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Short Communication

Theileria sp. in water buffaloes from Maranhão State, northeastern Brazil

Theileria sp. em búfalos do Estado do Maranhão, nordeste do Brasil

Hugo Luca Abate[1]; Nelson Jessé Rodrigues dos Santos[1]; Danilo Rodrigues Barros Brito[2];
Jessica Damiana Marinho Valente[3]; Thállitha Samih Wischral Jayme Vieira[3]; João Luis Garcia[1];
Rafael Felipe da Costa Vieira[3]*; Odilon Vidotto[1]

[1] Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina – UEL, Londrina, PR, Brasil

[2] Laboratório de Saúde Animal, Instituto Federal do Maranhão – IFMA, São Luís, MA, Brasil

[3] Departamento de Medicina Veterinária, Universidade Federal do Paraná – UFPR, Curitiba, PR, Brasil

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Abstract

Anaplasma marginale and piroplasm species are widespread among Brazilian cattle herds. Both of these tick-borne pathogens hamper livestock production and cause a significant economic impact. Although buffaloes have demonstrated a high level of adaptability, data on tick-borne pathogens are scarcely reported in Brazil. Thus, the aim of this study was to screen water buffaloes from the state of Maranhão for piroplasm and *A. marginale* occurrence using PCR assays. All samples were negative for *A. marginale*. One of the 287 (0.35%) water buffaloes tested was positive for *Theileria* sp. Sequencing of the 18S rDNA fragment (356 bp) showed that the *Theileria* sp. identified was closely related to the *T. buffeli/orientalis* group. Future studies on the clinical signs of infection and the main vector in this country are needed.

Keywords: Piroplasms, *Anaplasma marginale*, ticks, PCR.

Resumo

Anaplasma marginale e espécies de piroplasma são amplamente distribuídas no rebanho bovino brasileiro. Ambos os patógenos transmitidos por carrapatos dificultam a produção pecuária e causam um impacto econômico significativo. Embora os búfalos tenham demonstrado um alto nível de adaptabilidade, dados sobre patógenos transmitidos por carrapatos são raramente relatados no Brasil. Assim, o objetivo deste estudo foi investigar búfalos do estado do Maranhão para piroplasmas e *A. marginale* utilizando-se a técnica da PCR. Todas as amostras foram negativas para *A. marginale*. Um dos 287 (0,35%) búfalos testados foi positivo para *Theileria* sp. O sequenciamento de um fragmento do gene 18S rDNA (356 pb) demonstrou que *Theileria* sp. identificado estava relacionada ao grupo *T. buffeli/orientalis*. Estudos futuros sobre os sinais clínicos de infecção e o principal vetor neste país são necessários.

Palavras-chave: Piroplasmas, *Anaplasma marginale*, carrapatos, PCR.

Bovine piroplasmosis, caused by *Babesia* spp. and *Theileria* spp., is a tick-borne protozoan disease that infects cattle and buffaloes worldwide (SIVAKUMAR et al., 2014). *Babesia bovis*, *B. bigemina*, *Theileria parva,* and *T. annulata* are known to have a significant impact on the cattle industry (BOCK et al., 2004). Although clinical manifestations are not commonly observed in piroplasm-infected water buffaloes (*Bubalus bubalis*) (VINODKUMAR et al., 2016), a previous study reported a *Theileria* sp. related to the *T. buffeli/orientalis* group that infects water buffaloes in the Brazilian Amazon region (SILVEIRA et al., 2016).

In Brazil, cattle and buffalo live and graze together and thus may share pathogens. A previous study of a multispecies grazing system found a single *Anaplasma marginale* strain that infects coexisting cattle, buffalo and ticks (SILVA et al., 2014a). *A. marginale* is a tick-borne bacterium that causes bovine anaplasmosis (DUMLER et al., 2001), and it is widely spread throughout Brazil (POHL et al., 2013). However, data on *A. marginale* in buffaloes are scant (SILVA et al., 2014c).

In Brazil, approximately 89,945 out of the one million water buffalo in the country are located in the state of Maranhão in the northeastern region of the country (IBGE, 2017). Buffalo farming has increased as an alternative use of land that is unfit for cattle breeding; furthermore, it has become an economically profitable option due to the ability of buffaloes to adapt to climate change and produce high-quality milk and meat (BRASIL, 2007). Although

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***Corresponding author:** Rafael Felipe da Costa Vieira. Departamento de Medicina Veterinária, Campus Agrárias, Universidade Federal do Paraná – UFPR, Rua dos Funcionários, nº 1540, Juvevê, CEP 80035-050, Curitiba, PR, Brasil. e-mail: rvieira@ufpr.br

buffaloes have demonstrated a high adaptability, data on sanitation conditions is scarcely reported in the country (BRASIL, 2007). Thus, the aim of this study was to screen water buffaloes from the state of Maranhão for piroplasm and *A. marginale* occurrence using PCR assays.

A total of 287 water buffalo DNA samples were retrieved from a previous study (SANTOS et al., 2018). All samples were tested using a PCR for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to ensure successful DNA extraction, as previously described (BIRKENHEUER et al., 2003). DNA samples were screened by conventional PCR using a previously described primer set targeting the 18S rDNA gene of piroplasms (ALMEIDA et al., 2012). A cattle-positive blood sample for *B. bovis* and ultrapure water were used as positive and negative controls, respectively. DNA samples were also screened by conventional PCR using a primer set targeting the *A. marginale msp4* gene as described previously (JOAZEIRO et al., 2015).

A fragment (356 bp) of the 18S rDNA gene from one piroplasm isolate was sequenced. The PCR product was purified from the agarose gel (PureLink®, Quick Gel Extraction Kit, Invitrogen, Carlsbad, CA, USA), evaluated by spectrophotometry for concentration and purity (Pico100 Picodrop® Spectrophotometer, Picodrop Limited, Hinxton, UK), and sequenced from both directions by Sanger method (SANGER et al., 1977) using a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The assembled partial sequence of the 18S rDNA gene was compared with sequences deposited in the GenBank database using the basic local alignment search tool (BLASTn) (ALTSCHUL et al., 1990). The amplified nucleotide sequence of the *Theileria* sp. was submitted to GenBank (accession no. KY355137).

Theileria sp. 18S rDNA sequence was aligned using MAFFT 7 (KATOH & STANDLEY, 2013) on Guidance2 (SELA et al., 2015). The best-fit evolutionary model was estimated as F81+I+G using jModeltest 2.1.4 (DARRIBA et al., 2012). The Bayesian Information Criterion (BIC) and Maximum Likelihood (ML) algorithms were used to the phylogenetic inference. Reconstruction was visualized with FigTree 1.4.0 software.

All water buffalo's samples consistently amplified the GAPDH gene. One of the 287 (0.35%; 95% CI: 0.06 – 1.95) water buffalo tested was positive for *Theileria* sp. Sequencing of the 18S rDNA fragment (356 bp) showed that this gene region was ≥99% identical to multiple *T. buffeli* genes deposited in GenBank (EF126184, HQ840964, HM538205, HM538194, DQ104611). Phylogenetic 18S rDNA gene fragment analysis confirmed the close relationship of the water buffalo *Theileria* genotype of the present study with *T. buffeli* detected in India (Figure 1). All samples were negative for *A. marginale*.

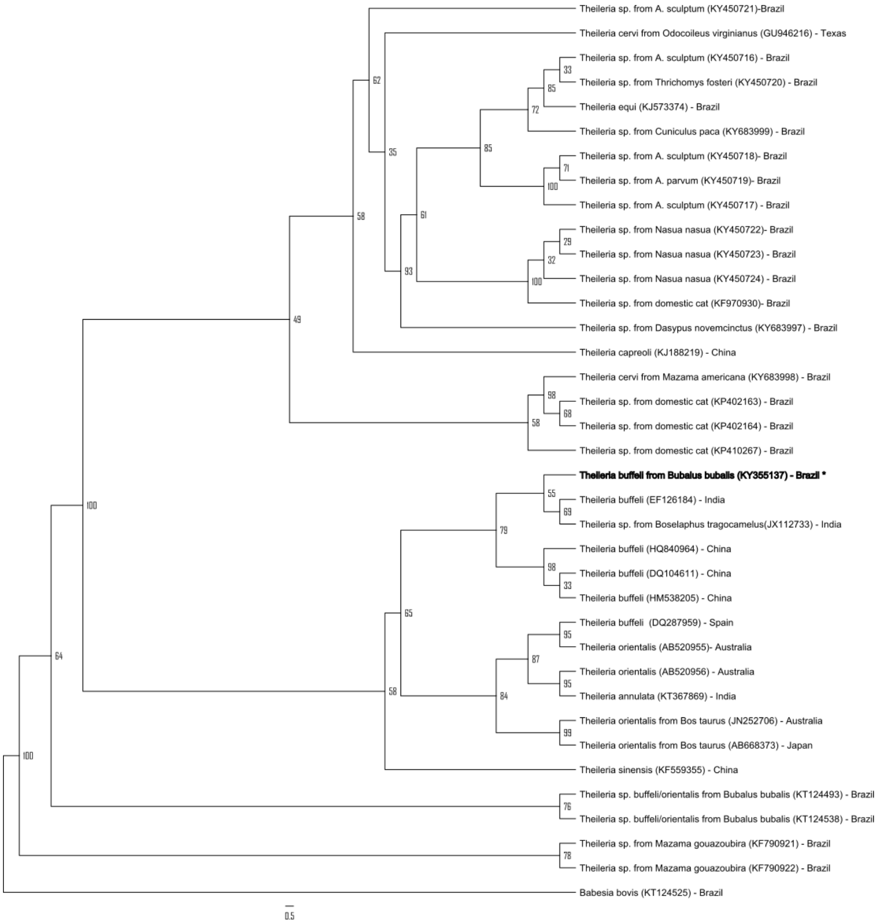

Figure 1. Phylogenetic tree based on partial sequences of the 18S rDNA gene, showing the relationship between the *Theileria* sp. detected in the water buffaloes (*Bubalus bubalis*) from this study and other *Theileria*. *Babesia bovis* was used as an outgroup. The GenBank accession number is in parentheses after the species name and origin of each bacterium. Maximum Likelihood analyses were carried out applying the F81+I+G model and 1000 bootstrap replicates for all analyses.

In the present study, a *Theileria* sp. closely related to the *T. buffeli*/*orientalis* group was detected in water buffaloes from the state of Maranhão in northeastern Brazil. *Theileira* sp. was found in only one animal, supporting that this protozoan has a low prevalence in water buffaloes in Brazil (SILVEIRA et al., 2016). *Haemaphysalis* spp. ticks are the main vectors of species belonging to the *T. buffeli*/*orientalis* group (UILENBERG et al., 1982). In Brazil, despite *Haemaphysalis juxtakochi* (Cooley, 1946) ticks have been found infesting small red brocket deer (*Mazama bororo* Duarte) (SZABÓ et al., 2006) and Brazilian dwarf brocket deer (*Mazama nana*) (MARTINS et al., 2007), this tick species has never been associated to buffaloes in the country. However, both tick species have never been associated as vectors of *T. buffeli*/*orientalis*, which may explain the low prevalence of infection found in this sample. Conversely, *Rhipicephalus microplus* ticks are endemic in Brazil (DANTAS-TORRES et al., 2009), hampering livestock production and causing annual economic losses estimated at 3.24 billion USD (GRISI et al., 2014). Additional studies are necessary to elucidate the putative tick vector of the *Theileria* sp. found in the present study.

Clinical signs of *Theileria* spp. infection in water buffaloes are still controversial (SIVAKUMAR et al., 2014), although fatal cases of theileriosis may occur during outbreaks (VINODKUMAR et al., 2016). In a previous study in water buffaloes from Brazil, *Theileria* infection was associated to lymphoproliferative disease (SILVEIRA et al., 2016). *T. orientalis* is distributed worldwide and is well known to cause clinical disease in cattle (WATTS et al., 2016). Considering that multispecies grazing is a common practice in this region of Brazil, further studies are needed to evaluate cattle from the same Brazilian region, as cross-species transmission may occur and may lead to important economic consequences for the cattle industry.

Anaplasma marginale is endemic in Brazilian cattle herds (JOAZEIRO et al., 2015; KOCAN et al., 2010). Although studies on *A. marginale* in water buffaloes are scarce in Brazil (SILVA et al., 2014a,b,c), previous studies have detected the same strains of *A. marginale* in water buffaloes and cattle (SILVA et al., 2014a). Thus, co-grazing of cattle and water buffaloes may be an important risk factor for *A. marginale* infection, and this hypothesis should be further evaluated.

Our results reinforce that *Theileria* sp. is closely related to the *T. buffeli*/*orientalis* group occurs in water buffaloes' samples from Brazil. Future studies are needed investigating clinical signs of infection and the main vector for disease transmission in this country.

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