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Original Article

Prevalence and risk factors associated with *Babesia bovis* infection in *Crioula Lageana* cattle

Mariana da Silva Casa[1], Julio de Matos Vettori[2], Ketriane Mota de Souza[3], Paulo Ricardo Benetti Todeschini[1], Luiz Claudio Miletti[3,4], Carla Ivane Ganz Vogel[3,4], André Luiz Ferreira Lima[5], Joandes Henrique Fonteque[1,2]

[1] Postgraduate Program in Animal Science, Santa Catarina State University, Lages, SC, Brazil
[2] Department of Veterinary Medicine, Santa Catarina State University, Lages, SC, Brazil
[3] Postgraduate Program in Biochemistry and Molecular Biology, Santa Catarina State University, Lages, SC, Brazil
[4] Department of Animal Production and Food, Santa Catarina State University, Lages, SC, Brazil
[5] Department of Animal Husbandry and Rural Development, Santa Catarina Federal University, Florianópolis, SC, Brazil

Abstract

Introduction: Bovine babesiosis caused by the protozoan *Babesia bovis* is a worldwide disease and causes great economic damage to livestock. There are no studies on the epidemiology of this disease in native breeds such as Crioula Lageana cattle raised in the South of Brazil.

Methodology: DNA samples from 311 animals were amplified by polymerase chain reaction (PCR) for the identification of the gene *rap-1* (*Rhoptry Associated Protein 1*) from *B. bovis*. An epidemiological questionnaire was used to determine the risk factors associated with infection.

Results: The prevalence of *B. bovis* infection was 72% (224/311). Age and tick infestation affected infection. The factors associated with infection were the breeding objective ($p = 0.042$; CI = 0.746-0.995; OR = 0.861), contact of cattle with other animal species ($p = 0.002$; CI = 0.517-0.860; OR = 0.484), absence of tick control ($p = < 0.001$; CI = 0.074-0.480; OR = 0.188) and timing of tick treatment ($p = 0.026$; CI = 0.673-0.975; OR = 0.810), and these were considered to be factors that can protect against the disease.

Conclusions: The Crioula Lageana cattle breed has near enzootic stability with regards to *B. bovis* infection.

Key words: babesiosis; southern Brazil; hemoparasites; molecular diagnosis.

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Introduction

Beef cattle ranching is an important industry in the world economy. In the fourth quarter of 2022 alone, more than 7.495 million cattle were slaughtered in Brazil, with 2,263,000 tons of beef destined for export to several countries [1]. Nevertheless, many diseases such as babesiosis, caused by *Babesia bovis* and *Babesia bigemina*, cause major losses in production.

Crioula Lageana is a native breed of cattle from the Santa Catarina plateau in Southern Brazil. It is derived from Bos Taurus and is appropriate for slaughtering. It displays great rusticity and tolerance to diseases, being even more resilient to *Rhipicephalus microplus* infestation than the Aberdeen Angus breed [2]. Furthermore, it produces high quality meat, with high intramuscular fat [3]. In addition to greater genetic diversity when compared to commercial breeds, native breeds are adapted to specific regional environmental conditions.

When clinically manifested, babesiosis leads to high morbidity and mortality, as well as loss of productive efficiency, leading to major economic losses [4]. In the case of babesiosis caused by *B. bovis*, blood stasis induced by the aggregation of infected erythrocytes in capillaries can lead to brain damage, culminating in a neurological condition with the presentation of signs such as incoordination, convulsions, limb paralysis and coma [5].

Bovine babesiosis agents are distributed throughout Latin America based on the presence of the tick vector *R. microplus* [6]. Brazilian climatic conditions are highly favorable for the development of many ectoparasites including *R. microplus* [7], which makes transmission of pathogenic hemoparasites easier.

In 2014 there was an outbreak in the Ponte Alta region, in Santa Catarina, southern Brazil where only 18.2% of the animals tested by polymerase chain reaction (PCR) were positive for *B. bovis* [8]. A recent

study determined the prevalence of *B. bovis* in the Santa Catarina plateau using PCR technology and concluded that 29.57% of the herd in this region was positive for the agent [9].

The diagnostic methods most used for the detection of *B. bovis* infection are microscopic detection, serology and molecular methods [10]. Currently, molecular techniques such as PCR have proved to be highly sensitive and specific [11].

This is the first molecular epidemiological study aiming to determine the prevalence and risk factors associated with *B. bovis* infection in Crioula Lageana cattle, an important breed in the Santa Catarina plateau due its productive qualities and rusticity. The aim of this study was to find the prevalence of *B. bovis* in the population of the Crioula Lageana breed.

Methodology

Sample size determination

In order to assess the prevalence of *Babesia bovis* in the Crioula Lageana cattle, the ensuing formula, proposed by the Pan American Health Organization (OPAS) [12], was used:

$$n_0 = \frac{1.96^2[p(1-p)]}{(d)^2}$$

in which n_0 corresponds to the number of samples, p to the expected prevalence, and d to the error margin. In order to estimate a disease prevalence of 50% with a 95% confidence interval and error margin of 5%, we needed 384 animals. However, considering a finite population, the following correction was applied:

$$n = \frac{N \times n_0}{N + n_0}$$

in which N is the total number of animals in the population. In the case of the Crioula Lageana breed this number equals 1500 organisms, according to data from the Brazilian Association of Crioula Lageana Ranchers (Associação Brasileira de Criadores da Raça Crioula Lageana, ABCCL). Using this equation, a final number of 306 animals to be sampled was reached.

Animals and blood collection

Blood samples were collected from 311 Crioula Lageana bovines, including both males and females, young and adult, all registered in the Brazilian Association of Crioula Lageana Ranchers (Associação Brasileira de Criadores da Raça Crioula Lageana, ABCCL). Samples were obtained from all in situ conservation properties of the breed and the animals were randomly selected. Clinical examination was carried out to identify clinical signs compatible with the disease manifestation. The heart rate (HR), respiratory rate (RR), rectal temperature, and ruminal motility (RM) were measured, and mucous membrane coloring were observed. The animals examined were in 6 in situ conservational unit estates, located on the plateau of the State of Santa Catarina, South of Brazil. The animals were selected randomly. All the samples were collected during autumn. This region, according to Koppen's climate classification, has "Cfb" temperate climate with mild summer, well distributed rainfall and no dry season. The animals were grouped as follows: 32 bulls (aged 2 years and over), 141 cows (aged 2 years and over), 66 heifers (aged between 1 and 2 years) and 72 calves (males and females up to 1 year old). The absence of males aged between 1 and 2 is due to exclusion from breeding and being sold afterwards. In the case of the gender related analysis, the bovines were separated disregarding age difference: 62 males and 249 females. Vacuum collection tubes with 10% EDTA anticoagulant were used to obtain the jugular vein blood samples, which were subsequently frozen at -20 °C for future DNA extraction.

DNA extraction

After thawing, the blood samples were immediately used for DNA extraction with the commercial kit ReliaPrepTM Blood gDNA Miniprep System (Promega, Wisconsin, USA), following manufacturer's instructions. After extraction, DNA concentration in each sample was measured by spectrophotometry using a Nano Drop 2000 (Thermo Fischer Scientific, Massachusetts, USA). Subsequently, each sample was diluted to maintain a minimal concentration of 20 ng/μL.

Molecular analysis

PCR and nested-PCR (n-PCR) techniques were used to amplify *rap-1* (*Rhoptry-Associated Protein 1*) gene from *B. bovis*. The primer sets used in the reactions are those described by Figueroa *et al*. [13]. All DNA samples were subjected to both PCR and n-PCR.

The PCR reaction was performed in 0.2 mL microtubes, where a final volume of 25 μL of solution containing 1U *Taq* Polymerase GoTaq® Hot Start Polymerase (Promega, Wisconsin, USA), 8.5 pmoles of each primer (BoF: 5'-CAC GAG GAA GGA ACT GAT GTT GA-3' and BoR: 5'-CCA AGG GTC TAC AAC GTA CGA GGT CA-3'), 0.2 mM of nucleotides (dNTPs); 3.5 mM magnesium chloride; 5 μL of 5X Green GoTaq® Flexi Buffer (Promega, Wisconsin, USA), 3 μL DNA (concentration between 20 and 100 ng/μL) and distilled water to complete final volume and

adjust reagent concentration. Positive and negative controls were used for each reaction. The negative controls were used to guarantee the quality and specificity of the technique, addressing the same parameters mentioned above, replacing only the use of genomic DNA with DNAse free distilled water. The same reaction mix was used for n-PCR, only replacing the animal DNA with 2 µL of the first PCR product and the specific primers (BoFN 5'-TCA ACA AGG TAC TCT ATA TGG CTA CC-3' and BoRN 5'-CTA CCG AGC AGA ACC TTC TTC ACC AT-3').

The temperature conditions applied to the thermocycler (BioCycler, BioSan, Riga, Latvia) for both reactions involved initial denaturation at 95 °C for two minutes, followed by 30 cycles of 94 °C for 1 minute, 54.2 °C for 1 minute and 73 °C for 1 minute and a final extension at 73 ºC for 7 minutes. These conditions were established after submitting the positive control to a temperature gradient, in order to obtain the best annealing temperature for the primers used.

The electrophoresis of the amplified products was performed in a horizontal vat using 2% agarose gel with the addition of 20,000x Unisafe Dye (Uniscience, São Paulo, Brazil). In the first gel well, a 100 bp molecular weight marker was used as a standard to determine the size of the sample bands. The electric source was 140 Volts and 400 mA and the procedure was conducted for 01 h 00 min. Visualization was done by exposure to ultraviolet light. Bands with size close to 350 bp were considered positive for *B. bovis* in the first reaction and bands with size approximate to 290 bp were considered positive in the second reaction.

To confirm that the fragments obtained in the electrophoresis originated from the *B. bovis rap-1* gene, positive samples were randomly chosen and sequenced by Ludwig Biotec (Alvorada, Brazil) using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Waltham, USA). The sequenced fragments were analyzed using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All the fragments had the same sequence and showed 99% identity with the sequence LC157854.1, corresponding to the *rap-1* gene in GeneBank (https://www.ncbi.nlm.nih.gov/genbank/).

Associated factors

In order to determine the factors associated with the development of babesiosis infection by *B. bovis*, an epidemiological questionnaire containing questions related to the property profile was applied to the owners of each estate analyzed.

Statistical analysis

A univariate analysis was performed to compare *B. bovis* infection rates, taking into account animal gender, age category and presence of ticks at the time of sampling. The Chi-squared test ($p \leq 0.05$) and odds ratio

Table 1. Physical examination values (mean ± standard deviation) of the Crioula Lageana animals, categorized by: bulls, cows, heifers and calves; positive and negative for *B. bovis* through polymerase chain reaction (PCR).

Variables	**Bulls (n = 32)**		
	Positive (n = 27)	**Negative (n = 5)**	***p* value**
HR (bpm)	80.15 ± 21.22	82.40 ± 23.26	0.831
RR (mpm)	27.26 ± 7.34	26.80 ± 6.26	0.897
RM (cont./5min)	5.11 ± 1.95	6.60 ± 3.21	0.167
T (°C)	38.67 ± 0.56	38.60 ± 0.55	0.806
	Cows (n = 141)		
	Positive (n = 104)	**Negative (n = 37)**	***p* value**
HR (bpm)	80.49 ± 23.18	69.65 ± 16.87	0.010*
RR (mpm)	26.73 ± 8.76	25.14 ± 6.71	0.315
RM (cont./5min)	3.57 ± 1.94	3.70 ± 2.34	0.730
T (°C)	38.76 ± 0.58	38.62 ± 0.64	0.230
	Heifers (n = 66)		
	Positive (n = 38)	**Negative (n = 28)**	***p* value**
HR (bpm)	94.63 ± 22.57	86.86 ± 19.27	0.147
RR (mpm)	31.74 ± 8.69	34.36 ± 7.60	0.207
RM (cont./5min)	4.00 ± 2.36	3.21 ± 2.43	0.191
T (°C)	39.11 ± 0.51	39.18 ± 0.39	0.527
	Calves (n = 72)		
	Positive (n = 55)	**Negative (n = 17)**	***p* value**
HR (bpm)	103.11 ± 23.08	103.53 ± 12.80	0.943
RR (mpm)	33.42 ± 8.90	30.82 ± 5.80	0.263
RM (cont./5min)	2.25 ± 1.64	2.65 ± 1.77	0.399
T (°C)	39.40 ± 0.63	39.18 ± 0.40	0.170

*Significant difference between groups according to Student's t test ($p \leq 0.05$). HR; heart rate; RR; respiratory rate; RM; ruminal movements; T; temperature.

analysis were used. The physical examination variables were analyzed through Student's t test ($p \leq 0.05$), in order to compare the mean values obtained from groups of positive and negative animals.

The statistical model applied to the questionnaire data was developed by a univariate analysis employing Chi-squared test ($p < 0.05$). In the case of questions with expressive results in the first analysis, multivariate analysis was carried out making use of logistic regression analysis ($p < 0.05$). The questions that presented multicollinearity on the second analysis were excluded from the evaluation in order to verify the association between the presence or absence of the agent and the associated factors.

Ethics committee approval

The study was approved by the Animal Experimentation Ethics Committee (Comitê de Ética em Experimentação Animal - CETEA) of Santa Catarina State University (Univeridade do Estado de Santa Catarina - UDESC) under protocol number 2461171115. It was also approved by the Ethics Committee in Research with Human Beings (Comitê de Ética em Pesquisa com Seres Humanos - CEPSH) of UDESC, under protocol number CAAE 2,068,771.

Results

The prevalence for *B. bovis* infection was 72% (224/311). All the animals positive for the condition were considered as carriers, as no clinically relevant change in the parameters evaluated upon examination was verified. The cows positive for *B. bovis* had heart rates significantly higher, yet remained within the reference values for the species (Table 1).

The assessment of gender and age category in relation to the presence or absence of infection concluded that 249 (249/311; 80%) of the total sampled animals were female and of these 174 (174/249; 70%) were positive for *B. bovis*. Of the 62 (62/311; 20%) males evaluated, 50 (50/62; 81%) tested positive. There was no significant difference ($p = 0.126$) in the proportion of parasitized animals among males and females.

Of the 32 (32/311; 10%) bulls evaluated, 27 (27/32; 84%) tested positive. From the 66 (66/311; 21%) heifers, 38 (38/66; 58%) were positive. Among the 141 (141/311; 45%) cows, 104 (104/141; 74%) tested positive for *B. bovis*, as did 55 (55/72; 76%) calves out of a total of 72 (72/311; 23%) (Figure 1).

There was a difference ($p = 0.016$) between bulls and heifers, and for this population, bulls were 3.979 (CI = 1.36-11.62) times more likely to have babesiosis infection than heifers. Similarly, between heifers and cows there was a difference ($p = 0.029$), with 2.071 (CI = 1.11-3.83) times more chance of cows having an infection compared to heifers. Among heifers and calves ($p = 0.030$), the chance of calves acquiring the infection was 2.384 (CI = 1.14-4.95) times higher than for heifers (Figure 1).

The relation between presence and absence of ticks in the animals at the time of sampling and the positivity or not for *B. bovis* infection was evaluated. This analysis showed a significant relationship ($p = 0.026$) between the variables in the Chi squared test, with 1.85 (CI = 1.11-3.23) times more chance of animals with the presence of *R. microplus* to acquire hemiparasites.

Analyses of the responses to the questionnaires applied to the cattle owners indicated a difference in the univariate analysis of the associated factors. These factors included breeding objective, contact of the cattle with other animal species, contact with cattle of other properties, period of greatest tick infestation, presence of hematophagous insects, tick control, type of acaricides used, and timing for tick treatments (Table 2).

The factors associated with infection were the breeding objective ($p = 0.042$; CI = 0.746-0.995; OR = 0.861), cattle's contact with other animal species ($p = 0.002$; CI = 0.517-0.860; OR = 0.484), absence of tick control ($p \leq 0.001$; CI = 0.074-0.480; OR = 0.188) and correct timing for tick treatment ($p = 0.026$; CI = 0.673-0.975; OR = 0.810), which were considered factors that protect against the disease (Table 3).

When checking the *p* values for each predictor, it was verified that the use of acaricides is not a major predictor for the model, while the other variables were considered significant.

Figure 1. Number and proportions of Crioula Lageana animals positive and negative for B. bovis polymerase chain reaction (PCR), per sampling category.

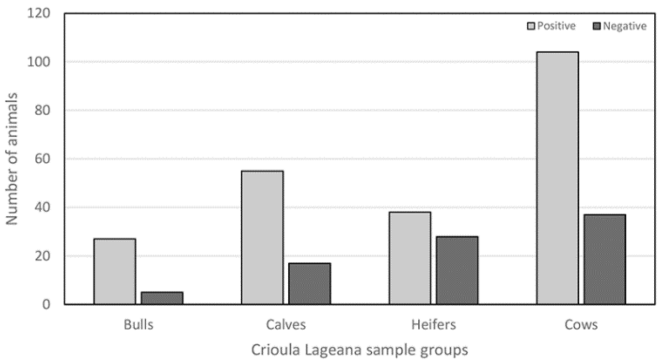

Table 2. Univariate analysis (Chi-square) of factors associated with *B. bovis* infection in Crioula Lageana cattle.

Variables	*B. bovis* infection				*p*
	Positive		Negative		
	N	%	N	%	
Number of animals on the property					
51-100	39	12.5	8	2.6	0.101
> 100	185	59.5	79	25.4	
Breeding objectives					
Meat, reproduction and animal trade (ref.)	103	33.1	62	19.9	
Meat	11	3.5	6	2	
Reproduction and animal trade	55	17.7	6	2	< 0.001
Meat and animal trade	27	8.7	11	3.5	
Animal trade	28	9	2	0.6	
Property size					
50-100 ha	11	3.5	6	2	0.679
> 100 ha	213	68.5	81	26	
Contact of cattle with other animal species					
Equine, dog and sheep (ref.)	52	16.7	20	6.4	
Swine	28	9	2	0.6	
Equine and dog	27	8.7	11	3.5	< 0.001
Equine, swine, dog and cat	55	17.7	6	2	
Equine, dog, cat, bird and sheep	51	16.4	42	13.5	
Equine, dog, cat and wild animals	11	3.5	6	2	
Contact with cattle from other properties					
Yes	134	43.1	37	11.9	0.009
No	90	28.9	50	16.1	
Replacement of animals					
Own herd	80	25.7	22	7.1	0.104
Own herd and other properties	144	46.3	65	20.9	
Veterinary care					
Yes	107	34.4	33	10.6	0.150
No	117	37.6	54	17.4	
Previous cases of anaplasmosis					
Yes	79	25.4	31	10	0.943
No	145	46.6	56	18	
Period of greatest infestation by ticks					
Autumn	66	21.2	12	3.9	
Summer	79	25.4	44	14.1	0.020
Fall and spring	27	8.7	11	3.5	
Summer and autumn	52	16.7	20	6.4	
Presence of hematophagous insects					
Yes	169	54.3	81	26	< 0.001
No	55	17.7	6	2	
Tick control					
Yes (ref.)	158	50.8	75	24.1	0.007
No	66	21.2	12	3.9	
Use of acaricides					
Pyrethroids (ref.)	90	28.9	37	11.9	
Organophosphates and avermectins	79	25.4	44	14.1	< 0.001
Avermectins	55	17.7	6	2	
Time of treatment for ticks					
Autumn (ref.)	66	21.2	12	3.9	
Spring and autumn	27	8.7	11	3.5	
Spring, summer and autumn	51	16.4	42	13.5	< 0.001
Summer	28	9	2	0.6	
Summer and autumn	52	16.7	20	6.4	
Most tick-parasitized categories					
Pregnant, lactating cow and calf	27	8.7	11	3.5	
Lactating cow	11	3.5	6	2	0.771
Calf	186	59.8	70	22.5	

The category used for reference for each variable is indicated in Table 1 as "ref."

Discussion

This study identified a prevalence of 72% (224/311) *B. bovis* infection in the population of the Crioula Lageana breed, indicating high prevalence as expected. Considering the definition of endemic stability/instability recommended by the Food and Agriculture Organization (FAO), the population evaluated is very close to endemic stability (from 61 to 80% of animals up to 9 positive months) [14].

All the animals were considered clinically healthy at the time samples were collected and clinical examination was carried out. Even though cows positive for *B. bovis* presented themselves with a statistically higher mean heart rate ($p = 0.010$), these values still fell between 60 to 80 beats per minute, as described for the species [15].

Elias *et al.* [16] found a high prevalence of *B. bovis* infection in sampled animals in the state of Paraná in southern Brazil, up to 95.5% positivity with enzyme linked immuno sorbent assay (ELISA) serological test. Osaki *et al.* [17] noted 64.2% seropositivity for *B. bovis* among the analyzed animals by utilizing the same test in the city of Umuarama, Northwest of the state of Paraná. In the Northern Plateau of Santa Catarina, the dairy herd showed 76.8% positivity with the indirect immunofluorescence test [18]. This result is close result to that found for the Crioula Lageana breed. However, immunological methods, such as ELISA and indirect immunofluorescence are used for looking for antibodies against these agents [10], not allowing for the comparison of the prevalence obtained by these methods with that obtained by PCR, as they are different methods.

Recently, an outbreak of babesiosis and anaplasmosis occurred in Ponte Alta, Santa Catarina, where 18.2% of the animals treated were confirmed positive for the presence of *B. bovis*, using the multiplex-PCR technique [8]. Another study evaluated the prevalence of tick-borne disease agents by employing the same multiplex-PCR technique and observed that 29.57% of Planalto Catarinense bovine herd, composed of several different breeds and their crosses, was positive for *B. bovis*. These indexes represent enzootic instability for babesiosis in the region, making the control of infectious vectors necessary [9]. Although the animals are not within the minimum value for the enzootic stability condition, no outbreaks of babesiosis have been reported on the properties raising Crioula Lageana breed. Hence, it raises the hypotheses regarding this breed's particularities, which may bring about its capacity for hemoparasite infection tolerance, as well as superior resistance to ticks when compared to the Aberdeen Angus breed [2].

The *B. bovis* infection in Crioula Lageana breed was not related to the gender factor. On the other hand, there was a difference ($p < 0.017$) in the age categories. Bulls and cows had more chance to acquire the parasite when compared to heifers, and this may be related to the age of the animals. This data may also be associated with the multiplicity of infections, so that older animals would be prone to more tick bites throughout their lives and thus would have contact with more agent genotypes, keeping the animals in constant challenge. This might explain why the infection would increase with age [19]. Trueman and Blight [20] observed that animals that were over 2 years old would be more affected by hemoparasites, reiterating the data found for the Crioula Lageana cattle. They also reported that young oxen, aged 1.5 years, reacted in an intermediary way between cows and calves. However, Crioula Lageana heifers presented lower chances of infection when compared to cows and calves. Calves present innate immunity, which is also a contributing factor for being less affected by clinical diseases when infected [21]. Nevertheless, in the present study, calves had statistically lower chance to acquire *B. bovis* infection when compared to other categories.

The presence of *Rhipicephalus* ticks parasitizing animals affects the prevalence of *B. bovis*. The sampling timing is also related to the higher presence of ticks parasitizing the animals. In Brazil, the season when the highest tick infestation rates occur is autumn [18]. Since the samples for this study were collected during this time of year, it can be inferred that this was the period of the greatest tick infestation in these animals and consequently may be the time when there was the highest rate of *B. bovis* infection. As described

Table 3. Multivariate analysis of factors associated with *B. bovis* infection in Crioula Lageana cattle.

Associated factor	*p*	OR	95% CI	Coefficient	SE
Breeding objectives	0.042	0.861	0.746 – 0.995	-0.149	0.074
Contact of cattle with other animal species	0.002	0.484	0.517 – 0.860	-0.405	0.130
Tick control	< 0.001	0.188	0.074 – 0.480	-1.670	0.477
Use of acaricides	0.154	1.384	0.885 – 2.163	0.325	0.228
Time of treatment for ticks	0.026	0.810	0.673 – 0.975	-0.210	0.094

Significance association at 5% level. OR; Odds ratio, CI; 95% confidence interval, SE; standard error (estimate). The category used for reference for each variable is indicated in Table 1 as "ref."

by Lorusso *et al.* [22], ticks parasitizing the animals are linked to *B. bovis* infection in the case of Crioula Lageana cattle. Yet, the number of ticks did not influence the level of parasitemia for *B. bovis*, since no study has established a correlation between the number of ticks and the number of copies of DNA of the protozoan in bovine blood [23].

In the analysis of the factors associated to *B. bovis* infection, the cattle farmers' answers to the specific questionnaire applied during the study revealed that the breeding objective (for beef production, reproduction or trade) was a factor that reduced the chances of infection by *B. bovis*. Animals intended only for beef production showed the lowest prevalence of infection. However, the age of these animals may influence the result. On average, Crioula Lageana cattle are slaughtered when they are 2 years old, and those kept for breeding end up living longer. While these animals are kept in breed conservation centers, several of them are also sold over the age of 2 years. Animals raised for meat production were slaughtered earlier and thus had less contact with ticks and different genotypes of *B. bovis*, which would explain the lower prevalence of infection in these animals [21].

Owing to an extensive raising system, most cattle encounter other species like equine, swine, canine, feline and sheep. *Rhipicephalus microplus* ticks can have horses and sheep as hosts which can harbor larvae and nymphs [24]. Coexistence between these species and consequent exchange of hosts by these ectoparasites may explain repeated tick infestations. This may result in the animals acquiring immunity to tick infestation and *B. bovis* infection.

Tick control and timing for tick treatments also influenced *B. bovis* infection. Proper tick control routines on farms can reduce the chances of *Babesia* spp. infection, as reported in Thailand [25]. The timing for treatment is also associated with reduced inoculation rates. Treatments carried out in spring, summer and autumn lead to lower rates for *B. bovis* positivity, as they coincide with the highest tick infestation seasons [18]. Nevertheless, more studies related to the dynamics of tick infestation in this population are needed.

Based on the results obtained, the owners of Crioula Lageana animals can be instructed to carry out better tick control, at the appropriate times, and to try to avoid contact between cattle and other animal species, in order to prevent possible outbreaks.

Conclusions

The prevalence for *Babesia bovis* infection tested using PCR technique in Crioula Lageana cattle was near the enzootic stability. With regards to the age of the cattle, the heifers were less prone to infection when compared to other groups, and this could be explained by their lower exposure to ticks due to the age. The factors associated with protection against infection include the raising system for beef production, since the animals are slaughtered up to the age of 2 years old; as well as contact with other species, like equine, swine, canine, feline and sheep, which can carry the vector *R. microplus*. The absence of tick control and the timing for tick treatment, which influences the number of ticks parasitizing animals, are also factors that have impact on the infection.

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Authors' contributions

MSC, JHF, ALFL, CIGV and LCM designed the study; MSC, JMV, PRBT and KMS performed the experiment; MSC, JHF, CIGV and LCM analyzed the data and wrote the manuscript.

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Corresponding author

Mariana da Silva Casa, PhD
Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias,
Av. Luiz de Camões, 2090, CEP 88520-000, Lages, SC, Brazil.
Tel: +55 49 999839025
Email: mari.casa@hotmail.com

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