetic analysis was performed, and a tree was constructed based on the neighbor joining method using the MEGA 6 program with a nonparametric bootstrap robustness of 1000 pseudo replicates.

After euthanasia of the animal, necroscopic and histopathological analyses were performed. Due to the appearance of clinical neurological signs, a multiplex PCR for bovine herpesvirus 1 and 5 was performed according to Claus *et al.* (2005) (primers displayed in Table 1) from brain fragments.

Table 1. List of primers used in this study

| Primer name | Sequence (5'-3') | Target | Amplicon size bp |
|----------------|--------------------------------|---------------------------------------------------|------------------|
| <i>Cybi</i> | GTTCCAGGAGATGTTGATTC | cytochrome b gene of <i>Babesia bigemina</i> | 263 |
| <i>Cbbr</i> | CTCCCCARTAACTCATTGT | | |
| <i>Cybo</i> | GTTCTGGAAGCGTTGATTC | cytochrome b gene of <i>Babesia bovis</i> | 263 |
| <i>Cbbr</i> | CTCCCCARTAACTCATTGT | | |
| <i>msp5-F</i> | ATGAGAATTTTCAAGATTGTGTCTAACCTT | msp5 gene of <i>Anaplasma marginale</i> | 714 |
| <i>msp5-R</i> | AGGAAAGCCCCCAAAGCCCCATACTT | | |
| <i>dsb-321</i> | TTGCAAAATGATGTCTGAAGATATGAAACA | <i>dsb</i> gene of <i>Ehrlichia</i> spp. | 378 |
| <i>dsb-671</i> | GCTGCTCCACCAATAAATGTATCYCCTA | | |
| <i>b1</i> | CAA CCG AGA CGG AAA GCT CC | glycoprotein C gene of bovine herpesvirus 1 and 5 | 354 (BoHV-1) |
| <i>b5</i> | CGG ACG AGACGC CCT TGG | | 159 (BoHV-5) |
| <i>Bcon</i> | AGT GCA CGT ACA GCG GCT CG | | |

The results of all PCR reactions were visualized in a UV transilluminator (BioRad®, USA) after electrophoresis in agarose gel 3% stained with GelRed (Biotium®, USA) following the manufacturer's instructions. For each PCR reaction, the respective positive control from the DNA bank of the *Laboratório de Biologia Molecular - FAMEZ/UFMS* was used. Nuclease-free water was used as the negative control.

DISCUSSION AND CONCLUSIONS

The physical examination revealed depression, cachexia, ticks, pale mucous membranes, severe dehydration, heart rate of 90bpm, normophonetic heart sounds, rectal temperature of 39.3°C, one low-intensity rumen movement every three minutes, and yellow-green pasty diarrhea. Normochromic microcytic anemia, hypoproteinemia, and hyperfibrinogenemia were identified during the hematological analysis, along with inclusions similar to *Babesia* sp. and *Anaplasma* sp. in erythrocytes and *Ehrlichia* sp. morulae in mononuclear cells.

Positive PCR results were found for *B. bigemina*, *A. marginale*, and *Ehrlichia* sp. After DNA sequencing, the amplicon obtained in the *Ehrlichia* PCR reaction demonstrated 99 to 100% identity with DNA sequences of *Ehrlichia* sp. found in the hemolymph of *R. (Boophilus) microplus* in the state of Minas Gerais and

Ehrlichia sp. isolated from a calf in the state of Mato Grosso, Brazil, respectively (GenBank accession numbers JX629808 and KF621012, respectively). The phylogenetic analysis showed a cluster among the DNA sequences of *Ehrlichia* sp. identified in the present study as well as isolates from cattle and ticks from Brazil. Moreover, common ancestry was observed with *Ehrlichia canis* isolates, supported by a high bootstrap value (100%) (Figure 1). The DNA sequence obtained in the present study was deposited in the Genbank under access number KX595269.

After the physical and hematological examinations, the calf was treated intramuscularly with diminazene diacetate (4mg/kg) and oxytetracycline (20mg/kg). Intravenous fluid therapy with Ringer's lactate was also performed. However, neurological signs, such as blindness and opisthotonos, were identified two days after the onset of the treatment protocol. Due to the deterioration of the clinical state, the animal was euthanized in accordance with current legislation and the body was submitted to necropsy and histopathological analysis. No macroscopic abnormalities were observed. In the microscopic analysis, laminar neuronal necrosis was found, with polioencephalomalacia in the parietal and occipital cortex. Multiplex PCR for bovine herpesvirus 1 and 5 presented negative results.

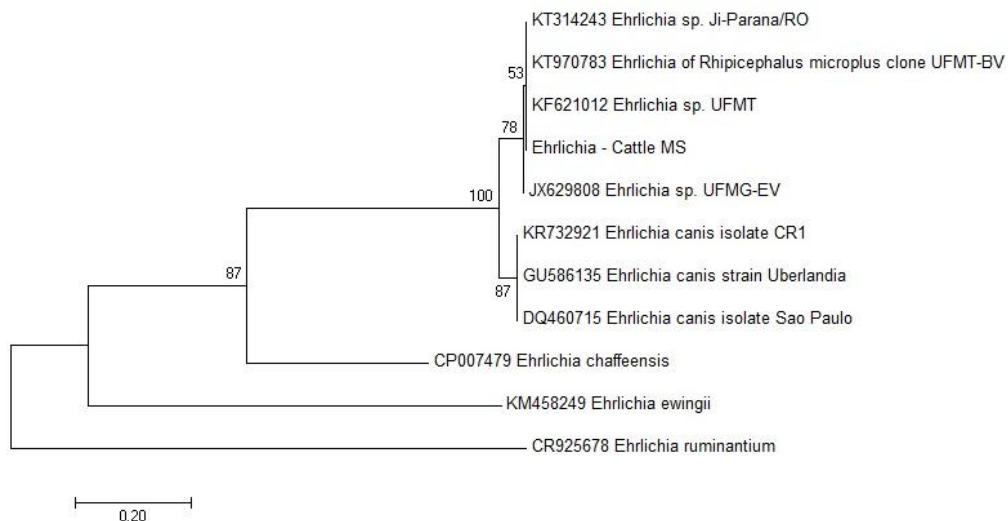


Figure 1. Phylogenetic tree based on a 317bp fragment of the dsb gene showing the relationship among cattle *Ehrlichia* sp. (arrow indicates *Ehrlichia* sp. found in the present study), *E. canis*, and other *Ehrlichia* species.

It was not possible to associate the clinical signs presented by the animal with *Ehrlichia* sp. infection due the presence of co-infection with *B. bigemina* and *A. marginale*. The lesions and clinical signs were consistent with polioencephalomalacia. According to a study by Sant'Ana *et al.* (2009), polioencephalomalacia is associated with several conditions, such as thiamine deficiency, poisoning by sulfur, salt poisoning, and bovine herpes virus. In the present report, based on the epidemiological characteristics (young animal feeding on sprouting pasture, isolated case, absence of bovine herpes lesions, and negative PCR results in brain tissue for *Ehrlichia* sp. and herpesvirus), polioencephalomalacia was most likely caused by thiamine deficiency.

This is the first report of *Ehrlichia* sp. in a bovine from the state of Mato Grosso do Sul, Brazil and in Cerrado biome. Although the importance of *Ehrlichia* sp. as causal agent of disease in cattle remains unclear, attention should be given to tick-borne diseases with the aim of accumulating evidence and achieving greater precision in the definition of the importance of this agent to the health and productivity of cattle herds.

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