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A small proportion of Zebu genetic background in crossbred calves may not be enough to improve resistance against natural bovine *Babesia* spp. infections

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ABSTRACT

The main objective of cattle breeders in tropical and subtropical regions is to acquire animals with taurine-productive traits adapted to the broad weather range of these regions. However, one of the main challenges on using taurine genetics in these areas is the high susceptibility of these animals to tick-borne diseases. Consequently, the present study evaluated from 10 November 2021–19 April 2022, the over 13 assessments, the *Babesia bovis* and *Babesia bigemina* DNA loads and the IgG anti-*B. bovis* and anti-*B. bigemina* levels in Angus (n = 17, 100% Taurine) and Ultrablack (n = 14, ~82% taurine and 18% Zebu) calves. Data were analyzed using a multivariate mixed model with repeated measures of the same animal including the fixed effects of evaluation, genetic group, sex, *Babesia* spp., and their interactions. The repeatability values were estimated from the (co) variances matrix and expressed for each species. The correlations between the DNA loads (CN_{log}) and IgG titers (S/P) values for the two species were also estimated using the same model. Regarding the specific IgG antibody titers for both *Babesia* spp., no significant differences were observed between the two genetic groups. However, for *B. bovis* and *B. bigemina* DNA loads, Ultrablack calves presented significantly higher values than Angus calves. Under the conditions evaluated in this study, our findings suggest that the low percentage of Zebu genetic in the Ultrablack breed was insufficient to improve resistance against babesiosis. Further studies must demonstrate if the low percentages of Zebu genetics in Taurine breeds can modify the susceptibility to babesiosis infections.

1. Introduction

Cattle producers utilize crosses between *Bos taurus taurus* and *Bos taurus indicus* as a strategy to achieve greater productive capacity. F1 crossbred cattle between Zebu x taurine cattle exhibit an increase in heterosis related to resistance to parasites and increased productivity, presenting better traits than the zebuine or taurine pure breeds (Seifert, 1971; Peacock et al., 1981). However, one of the main obstacles to using cattle with a high degree of taurine genetic is the higher susceptibility of these animals to parasites. Ticks and tick-borne agents are responsible for one of the main diseases that affect cattle herds and cause substantial economic losses in the livestock industry in tropical and subtropical regions (Barros et al., 2005). The direct and indirect losses caused by infestations with *Rhipicephalus microplus* were estimated at 3.24 billion dollars (Grisi et al., 2014).

In Brazil, bovine babesiosis is caused by *Babesia bovis* and *Babesia bigemina*, which are exclusively transmitted by the one-host tick *R. microplus* (Guglielmone, 1995; Oliveira-Sequeira et al., 2005). The most common manifestations of acute cases of babesiosis include fever, hemolytic anemia, icterus, weakness and haemoglobinuria, whereas chronic infections are usually subclinical (Bock et al., 2004; Schnittger et al., 2012). The most significant losses are attributed to *B. bovis* due to

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its higher pathogenicity than *B. bigemina*. Erythrocytes infected by *B. bovis* accumulate in the capillaries of various organs, including the brain and lungs, leading to often fatal clinical complications such as cerebral babesiosis, respiratory distress and multi-organ failure (Gohil et al., 2013).

The occurrence of these hemoparasites in cattle usually depends on the tick's population dynamics, which require favorable climatic conditions. Most Brazilian regions are predominantly tropical, with a small proportion subtropical, favoring the vector ticks' development and consequently blood parasite infections (Frabetti et al., 2023).

The use of quantitative PCR (qPCR) may provide an estimate of the quantification of bovine *Babesia* spp. DNA levels, and the obtained results are frequently used to indicate the resistance/susceptibility of cattle from different genetic groups to these infections (Bilhassi et al., 2014; Giglioti et al. 2016, 2018, 2020). Furthermore, several studies have combined immune responses and molecular tests to detect *Babesia* spp., aiming to better elucidate the mechanisms of host-parasite interactions involved in the resistant phenotype (Jaffer et al., 2010; Ramos et al., 2011; Machado et al., 2012; Shebish et al., 2012; Hüe et al., 2013; Ibrahim et al., 2013; Rosales et al., 2013; Giglioti et al., 2017).

The inclusion of Zebu breeds in breeding programs with taurine breeds has been applied to increase heterosis, especially regarding traits associated to enhanced parasitic resistance and adaptability to tropical climates. According to the Australian Brangus Cattle Association (ABCA), "Ultrablack" or "Ultrared" cattle were developed by crossing Brangus (Brahman x Angus) with Angus breeds that are black or red, respectively. To be eligible for registration in the Ultra Register, these cattle breeds must have Brahman genetic content ranging from 8% to 25%. According to the International Brangus Breeders Association (IBBA), the small proportion of Zebu cattle genetics in the Ultra breed can enhance the slicking hair ability of the animals, improve the environmental adaptability and reproductive performance of the resulting offspring. Additionally, this breed combines the environmental adaptability and maternal excellence of the Brangus breed with exceptional meat marbling and calving ease.

Therefore, the present study evaluated the levels of *Babesia* spp. infection, tick loads, and specific antibody titers for *B. bovis* and *B. bigemina* in Angus and Ultrablack calves raised under tropical conditions in Brazil, using qPCR and ELISA assays, and verified the effect of a small amount of Zebu genetics on Ultrablack resistance to these parasites.

2. Material and methods

2.1. Experimental animals

Thirty-one calves, from the municipality of José Bonifácio (São Paulo, Brazil; coordinates 21° 2′ 23″ S, 49° 41′ 28″ W) and representing two breeds, were used: Angus (n=17; 12 females and five males) and Ultrablack (n=14; six females and eight males). At the beginning of the experiment, the average ages of calves from the Angus and Ultrablack genetic groups were 2.8 ± 1.6 months and 2.1 ± 1.3 months, respectively. In the same order, the Angus group had maximum and minimum ages of 150 days and 13 days, while the Ultrablack group had maximum and minimum ages of 137 days and 13 days, respectively. According to the breeder's records, the Ultrablack breed was produced by crossing Brangus (3/8 Zebuine and 5/8 Angus), resulting in a zebu genetic composition representing approximately half of that of the Brangus breed parents (approximately 18%). Thirteen samplings were conducted at average intervals of 12 days between 10 November 2021 and 19 April 2022 (1st to 13th: 10-Nov-21, 17-Nov-21, 24-Nov-21, 8-Dez-21, 21-Dez-21, 5-Jan-22, 19-Jan-22, 31-Jan-22, 15-Fev-22, 2-Mar-22, 16-Mar-22, 30-Mar-22, 19-Abr-22), resulting in a total of 403 observations. Previous studies have confirmed that this region evaluated was considered endemic for babesiosis (Bilhassi et al., 2014; Giglioti et al., 2018). During the experimental period, the animals were constantly exposed to natural infestation by the tick vector *R. microplus*, and at all evaluations, the animals presented a score of tick infestations ≥ 1 (Frabetti et al., 2023). The mean rainfall levels for the 6 months of the experimental period were 2.9, 2.8, 9.1, 10.2, and 2.7 mm, respectively (National Institute of Meteorology - INMET, José Bonifácio station, São Paulo). The calves were kept in rotated paddocks comprising coast-cross grass (*Cynodon dactylon* (L.) Pers). Tick controls were performed every 21 days by the application of fipronil pour-on (Topline®). Hemoparasite controls were performed when animals presented clinical signs. This experiment adhered to the ethical principles of animal experimentation of the Instituto de Zootecnia Ethics Committee on Animal Experimentation (Protocol Nr. 328–2021).

2.1.1. Tick counts and sample processing

For each animal, *R. microplus* counts were based on the tick infestation scores (IS), which were assigned for all stages of parasite development, including adult females. This methodology was originally developed by Fraga et al. (2003) and subsequently modified by Frabetti et al. (2023): IS=0 (no visible tick instars), IS=1 (up to 20 ticks), IS=2 (20–60 ticks), IS=3 (60–100 ticks), and IS=4 (>100 ticks). At the time of each tick count, two blood samples were collected from the jugular vein of each animal using a vacuum system (Vacutainer®, Becton Dickinson), one containing the anticoagulant EDTA for DNA extraction, and the other, without anticoagulant, for serum separation.

Blood samples with EDTA underwent DNA extraction using the Wizard® Genomic DNA Purification Kit, following the protocol for isolating genomic DNA from 300 μL of whole blood (cat. No. A1620, Promega®, Madison, WI, USA). The DNA samples were assessed for purity and concentration using a BioDrop spectrophotometer (BioDrop uLITE, Biochrom Ltd, UK) and then diluted in TE buffer (Tris-EDTA, pH 7.8) at a ratio of 1:4 (DNA: TE). The serum from blood samples without anticoagulant was transferred into microtubes. Both DNA and serum samples were frozen at −20°C until analysis.

2.2. qPCR assays

The DNA copy number (CN_{log}) of *B. bovis* and *B. bigemina* was estimated by absolute quantification as described by Okino et al. (2018), using primers and probes that flank a 98-nucleotide fragment located in the gene encoding the cytochrome b mitochondrial gene (*cybmt*).

The PCR assays were performed using the CFX™ Real-Time PCR Detection System (BioRad, Hercules, CA, USA), with a reaction volume of 10 μL: 2 μL 5x HOT FIREPol Probe Universal qPCR Mix (Solis BioDyne, Tartu, Estonia), 0.5 μL of each primer (10 μM), 0.5 μL of probe (2.5 μM), 4.0 μL of nuclease-free water, and 2.0 μL of the DNA sample. The thermal conditions were set to 10 minutes at 95°C, followed by 40 cycles of 95°C (denaturation) for 15 seconds and 60°C (annealing/extension) for 1 minute. The samples were analysed in duplicates, including positive and negative controls. The calibration curve for the quantification of *B. bovis* and *B. bigemina* DNA was constructed using synthetic DNA, gBlocks® gene fragments (IDT, IA, USA). The gBlocks® fragments representing each target sequence of *B. bovis* and *B. bigemina cybmt* were subjected to 10-fold serial dilutions (10^{-1} to 10^{-10}).

2.3. Recombinant indirect ELISA assays

Serum samples were analysed by the recombinant indirect ELISA assay following the methodology described by Terkawi et al. (2011) and modified by Okino et al. (2020). In brief, polystyrene high-bind microplates (Cat. CLS3590, Sigma, St. Louis, USA) were coated overnight at four °C with 0.19 μg of recombinant protein BbSBP4 (*B. bovis*) or BbigRAP (*B. bigemina*) (Terkawi et al., 2011) (Genscript, USA synthesized both proteins), using delimited protein sequences diluted in 50 mM carbonate-bicarbonate buffer pH 9.6. The plates were washed (all washing steps were performed using 0.05% Tween 20 PBS, repeated six times) and incubated with 100 μL of blocking solution (3% skimmed

milk in PBS) at 37°C for 1 hour. After that, the plates were washed and incubated with 50 μL of serum samples (diluted 1:100 in a blocking solution) at 37°C for 1 hour, followed by washing and incubating with sheep anti-IgG bovine antibody conjugated to horseradish peroxidase (HRP) (Cat.AAI23P, Bio-rad) diluted at 1: 4000 in a blocking solution. After this, the plates were washed and incubated with 100 μL of SIGMAFAST OPD (Cat. P9187, Sigma) at room temperature (20–25°C) for 15 min. The enzymatic reaction was blocked by adding 50 μL of 1 M HCl solution. All samples were tested in duplicates, and standard controls (positive and negative serum) were included in each plate. Optical density (OD) at 490 nm was measured using Model 550 of the microplate reader (Biorad). The (OD) values were transformed into sample/positive (S/P) using the following equation: (OD of the sample – OD of negative control serum)/(OD of positive control serum – OD of negative control serum).

2.4. Statistical analysis

The CN_{log} and S/P values were analyzed using mixed model methods, employing two different models: (i) to estimate repeatability and compare means, and (ii) to estimate associations between variables (CN_{log}, S/P, and IS values). Model (i) was applied to each variable separately and included repeated measurements of the same animal. The effects of evaluation (EV), genetic group (GG), sex, and the interaction between EV and GG were considered as fixed effects. The covariance matrix assumed a first-order autoregressive structure (AR(1)). The repeatability levels were estimated from the (co)variance matrix, expressed for each species as the correlation between measures taken from the same individual across evaluations. Model (ii) was multivariate with repeated measurements in the same animal, including the fixed effects of EV, GG, variable analysis (VA) (CN_{log} and S/P values for each *Babesia* spp., and tick score values), sex, and the interactions between EV x GG and EV x GG x VA. This model used a structure for the (co)variance matrix of direct product structures (UN@CS) designed for multivariate repeated measures. For both models, all analyzed variables were precorrected for the animal's age effect for each evaluation, using the fixed effects of GG and sex in the model. A p-value ≤ 0.05 was considered statistically significant for both models.

3. Results

For all analyzed variables (CN_{log}, S/P, and IS), the evaluation effect (EV) was significant ($p < 0.001$), whereas the sex effect was not significant ($p > 0.05$), except for IS, where males presented higher infestation than females.

For IS, there was no significant effect of GG ($p = 0.8157$). However, significant effects of the interaction between GG and EV ($p < 0.0001$) were observed. The distribution of mean scores along with their standard errors for each assessment is shown in Fig. 1. The average IS in females (1.65 ± 0.06) was significantly lower ($p = 0.0379$) than males (1.85 ± 0.07).

Regarding the general means of DNA loads (CN_{log}), the Ultrablack group showed significantly higher values compared to Angus for both *B. bovis* ($p = 0.0174$) and *B. bigemina* ($p < 0.001$). The CN_{log} means, followed by standard errors for *B. bovis* and *B. bigemina* and for Angus and Ultrablack breeds, were 1.43 ± 0.09, 1.76 ± 0.09, 3.18 ± 0.11, and 4.10 ± 0.11, respectively. The interaction effect of EV x GG was also significant ($p < 0.01$) for both *Babesia* spp. However, only one evaluation (8-Dec-21) showed significantly higher levels of *B. bovis* DNA in the Ultrablack group, while during the first five evaluations (10-Nov-20, 17-Nov-20, 24-Nov-20, 8-Dec-20, and 21-Dec-20), the levels of *B. bigemina* DNA were significantly higher in this group (Fig. 1).

There were no significant differences ($p > 0.05$) in the general mean of specific IgG antibody titers between the two genetic groups for both *Babesia* spp. However, the interaction EV x GG was significant ($p < 0.01$). At the 2nd and 3rd samplings for anti-*B. bovis* titer levels, and from the 2nd to the 6th samplings for anti-*B. bigemina*, the Ultrablack calves exhibited significantly higher levels than the Angus calves (Fig. 2). At no point in the evaluations did the Angus calves exhibit anti-*Babesia* spp. titers higher than those observed in Ultrablack calves.

The positive frequencies for the CN_{log} and S/P values for *B. bovis* and *B. bigemina* did not differ among the genetic groups ($p > 0.05$), although there was a significant ($p < 0.05$) effect of EV for both species. For the CN_{log} data, the positive frequencies of *B. bigemina* started lower in the initial evaluations and increased over time, while the positive frequencies of *B. bovis* also showed the lowest frequency in the initial evaluations, with variable increases and decreases throughout the evaluations. The frequencies of *B. bovis* and *B. bigemina* positive values were initially high, above 80%, in the first evaluations but declined from

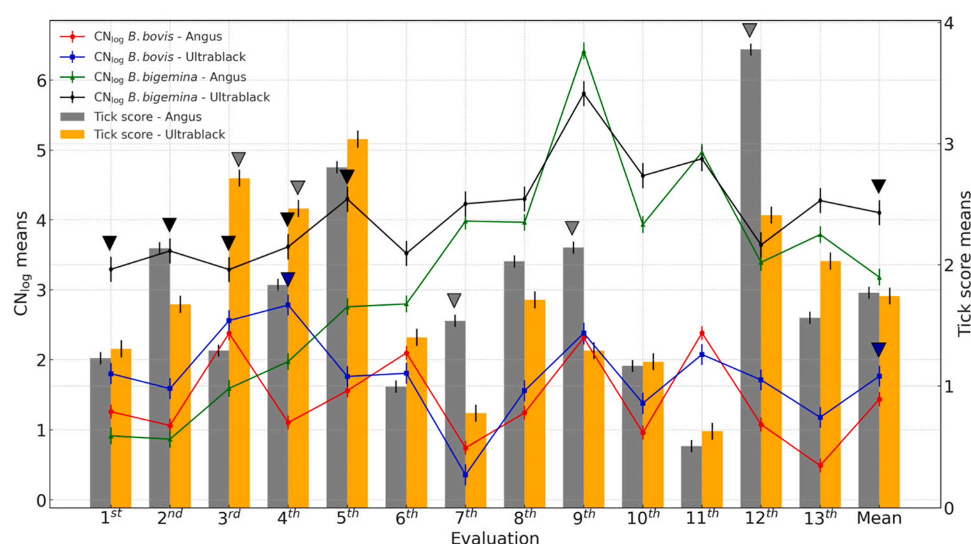

Fig. 1. Means (± error standard) of DNA copy number (CN_{log}) of *Babesia bovis* (blue and red lines) and *Babesia bigemina* (black and green lines), as well as the tick score (gray and orange bars) in Angus and Ultrablack calves for 13 evaluations (1st to 13th: 10-Nov-21, 17-Nov-21, 24-Nov-21, 8-Dez-21, 21-Dez-21, 5-Jan-22, 19-Jan-22, 31-Jan-22, 15-Fev-22, 2-Mar-22, 16-Mar-22, 30-Mar-22, 19-Abr-22) and general means. Triangles indicate significant differences ($p \leq 0.05$): The black triangle represents the CN of *B. bigemina* between Angus and Ultrablack; the blue triangle represents the CN of *B. bovis* between Angus and Ultrablack; and the orange triangle represents the tick score between Angus and Ultrablack.

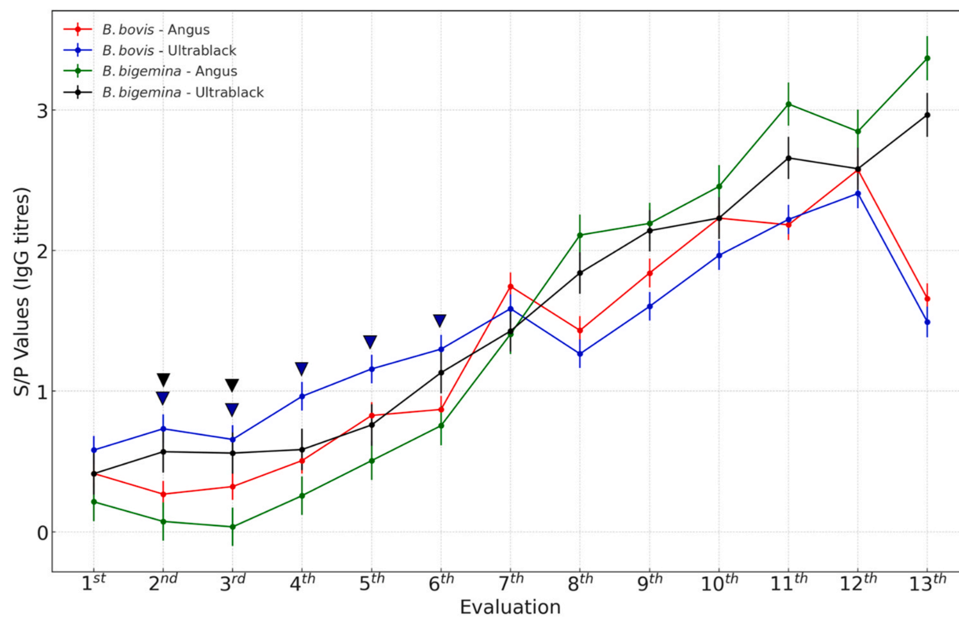

Fig. 2. Means (± SE) of specific IgG titers (S/P), anti-*Babesia bovis* (red and blue lines) and anti-*Babesia bigemina* (black and green lines) in Angus and Ultrablack calves for 13 evaluations (1st to 13th: 10-Nov-21, 17-Nov-21, 24-Nov-21, 8-Dez-21, 21-Dez-21, 5-Jan-22, 19-Jan-22, 31-Jan-22, 15-Fev-22, 2-Mar-22, 16-Mar-22, 30-Mar-22, 19-Abr-22). Triangles indicate significant differences ($p \leq 0.05$): The black triangle represents the S/P of *B. bigemina* between Angus and Ultrablack; the blue triangle represents the S/P of *B. bovis* between Angus and Ultrablack.

evaluation 11 onwards (Fig. 3).

The estimated repeatabilities for the CN_{log} and S/P values of *B. bovis* and *B. bigemina* were 0.21 and 0.31, and 0.71 and 0.82, respectively (Table 1). The correlation between the CN_{log} and S/P values of *B. bovis* and *B. bigemina* was low, 0.05 and 0.14, respectively, as were other associations (Table 1).

4. Discussion

Babesiosis monitoring by quantitative PCR may be utilized to characterize phenotypic or genetic resistance in cattle and can be implemented in a selection system. The present study compared babesiosis DNA loads in calves from two genetic groups: Angus (100% Taurine) and Ultrablack (~18% Zebuine, according to the breeder's records). Our results showed that Ultrablack calves had higher DNA loads of both *B. bovis* and *B. bigemina* than Angus calves. During the experimental

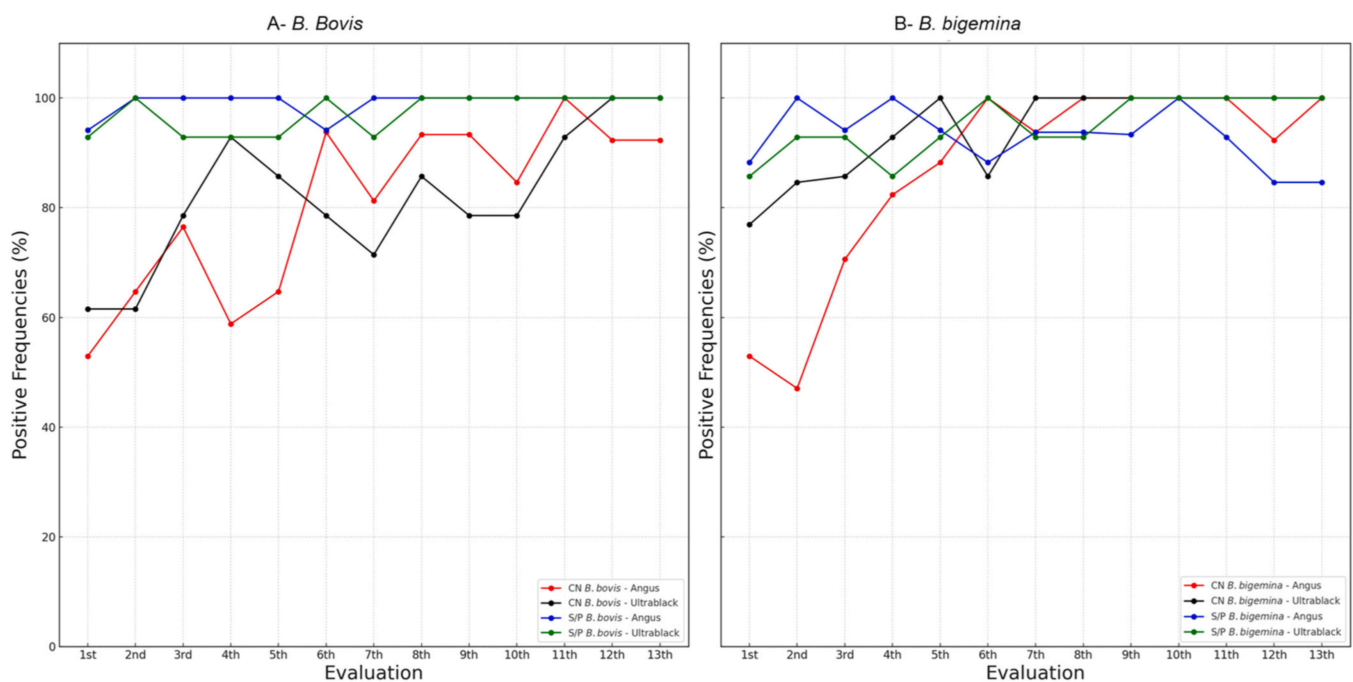

Fig. 3. Distribution of positive frequencies of qPCR (CN – DNA copy number) and ELISA (S/P- sample/positive) values for *Babesia bovis* (A) and *Babesia bigemina* (B) in Angus and Ultrablack calves for 13 evaluations (1st to 13th: 10-Nov-21, 17-Nov-21, 24-Nov-21, 8-Dez-21, 21-Dez-21, 5-Jan-22, 19-Jan-22, 31-Jan-22, 15-Fev-22, 2-Mar-22, 16-Mar-22, 30-Mar-22, 19-Abr-22).

Table 1
Estimated repeatabilities and correlations of the CN and S/P values for *B. bigemina*, *B. bovis*. Repeatabilities are in diagonals (in bold), and correlations are above the diagonals.

		Copy number (CN)		Antibody titers (S/P)	
	Species	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*
CN	*B. bovis*	**0.21**			
	B. bigemina	0.06	**0.31**		
S/P	*B. bovis*	0.02	0.03	**0.71**	
	B. bigemina	0.05	0.00	0.11	**0.82**
	Tick score	-0.09	0.08	0.03	0.00

period, all animals presented tick infestations with a score ≥ 1 (≥ 20 tick counts), which allowed the frequent inoculation of tick fever agents. Moreover, throughout the study, all animals shared the same paddocks, ensuring uniformity in tick infestations and, consequently, in babesiosis inoculations. Here, the 31 calves were monitored through 13 consecutive evaluations, with repeated measures over time (mean interval of 12 days). According to Vickers (2003), several studies estimate a continuous endpoint repeatedly over time since researchers aim to observe the time course of a clinical sign or evaluate how the effect of a treatment evolves over time.

As expected, significant effects of evaluation on tick scores and levels of babesiosis infection were identified. It is well-established that climatic conditions influence the development of free-living tick stages in pastures, indirectly affecting the transmission dynamics of babesiosis, as reported by Jongejan and Uilenberg (2004). The study area's tropical climate, with hot and humid summers and moderately cool and dry winters, supports the tick life cycle in pastures throughout the year, including the drier months. This area has been studied three times and is recognized as endemic for babesiosis (Bilhassi et al., 2014, Giglioti et al., 2016, Frabetti et al., 2023). The prevalence of hemoparasites in cattle is largely determined by the dynamics of the tick population, which thrives under favorable climatic conditions (Mahoney and Ross, 1972) have noted. Areas with constant tick occurrences are defined as regions of endemic stability, a term initially coined by Mahoney (1962) and further detailed by Mahoney and Ross (1972). In such environments, cattle are constantly exposed to these pathogens, leading to the development of enhanced immunity and the emergence of healthy, asymptomatic carriers (Bock et al., 2004).

This study was the first to compare *Babesia* spp. infection levels between Angus and Ultrablack animals. We initially hypothesized that even a small proportion of Zebu genetics added to Taurine animals could enhance resistance against babesiosis. Contrary to our expectations, for both *Babesia* spp. the general mean of the 13 evaluations showed higher infection rates in Ultrablack calves. However, for *B. bovis*, only one evaluation revealed a higher infection rate in Ultrablack, indicating that both Angus and Ultrablack breeds exhibited similar resistance levels to *B. bovis* infections. The interaction effect between genetic group and evaluation was more pronounced for *B. bigemina*, where, in the first five evaluations, Ultrablack calves exhibited higher infection rates than the Angus breed. The Angus breed's higher resistance to *B. bigemin*a infection may be attributed to the calves' younger age during the initial evaluations. According to Zintl et al. (2005), though both young and adult cattle are susceptible to *Babesia* spp. infections upon first exposure, younger animals display stronger innate resistance, also known as inverse age resistance, wherein clinical signs are milder if the first infection occurs at a very young age; after recovery, low levels of parasitemia persist for extended periods without causing apparent harm to the animals (Zintl et al., 2005). This phenomenon was not observed in Ultrablack calves, despite their younger age during the initial evaluations.

Studies have revealed divergences in bovine resistance to hemoparasite infections among Taurine, Zebu, and their crossbreeds using the qPCR method. Bilhassi et al. (2014), evaluating calves and cows from the Angus, Nellore, and Angus x Nellore genetic groups, found that only the Angus breed exhibited higher *B. bovis* infection levels in both calves and cows. In this study, the crossbred (Angus x Nellore) animals did not differ from the Nellore breed in either calves or cows. Maiorano et al. (2018) assessed resistance to *B. bovis* infection in beef heifers from *Bos taurus taurus* (Caracu, n = 20) and *Bos taurus indicus* (Nelore, n = 20) and found no differences in resistance between the two groups. Martins et al. (2020) examined *Anaplasma marginale* infection levels between Brangus and Nelore calves aged 8–10 months and found that infections in Brangus cattle had six times more DNA copies than in Nellore cattle.

Significant differences were observed in some evaluations regarding the specific antibody titers for both *Babesia* spp. For *B. bigemina*, at the 4th and 5th evaluations, the parasite DNA loads and antibody levels were significantly higher in the Ultrablack breed. Although antibody titers against *B. bovis* and *B. bigemina* in calves were lower in the initial evaluations, the positive frequencies in the first evaluation were already high (> 80%). Moreover, during the evaluations, no clinical cases of *Babesia* spp. infections were observed. According to Pérez et al. (1996), the absence of clinical cases can be explained by a high number of seropositive animals; under these conditions, it is expected that most animals will become seropositive over time, and clinical cases will be rare. In the current study, as the animals were frequently exposed to *R. microplus*, high inoculation for babesiosis was anticipated based on the high antibody titers in the initial evaluations.

The correlation analysis between babesiosis infection levels determined by qPCR and antibody levels quantified by ELISA revealed no association between these two measures. The low correlations (or absence thereof) across the 13 evaluations demonstrated that the variation in CN_{log} value was independent of the level of antibodies; in other words, higher levels of antibodies do not necessarily indicate a better ability to maintain low levels of infection (Giglioti et al., 2017). As the repeatability coefficients estimated for the S/P values for both *Babesia* spp. were high, it is suggested that even an animal positive at one evaluation and negative at another by qPCR can present high IgG anti-*Babesia* spp. levels at both evaluations, as antibody levels can remain high between or during the evaluations. Although Ultrablack calves may have exhibited an earlier immunological response compared to Angus calves, we cannot infer that they demonstrated greater resistance to babesiosis infections. Despite having higher anti-babesia titers, Ultrablack calves displayed a phenotype more susceptible to babesiosis, as verified by DNA copy levels. Thus, based on our study, the ELISA assay could not discriminate between resistance/susceptibility to *B. bovis* and *B. bigemina*, as previously predicted by Giglioti et al. (2017).

The low estimated correlation between the babesiosis infection levels measured by qPCR and tick infestation, determined by the tick score, demonstrates that, as in other previous studies, in regions endemic to tick occurrence, the level of babesiosis infection is independent of the tick infestation level (Giglioti et al., 2016, 2018; Maiorano et al., 2018). The estimated repeatability for *B. bovis* CN_{log} was low (0.21), whereas for *B. bigemina*, it was moderate (0.31). The lower repeatability estimated for *B. bovis* CN_{log} may explain this species' greater fluctuation during evaluations compared to *B. bigemina*. According to Giglioti et al. (2018), lower repeatabilities indicate that variations in parasitic loads depend more on factors specific to each evaluation rather than on the animal's intrinsic factors. Consequently, the permanent environmental effect may have exerted a greater influence on the fluctuation of *B. bovis* levels than on *B. bigemina* levels. Our findings contrast with those by Giglioti et al. (2018), who reported repeatability estimates for *B. bovis* and *B. bigemina* infections in Canchim calves from the weaning phase between 0.17–0.35 and 0.04–0.32, respectively. These authors suggested that it is possible to identify animals with the most resistant phenotype to both hemoparasites. However, the age of the animal may be a crucial factor in resistance against these parasites.

High values were observed for the estimated repeatabilities of the titers of IgG against anti-*B. bovis* and anti-*B. bigemina* (0.74 and 0.85, respectively). However, these high values merely indicate that the higher levels of antibodies against *B. bovis* and *B. bigemina* in one

evaluation remained high in subsequent evaluations. Therefore, the absence of a correlation between the CN_{log} and S/P values indicates that high anti-*B. bovis* titers and anti-*B. bigemina* do not determine the decrease or increase in infection by these hemoparasites.

Ultrablack (or Ultrared) is a synthetic breed created by crossing existing breeds, such as Brangus and Angus. This results in a 50% reduction in the Zebu genetic composition derived from the Brangus breed. Compared to terminal crosses, the disadvantages of synthetic breeds include a lower degree of heterozygosity and the loss of epistatic superiority in the gametes produced by the crossed parents (Alencar, 1997). Therefore, due to Mendelian segregation of gametes and crossing-over processes, the estimated proportion of Zebu genetics derived from mating between Ultrablack animals may differ from the actual proportion (~18%). Further studies should try and establish a threshold value for sufficient Zebu genotype to ensure a reliable degree of babesiosis resistance. Such studies would need to increase the animal sampling size to assess the possible genetic and environmental effects on the herd and apply genomic and functional genetic analyses to identify genes related to resistance to babesiosis.

5. Conclusions

Levels of *B. bigemina* infection were significantly higher in Ultrablack calves compared to Angus calves, especially in the initial evaluations. Although *B. bovis* infection was also significantly higher in Ultrablack animals, the differences between the two genetic groups across various measurements were not pronounced. The IgG antibody titers against *B. bovis* and *B. bigemina* were not correlated with the infection levels determined by the number of DNA copies of each *Babesia* spp. Our results suggest that under the conditions of this study, the low proportion of Zebu genetics in Ultrablack calves was insufficient to enhance resistance against infection and reduce the parasite load.

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CRediT authorship contribution statement

Henrique Nunes de Oliveira: Writing – review & editing, Supervision, Methodology, Investigation. **Bianca Tainá Azevedo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anibal Eugênio Vercesi Filho:** Writing – review & editing, Supervision, Methodology, Investigation. **Luciana Morita Katiki:** Writing – review & editing, Supervision, Methodology, Investigation. **Sandra Antunes:** Writing – review & editing, Supervision, Methodology, Investigation. **Ana Gonçalves Domingos:** Writing – review & editing, Supervision, Methodology, Investigation. **Cintia Hiromi Okino:** Writing – review & editing, Supervision, Methodology, Investigation. **Adriana Mércia Guaratini Ibelli:** Writing – review & editing, Supervision, Methodology, Investigation. **Márcia Cristina de Sena Oliveira:** Writing – review & editing, Supervision, Methodology, Investigation. **Rodrigo Giglioti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Data availability

All experimental data may be available if requested.

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