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Correlation between *Rhipicephalus microplus* ticks and *Anaplasma marginale* infection in various cattle breeds in Brazil

Kauê Rodriguez Martins[1] · Marcos Valério Garcia[2] · Paulino Bonatte-Junior[1] · Pãmella Oliveira Duarte[3] · Leandro Oliveira Souza de Higa[3] · Bárbara Guimarães Csordas[2,3] · Jacqueline Cavalcante Barros[4] · Renato Andreotti[4]

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Abstract
The tick *Rhipicephalus microplus* is responsible for the transmission of *Anaplasma marginale*, which causes hemolytic anemia, abortion, decreased production, and mortality in cattle in Brazil. However, *A. marginale* can also persist in cattle herds without any clinical signs. This study investigated the relationship between the number of ticks present on each cattle and the circulating number of *A. marginale msp1β* gene copies in the blood of Brangus and Nellore cattle reared in the Brazilian Cerrado through a year period. Twenty-three animals (11 Brangus and 12 Nellore) were raised for 12 months with ticks counted every 18 days, and blood collected every 36 days. Blood sera was used for total antigen iELISA, genomic DNA was extracted from whole blood by the phenol/chloroform method and then analyzed by PCR to confirm *A. marginale* presence with the *msp5* gene. Positive samples were quantified by qPCR using *msp1β* gene. Brangus cattle presented 4.5 fold more ticks than Nellore group. Although Brangus cattle carried a higher overall *A. marginale msp1β* gene presence than Nellore cattle, no relationship of tick count and copy number could be achieved due to high variability in copy number. Moreover, both breeds showed similar weight gain and a similar serological pattern throughout the year. None of the animals showed any clinical signs of anaplasmosis during the experimental period, indicating that a low level of tick infestation may be sufficient to maintain a stable enzootic situation.

Keywords Tick · Anaplasmosis · qPCR · iELISA · Cerrado

✉ Renato Andreotti
renato.andreotti@embrapa.br

[1] Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

[2] Bolsista DCR, Fundapam - Governo do Estado de Mato Grosso do Sul/Laboratório de Biologia do Carrapato, Embrapa Gado de Corte, Campo Grande, Mato Grosso do Sul, Brazil

[3] Programa de Pós-Graduação em doenças Infecciosas e Parasitárias, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

[4] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Corte, Avenida Radio Maia, 830, Mato Grosso do Sul, Campo Grande, Brazil

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Introduction

The Brazilian Cerrado biome is rich in diversity, although over the past decades, it has been fragmented by the rapid expansion of agribusiness (reviewed by Ratter et al. 1997), thereby generating billions of dollars for Brazil. In the year 2016, according to the Brazilian Institute of Geography and Statistics (IBGE 2017), 216 million cattle were raised across the country, with the mid-western states accounting for 74 million of this total. Grisi et al. (2014) estimate an annual economic loss of $13.96 billion when considering the various parasites that affect the cattle production chain, with the tick *R. microplus*, a vector of many diseases, accounting for $3.24 billion of these losses.

One of those diseases is anaplasmosis, which is caused by the gram-negative bacterium *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) (Dumler et al. 2001), an obligate intracellular parasite that chronically infects cattle and wild animals. *Anaplasma marginale* spreads through the bites of infected ticks, flies, and mosquitoes, as well as through needles and surgical instruments (Aubry and Geale 2011). *Anaplasma marginale* is part of the bovine tick-borne diseases (TBDs) complex, together with *Babesia bigemina* and *Babesia bovis*, which exert an economic impact through direct costs related to mortality and morbidity and indirect costs related to disease treatment and prevention (Madruga et al. 1986).

The ability of *A. marginale* to evade the immune system allows for a subsequent reinvasion of the host erythrocytes, which in turn allows the infection of new vectors; according to Futse et al. (2003), the rate of infection of *R. microplus* by *A. marginale* may reach 92% because they feed on the chronic carriers of the disease (Kocan et al. 1992). However, an equilibrium can be maintained in persistently infected animals that show no clinical signs of the disease. This concept is known as enzootic stability and was proposed by Mahoney and Ross (1972) for babesiosis.

The enzootic stabilities of *(A) marginale*, *(B) bovis*, and *B. bigemina* have been studied in several regions of Brazil (Almeida et al. 2006; Costa et al. 2018), where most of the populations show enzootic stability for at least one of the three etiological agents of TBDs. The objective of this study was to estimate *A. marginale* infection levels and the relationship between the number of ticks and the number of circulating copies of *A. marginale msp1β* gene of Brangus and Nellore breeds in the rearing phase in the Cerrado.

Materials and methods

Area and climate

The study was conducted at the farm of the Agropecuária Sanyo group, which is located in the municipality of Água Clara, Mato Grosso do Sul, Brazil (20° 46′ 24″ S, 52° 32′ 24″ W, 309 m altitude). The pasture was cultivated with *Urochloa* (*Brachiaria*) *decumbens* in sandy soil. The state of Mato Grosso do Sul has a humid tropical climate; the dry season lasts 1–3 months and has an average temperature above 18 °C throughout the year. Água Clara is located in the Bolsão region of Mato Grosso do Sul. According to Flumigan et al. (2015), it has a rainy season from December to February, with rainfall decreasing between March and May, after which the dry season starts; the dry season ends in August, as the rainy season gradually begins and progresses until December. Meteorological data

were obtained from the Weather and Climate Monitoring Center of Mato Grosso do Sul (CEMET/MS) from June 2016 to June 2017.

Animals and collections

All of the procedures with the animals were performed according to the standards that have been published by the National Council for the Control of Animal Experimentation (CONCEA), and the project was approved by the Ethics Committee on Animal Use (CEUA) of Embrapa Beef Cattle, protocols 01/2016 and 08/2014. In total 23 growing bulls were used (11 Brangus and 12 Nellore), of ca. 8–10 months old; the bulls were naturally infested and kept at a density of 0.6 animals per ha; no acaricides and prophylactic treatment (for anaplasmosis and babesiosis) were performed. The collections occurred from June 2016 to June 2017 at intervals of 18 days for tick count and 36 days for blood collection. Blood was collected from the caudal vein using 4 ml Vacutainer (BD Biociences, São Paulo, Brazil) tubes with or without ethylenediaminetetraacetic acid (EDTA). The samples were stored at 4 °C and then sent to the laboratory for processing and analysis.

Tick count

Tick count was performed according to Wharton and Utech (1970), where ticks of 4.5–8 mm were counted along the entire body on both sides of each animal. Subsequently, the counts from both sides of the animals were summed and bulls were divided into two homogeneous groups at the beginning of the experiment. Every 18 days, the ticks on each side were counted, and the bulls were weighed on a digital scale (Coimma, Dracena - SP) from the modal day until the end of the experiment.

gDNA extraction

Genomic DNA was extracted based on Di Pietro et al. (2011) with modifications. Briefly, 300 µl of bovine blood was used, to which 2 µl of proteinase K (20 mg/ml) and 500 µl of 20% sodium dodecyl sulfate (SDS) were added in a 2-ml tube. The samples were incubated for 1 h in a water bath at 65 °C. After the incubation period, 800 µl of chloroform was added, and the samples were vigorously vortexed for homogenization. Then, 350 µl of protein precipitation solution was added (6 ml of potassium acetate, 1.1 ml of glacial acetic acid and 2.9 ml of water), and the samples were centrifuged for 10 min at 18,000 g. The aqueous phase was transferred to a new tube, 1 ml of 100% ethanol at 4 °C was added, and the samples were stored overnight in a freezer at − 20 °C for DNA precipitation. After this period, the samples were centrifuged at 13,000 rpm for 5 min, after which the supernatant was discarded, and 1 ml of 70% ethanol was added, and the samples were centrifuged again at 13,000 rpm for 2 min. Then, the pellet was dried in a digital dry bath (D130XX series, Labnet International) at 37 °C, and the DNA was resuspended in 50 µl ultrapure water and eluted in a water bath for 30 min at 65 °C.

The samples were quantified by spectrophotometry in a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). Only samples with $A_{260} > 1.8$ and $A_{280} = 2.0–2.2$ were used for the final dilution to 100 ng/µl and were stored in a freezer at − 80 °C.

PCR

The samples were screened for *A. marginale* with quantitative polymerase chain reaction (PCR) according to the technique of Echaide et al. (1998) for the *msp5* gene. The following primers were used for the *msp5* gene: forward, 5'-GCATAGCCTCCCCCTCTTTC-3' - *msp5* position 254–273, and reverse, 5'-TCCTCGCCTTGCCCCTCAGA-3' - *msp5* position 710–692. The reaction mixture consisted of 2.5 µl of 10x buffer (1x), 0.75 µl of $MgCl_2$ (50 mM), 0.5 µl of dNTPs (2.5 mM/Invitrogen by Life Technologies), 0.5 µl of forward and reverse primers (10 pmoles), 0.3 µl of Taq (Ludwig Biotec), 1 µl of DNA (100 ng/µl) and ultrapure water that was added to a final volume of 25 µl. The reaction followed the following parameters: 95 °C/3 min, followed by 40 cycles of 95 °C/30 s, 65 °C/1 min, 72 °C/45 s, and a final extension step of 72 °C/10 min. The PCR product was visualized on a 1.5% agarose gel and was stained with ethidium bromide (EtBr) using a 100 bp marker (Bio-Rad, Hercules, CA, USA).

qPCR

The qPCR analysis was performed as previously reported (Carelli et al. 2007). Species-specific fluorescent primers and probes for the *msp1β* gene of *A. marginale* were designed in primer3 software (forward 5'-TTGGCAAGGCAGCAGCTT-3', probe 5'-/56-FAM/TCG GTCTAA/ZEN/CATCTCCAGGCTTTCAT and reverse 5'-TTCCGCGAGCATGTGCAT -3') and were produced by Integrated DNA Technologies (IDT, Coralville, IA, USA). A 10-µl aliquot of the reaction mixture was loaded into each well (5 µl of iTaq, 0.5 µl of F primer, 0.5 µl of R primer, 3 µl of H_2O and 1 µl of diluted sample for a final concentration of 100 ng/µl of DNA) in duplicate. Negative controls contained ultrapure water or mix instead of the sample. The calibration curve was calculated using gBlocks gene fragments (IDT) that had the same size as the target fragment (95-bp sequence of the *msp1β* gene of *A. marginale*), which was diluted following manufacturer's recommendations, and the standard curve was optimal from 10^{-1} to 10^{-10} ng/µl. After a preliminary test, concentrations of 10^{-5} to 10^{-10} were used in triplicate as internal controls in each 98-well plate. Samples were analyzed using the StepOne Plus Real Time PCR System (Thermo Fisher Scientific) using an activation cycle of iTaq DNA polymerase at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 45 s and an annealing/extension cycle at 60 °C for 1 min. The reaction signal was recorded during the extension step, and the data were analyzed using the StepOne software v.2.3.

iELISA

For antigen detection of IgG class anti-*A. marginale*, the indirect ELISA (iELISA) technique was used, following the protocol described by Machado et al. (1997) for *B. bovis* and modified for *A. marginale* by Andrade et al. (2004). Total antigen from *A. marginale*, produced by Laboratório de Imunoparasitologia da FCAV/UNESP Jaboticabal, in an optimal concentration of 10 µg/ml was diluted in 0.5 M carbonate/bicarbonate buffer, pH 9.6. After 12 h incubation at 4 °C, the plate block was created with PBS Tween 20 (pH 7.2), adding 6% powdered skim milk (Molico, Nestlé, Brazil). The 96-well plates (Maxisorp; Nunc, Thermo Scientific, Brazil) were incubated for 90 min at 37 °C within a moist chamber. After three washes with PBS Tween 20 buffer, the positive, negative, and reference sera were added (all diluted 1:400 in PBS Tween + 5% rabbit normal sera). The plates were then

incubated at 37 °C for 90 min in a moist chamber. After three washes with PBS Tween 20, the bovine anti-IgG conjugates linked to alkaline phosphatase (Sigma, St. Louis, MO, USA) and diluted 1:30000 in PBS Tween + normal 5% rabbit sera were added to the plates and washed again. Then, the alkaline phosphatase subtract p-nitrophenil phosphate (Sigma) was diluted in 1 mg/ml diethanolamine pH 9.8 buffer. The plates were sealed in aluminum foil and incubated for 30 min at room temperature. The plates were then read at 405 nm wavelength on a micro-ELISA reader (B.T.-100; Embrabio, São Paulo, Brazil).

DNA copy number calculation

Using the qPCR results, the number of target DNA molecules in each reaction was calculated according to the following formula described by Ke et al. (2006): NC = [6.022×10^{23} (copies/mol) × concentration (g/mol)] / molecular mass (g/l), where 6.022×10^{23} is Avogadro's number, and the molecular mass is the average molecular weight of double-stranded DNA (330 × 2) multiplied by the size of the cloned fragment.

Statistical analysis

R v.3.6.1 software was used for statistical analysis. A Kolmogorov-Smirnov test was performed to check data normality, and then a Mann-Whitney U test was used to compare the weight, number of ticks and copy number of *A. marginale msp1β* for both breeds as the data did not present normal distribution. The frequencies of positive animals obtained in the iELISA and qPCR for both breeds were analyzed using a χ^2 test. For all tests, $\alpha = 0.05$.

Results

Field data

Only *R. microplus* ticks were observed in both breeds throughout the year, and the Brangus group had an average tick count of 4.5 more than the Nellore group (mean ± SD = 45.51 ± 20.91 vs. 10.08 ± 2; Mann-Whitney U test: $p < 0.01$) (Figs. 1 and 2a). The Brangus bulls had a mean (± SD) weight of 231 ± 18.1 kg at the beginning and 381 ± 29.6 kg at the end of the experimental period (Fig. 1). The Nellore cows weighed 208 ± 23.4 kg at the beginning and 389 ± 38.4 kg at the end of the experimental period (Fig. 1). These weights did not differ between breeds (Mann-Whitney U test: $p > 0.05$). The Brangus bulls had a mean (± SD) monthly weight gain of 13.7 ± 8.1 kg, whereas for Nellore weight gain was 16.5 ± 8.7 kg.

Mean (± SD) temperature was 24.9 ± 2.8 °C, humidity was 66.9 ± 4.5%, and rainfall was 33.4 ± 18.6 mm during the sampling period (Fig. 1).

PCR, qPCR and ELISA

The screening of PCR samples from all bulls showed the presence of the *msp5* gene of *A. marginale*. Of the 276 qPCR samples, 275 tested positive for *A. marginale*, and the Brangus group had sixfold more copies than the Nellore group (mean ± SD = 2758.9 ± 1205.2 vs. 431.1 ± 425.9; Mann-Whitney U test: $p < 0.01$) (Table 1; Fig. 2b). The overall

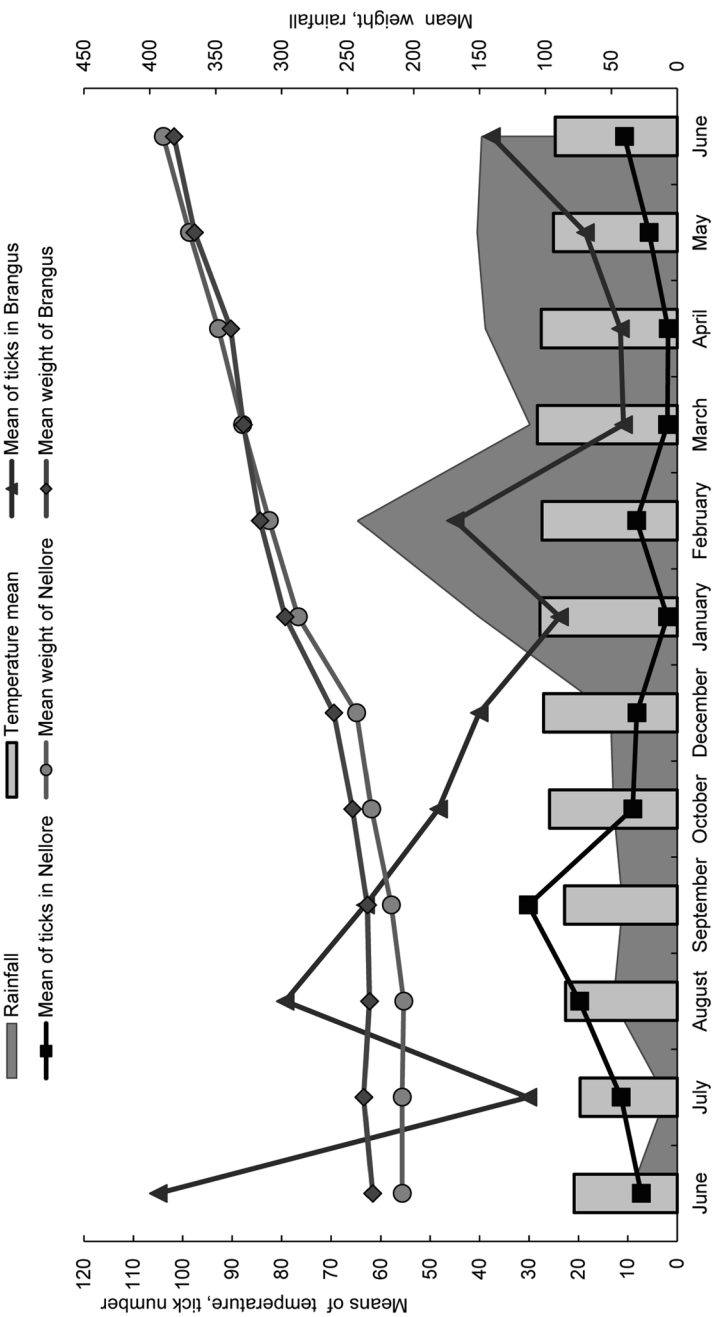

Fig. 1 Mean temperature (°C), rainfall (mm), number of ticks, and weight (kg) of Brangus and Nellore cows from June 2016 to June 2017

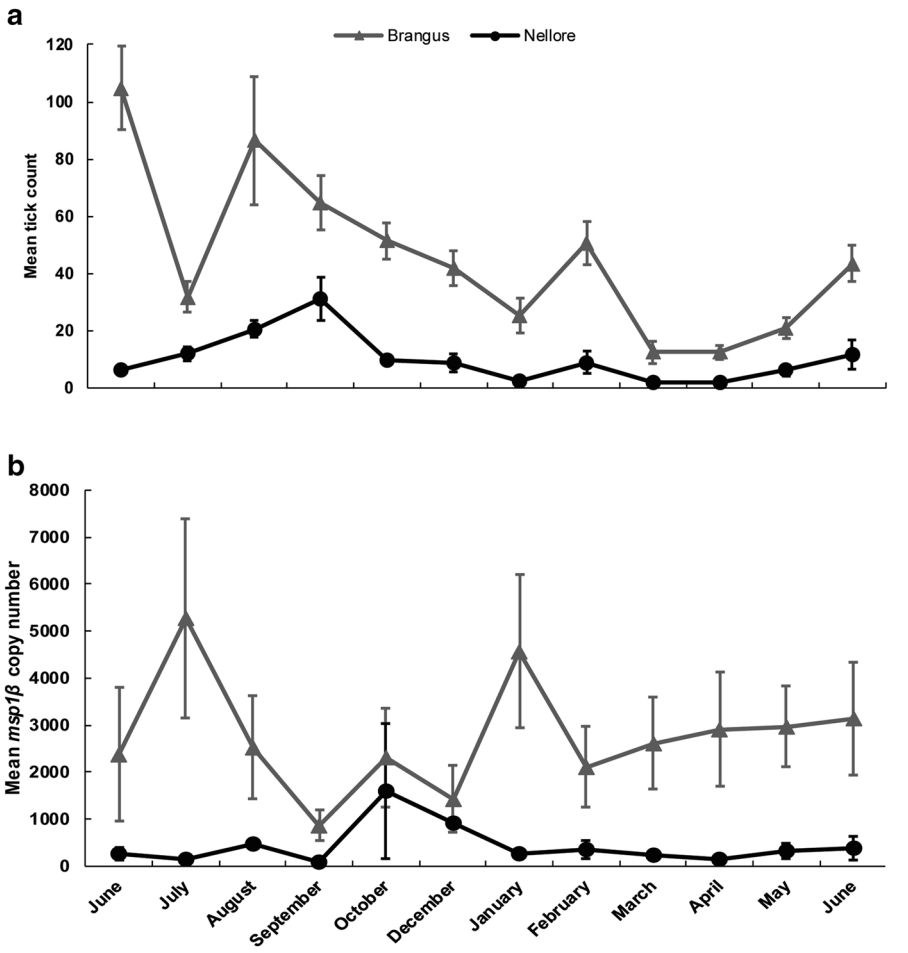

Fig. 2 Mean (± SE) number of **a** ticks and **b** DNA copies (per cattle breed) of Brangus and Nellore cattle from June 2016 to June 2017 at 36-day intervals

Table 1 Parameters for qPCR analysis of *Anaplasma marginale msp1β* gene obtained from gDNA extracted from Brangus (n = 132) and Nelore (n = 144) cattle in the period ranging from June 2016 to June 2017 in the Cerrado-MS

	E	R^2	Slope	y-intercept	Cq		SQ	
					Nellore	Brangus	Nellore	Brangus
Max	105.82	0.995	− 3.491	41.891	36.20	38.89	1.75×10^4	2.10×10^4
Min	93.4	93.4	− 3.19	33.285	20.84	0.0	0.218	
Mean	98.75	98.75	− 3.374	38.032	26.87	26.12	545	2870

E efficiency of amplification, R^2 determination coefficient, *Cq* quantification cycle, *SQ* starting DNA quantity: number of copies of a 95-bp fragment from *A. marginale msp1β* gene

prevalence of *A. marginale* determined by antibody detection was 11.4% for Brangus and 11.8% for Nellore cattle.

Discussion

This study observed the development of Brangus and Nellore cows in the growing phase during a 1-year period in the Brazilian Cerrado and determined the presence of the agent *A. marginale*, the number of circulating copies, the host's immune response, and the relationship of these factors to the number of ticks.

Climate and ticks

The distribution of many invertebrates is known to be regulated by temperature and rainfall, and ticks are no exception (Estrada-Peña et al. 2005; Korotkov et al. 2015). In particular, the tick *R. microplus*, which has a monoxenous cycle, is distributed between 32° N and 32° S, with some sporadic outbreaks in the 35° parallel. These regions have a tropical climate, and Brazil is characterized as an endemic region for the tick *R. microplus* and the diseases it transmits. The mean temperature and humidity throughout the period were 25 °C and 66%. In the Cerrado region annually, 3–4 tick generations are observed, three of them in the rainy season from October to April, when greater infestations occur (Gomes et al. 1988). However, we also observed high tick infestations in Brangus cows within a period that coincides with the onset of the rearing phase and associated stress factors (Bianchin et al. 2007), along with the dry season (Flumigan et al. 2015), possibly contributing to sufficient maintenance of the tick population during the most challenging months for its survival.

Weaning, age of growing bulls in the rearing phase and the cerrado conditions

In areas of enzootic stability where cows are constantly infected, calves are presumed to have a greater resistance to developing TBDs because they have acquired enough antibodies through the colostrum to fight it (Madruga et al. 1987), they have greater cellular immunity and the presence of serum resistance factors (Madruga et al. 1985), and transplacental transmission could also occur (Costa et al. 2016). Although the Mato Grosso do Sul region is characterized as an area of enzootic stability, critical periods of low humoral resistance may occur (Madruga et al. 1983). Moreover, it is possible that clinical cases of babesiosis can occur, which is a condition that is associated with anaplasmosis in the TBD complex.

However, the weaning period can generate stressful conditions that may result in immune suppression in calves (Hulbert et al. 2011). In addition, the early rearing phase, which is often accompanied by cattle transport stress (Trunkfield and Broom 1990; Broom 2003), is concomitant with the beginning of the dry season in the Cerrado, especially in the state of Mato Grosso do Sul, where pasture availability is of low quality due to degradation (Andreotti et al. 2018); this factor may cause nutritional stress to the animal, suppressing its immune system even further (Carroll and Forsberg 2007). In addition to the breed of the cattle, these factors that are associated with the early phase were evaluated when there is a higher occurrence of ticks, and this choice may have contributed to the large number and infestation of animals of the Brangus group. Thus, previous evaluation of tick resistance to insecticides (Drummond et al. 1973), followed by a proper method of

application and product choice (Higa et al. 2019), should be considered for strategic treatment in 21-day intervals during the dry period in the Cerrado region (June to August), as suggested by Bonatte-Junior et al. (2019), to ease such effects for these animals during the growing phase.

Detection by PCR, quantification by qPCR and serology

Antibody levels detected for each animal and sample every 36 days in a 1-year period showed a low percentage of positive samples and did not reflect the results obtained by PCR and qPCR analysis. All animals of both breeds had *A. marginale* detected by the PCR technique as well as by the qPCR technique, which proved to be extremely sensitive for the detection of *A. marginale*, in a result similar to that of Carelli et al. (2007) and Giglioti et al. (2018). The mean number of circulating *A. marginale* copies was sixfold higher in the Brangus than in Nellore individuals. One possible explanation for the difference in tick count in each breed could be if the Nellore animals had a rapid response against the ticks and were thus exposed to fewer *A. marginale*, whereas the Brangus animals may have had a higher tick load and consequently showed greater inoculation of rickettsial agents.

However, similar to the results observed by Giglioti et al. (2016), it was possible to observe substantial fluctuation in the number of copies of *A. marginale* in the blood of growing bulls over time when comparing the tick infestations between blood collections (36 days). Previous studies have shown that there is no association between the number of ticks and the circulating number of pathogen copies (Giglioti et al. 2016, 2017), indicating that this factor cannot be attributed as a cause of variation. *Anaplasma marginale* infects the epithelial cells of the tick gut, then migrates through the hemolymph and colonizes the salivary glands (Kocan et al. 1992). After the primary infection and an incubation period of 7–60 days in the host (Kocan et al. 2003, 2004), a replication cycle occurs in the erythrocytes, and the infected erythrocytes are removed through the mononuclear phagocyte system. In cattle, acute infection is characterized by increasing bacteremia in infected erythrocytes, peaking at 2–6 weeks after infection and reaching $\geq 10^6$ bacteria per ml of blood (Eriks et al. 1993; de la Fuente et al. 2002). Those fluctuations could be attributed to *A. marginale* antigenic variation responsible for immune evasion (Graça et al. 2015) and different strains (Quiroz-Castañeda et al. 2016) within the herd and/or animal (Silva et al. 2015; Ramos et al. 2019). However, the persistent infection in hosts throughout their lifespans can be kept in circulation within the herd by other well-known agents as mechanical vectors, such as tabanid horse flies (Hornok et al. 2008) and mosquitoes (Ristic 1977). *Anaplasma marginale* transmission by flies such as *Stomys calcitrans* cannot be discarded as they were observed during the experimental period but not collected; however, their ability to transmit this infection mechanically is questionable (Scoles et al. 2005).

The role of ticks as a vector of *A. marginale* is unquestionable, but there remains a gap in the cycle, as transtadial and interstadial transmissions have been reported (Aguirre et al. 1994), but not transovarial transmission (Raoult and Roux 1997), and newborn larvae of *R. microplus* are not infected with this *Rickettsia*. Moreover, when larvae feed, they rarely change hosts; typically, such change behavior is performed by adult males (Kessler 2001), making the tick a multiplier of *A. marginale* through feeding on persistently infected hosts in zones of enzootic stability.

Ticks are known to promote immunosuppression at the site of their bites through secreting substances in their saliva (Kazimírová and Štibrániová 2013; Perner et al. 2018) and by stimulating animals' inappetence; these factors are potentiated by the number of ticks

during infestation. Few bulls had specific antibodies against *A. marginale*. This may be attributed to the antigenic variation of *A. marginale* (Graça et al. 2015) which enables it to evade the immune system and chronically infect its hosts, making them a reservoir for other vectors.

According to Eriks et al. (1993), who studied *A. marginale* in *Dermacentor andersoni*, persistently infecting cattle showed fluctuating *Rickettsia* levels during the 5th week of a 24-week period, with between $< 10^4$ and 10^7 infected erythrocytes per ml of blood, and levels $< 10^4$ were maintained for 4–8 days in each 5-week cycle. This fact may also be related to the highly fluctuating number of copies that were observed in this study.

Despite showing natural resistance against tick infestations, the Nellore cattle presented similar rates as the Brangus cattle in the ELISA test, thus requiring a smaller number of ticks to generate a response similar to a Brangus individual with on average 4.5-fold more ticks. Frisch (1999) attributed this difference to the fact that certain cattle species have already shared their environment with ticks for a long time and have consequently developed a large number of genes with smaller effects. In addition, such differences can also be explained by the long historical association between *Bos indicus* and ticks (Sutherst and Utech 1981), self-grooming behavior (Snowball 1956), and morphological differences (Verissimo et al. 2015).

Piper et al. (2009) showed that *B. indicus* developed a stable T-cell-mediated response to infestation by ticks and reported its cell and leukocyte profile. *Bos taurus* cows showed cellular and gene expression patterns that were consistent with an innate response, inflammatory response to infestation, and high tick-specific IgG1 titers, suggesting that these animals also develop a T-cell response to infestation. The immune responses of frequently infected cows that are exposed to antigen inoculants are sufficient to keep blood parasites at low levels without eliminating them completely from the organism (Giglioti et al. 2017). Despite the natural resistance exhibited by the Nellore animals, the present study also found variation in individuals of the same breed, as observed by Piper et al. (2009) and Andreotti et al. (2018).

Enzootic stability and breeds reared in the Cerrado

Integrated pest management can be used to achieve enzootic stability because it combines strategies of environmental manipulation and chemical and biological control and can reduce arthropod populations that are in disequilibrium (Zucchi 1990). Some strategies, such as immersion baths, do not disturb the enzootic equilibrium (Smith et al. 2000); however, their misuse can cause a decline in the tick population for a prolonged period that is sufficient to reduce the inoculation rate necessary to maintain enzootic stability but not enough to eliminate the TBD parasites.

According to the predictions of Gomes et al. (1988), an ideal number of ticks to achieve enzootic stability in purebred Nellore cows would be an annual population of 3–4 engorged females/day. Our study observed an average of 10 engorged females/day. In turn, crossbred animals (4/8 Nellore and 4/8 European) were expected to have mean annual populations of 20–25 engorged females/day could lead to a 5.5 kg/animal/year weight loss in terms of herd that could be worrying from a sanitary and economic point of view. Also Ibagé animals (3/8 Nellore and 5/8 Angus) exhibited populations of 60 engorged females/day under the same conditions (Gomes et al. 1988). The Brangus animals (3/8 Nellore and 5/8 Angus) in our study had an average of 45 engorged females/day. At the end of the study period, no severe weight loss was observed, and both breeds had similar weight averages (381 kg for

Brangus and 389 kg for Nellore). In addition, location and climatic variation can greatly affect enzootic stability, as demonstrated by previous researchers (Smith et al. 2000) who observed that sites with fewer generations of ticks per year may present moments of instability because there are periods when the tick population is relatively small and, thus, there is no minimum level of parasite inoculation.

Andreotti et al. (2018) showed that crossbred animals (Brangus) have higher rates of infestation by ticks, even when they are reared with Zebu cows. Throughout the year, the infection rate by *A. marginale* remains constant, as corroborated by data presented by Giglioti et al. (2018); thus, maintaining tick infestation at low levels may be advantageous for the herd when the animals are exposed at early ages, as this is the period when the immune system is maturing and responds more efficiently (Madruga et al. 1983). However, in situations of stress—such as unfavorable environmental conditions, the weaning phase, and poor nutrition due to poor quality pastures—this strategy may have the reverse effect and lead to the development of anaplasmosis and other diseases.

Conclusions

Despite the higher number of ticks, no relation of a standard number of circulating copies of *A. marginale msp1β* gene per tick could be achieved, possibly due to interactions of the host and bacteria evasion mechanisms. No relation was found between the number of ticks and the number of *A. marginale msp1β* gene copies in naturally infested and untreated Brangus and Nellore bulls in the rearing phase in the Cerrado. There were no clinical signs of disease and no apparent losses in population weight gain throughout the year. Therefore, the maintenance of enzootic stability by maintaining a low number of ticks in contact with the bovine population and combining this stability with the strategic control of the TBD vectors could help maintain enzootic equilibrium, preventing moments of enzootic instability and the emergence of clinical signs of TBDs. Although the literature about certain vectors (primarily mosquitoes and flies) and *A. marginale* transmission goes back a long time, new studies should be conducted due to changes in habitat (such as degradation), climate, and vector population dynamics.

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Author contributions RA led the study conception and fund raising. All authors contributed to the study design. Material preparation, data collection, and analysis were performed by KRM, LSOH, PBJR, MVG, POD, BGC, and JBC. The first draft of the manuscript was written by KRM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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