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Neither quantification by qPCR nor quantitative Elisa can be used to discriminate Angus cattle for resistance/susceptibility to *Babesia bovis*

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ABSTRACT

With the aim of finding quantitative phenotypic traits that can be used to discriminate the levels of resistance/susceptibility to *Babesia bovis*, we estimated the repeatability and correlation between the level of infection, determined by the number of copies of a fragment of the gene that encodes cytochrome B (NC mt-cyB) of *B. bovis*, and the levels of the anti-*B. bovis* antibodies, in blood samples collected from 51 Angus cattle on two different occasions. Samples with the anticoagulant EDTA were used for DNA extraction and without anticoagulant for separation of the blood serum. The quantification of the NC mt-cyB of *B. bovis* was carried out by the quantitative PCR technique (qPCR), while the anti-*B. bovis* IgG antibody titers (S/P) were quantified by the ELISA method. The NC and S/P data were log10-transformed to improve the approximation to the normal distribution and were analyzed using mixed models. The correlations between NC mt-cyB and S/P were estimated, as well as the repeatability values for each trait. The results obtained showed the high sensitivity of the techniques, with 100% of the animals being positive for *B. bovis,* detected by both the serological and molecular tests. The correlations estimated between NC and S/P were low, 0.10 and 0.12, in the first and second collection, respectively. The repeatability estimated for NC was 0.06, whereas for the S/P it was 0.42. The low correlations between S/P and NC in the two collections demonstrated that the variation in the NC value is independent of the level of antibodies. This results indicated that animals with a higher levels of antibodies against *B. bovis* in the first collection continued to have a higher levels in the second one. However, the very low values for the repeatability value of NC, and for the correlations between S/P and NC, demonstrates that neither NC or S/P could be used to discriminate animals for resistance/susceptibility to *B. bovis*.

1. Introduction

Bovine babesiosis is a disease caused by protozoa of the genus *Babesia,* hemoparasite transmitted exclusively by ticks, causing losses to stock breeders throughout the world (McCOSKER, 1981). In Latin America, this disease affects both native cattle and animals imported from disease-free areas, causing significant losses to the producers (Guglielmone, 1995).

Babesia bovis is highly pathogenic and the infective forms, called sporozoites, are produced around two to three days after attachment of the tick larvae on the host (Riek, 1966). These evolutive forms invade the animals' erythrocytes and multiply by merogony, provoking hemolysis and penetrating new cells until the host dies or develops immunity (Hunfeld et al., 2008). Erythrocytes infected by *B. bovis* accumulate in the capillaries of various organs, among them the brain, causing cerebral babesiosis (Gohil et al., 2013). Cattle born in areas that are endemic for babesiosis often show a certain degree of natural resistance to the disease (Bock et al., 2004).

Greater resistance to babesiosis of zebu breeds (*Bos taurus indicus*) in comparison with taurine breeds (*Bos taurus taurus*) has been observed, mainly in the absence of clinical symptoms or the occur-

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rence of less severe symptoms (Bock et al., 1997, 1999; Jonsson et al., 2008; Piper et al., 2010). This pattern has prompted the great majority of Brazilian producers to choose zebu animals, as also occurs in Australia, in areas with high rates of tick infestation (Jonsson, 2006). Nevertheless, higher resistance to babesiosis appears not to be an exclusive trait of zebu animals, since Benavides and Sacco, (2007), studying challenge of naïve animals with *B. bovis*, found the occurrence of three distinct phenotypes in the taurine breeds Hereford and Aberdeen Angus: resistant, intermediate and susceptible animals. This variation between and within breeds suggests that resistance to babesiosis is a genetically determined trait and also that it can respond to selection. However, the main issue is to identify a quantitative phenotypic trait that enables to distinguish animals that are more resistant or sensitive to babesiosis.

Serological tests, such as the enzyme-linked immunosorbent assay (ELISA), can quantitatively determine the levels of antibodies against babesiosis (Böse et al., 1990; Machado et al., 1997) and can be used to indicate resistance to the disease in genetic selection studies. On the other hand, techniques based on polymerase chain reaction (PCR) allow amplification of the DNA of the protozoa in cattle blood samples with high sensitivity and specificity (Oliveira et al., 2005; Oliveira-Sequeira et al., 2005; Buling et al., 2007; Guerrero et al., 2007). Bilhassi et al. (2014) used the qPCR technique to study the level of infection by *B. bovis* in cattle of different genetic groups and found that blood samples from taurine animals had a significantly higher number of copies of the parasite's DNA than samples from zebu and crossbred animals. Recently, several research groups have conducted experiments involving the association of immunological and molecular tests to detect *Babesia* (Ferreri et al., 2008; Hüe et al., 2013; Ibrahim et al., 2013; Jaffer et al., 2010; Machado et al., 2012; Mousa et al., 2013; Ramos et al., 2011; Rosales et al., 2013; Shebish et al., 2012). These associations have shown excellent efficiency in detecting infected animals, going a long way toward resolving the problems of predicting the occurrence of babesiosis outbreaks.

No studies have been published reporting the repeatability and correlation between the *Babesia* infection levels quantified by qPCR and ELISA. The study by Giglioti et al. (2016) was the first to estimate the repeatability of *B. bovis* infection levels in Angus cattle quantified by qPCR. The authors observed low repeatability for both collections analyzed.

In light of the foregoing, we conducted the present experiment to evaluate the phenotypic traits that can discriminate the levels of resistance/susceptibility to *B. bovis*, to be used in genetic selection studies. Blood samples from Angus cattle (*Bos taurus taurus*), reared in a stable endemic area for babesiosis, were collected on two occasions and were used to determine the NC mt-cyB of *B. bovis,* using the qPCR technique, and the levels of anti-*B. bovis* antibodies, employing the ELISA method. The resulting data were analyzed to estimate the repeatability of each trait and the correlations between them, within and between collections.

2. Material and methods

2.1. Animals and sample collection

The animals used in this experiment were reared on a farm located in the municipality of José Bonifácio (21° 03′ 10″ S and 49° 41′ 18″ W), in the state of São Paulo, where the cattle tick *Rhipicephalus microplus* infests herds in all months of the year. The area is considered to have endemic stability for babesiosis (Bilhassi et al., 2014). The climate in the region is classified as AW, with average temperatures in May of 20 °C (minimum of 13 °C and maximum of 27 °C), with rainfall of 40 mm, and 21 °C in July (minimum of 11.7 °C and maximum of 26.0 °C), with rainfall of 23 mm. We used 51 clinically healthy Angus males, with ages near two years at the time of collection. Blood was drawn from each animal on two occasions in 2012, in Brazilian autumn (May) and winter (July). The samples were obtained by puncture of the jugular vein using the Vacutainer® system in tubes containing the anticoagulant EDTA for extraction of DNA and without anticoagulant for separation of the serum. All the animals were infested by ticks, as confirmed by observation and palpation of the entire right side of the body. The animals were all considered healthy because they did not show signs of the disease or of hyperthermia.

This experiment was in accordance with ethical principles for animal experimentation of the Embrapa Pecuária Sudeste ethics committee for animal experiments (CEUA-EMBRAPA/CPPSE).

2.2. Extraction of DNA and preparation of serum

The DNA was extracted from the blood samples using the illustra blood genomic Prep Mini Spin kit (GE Healthcare, Little Chalfont, UK), according to the manufacturer's recommendations, using a blood volume of 300 μL. The blood samples without anticoagulant were centrifuged to separate the serum, which was placed in microtubes and frozen at −20 °C until the analysis.

2.3. ELISA for Babesia bovis

The ELISA protocols were conducted in the Immunoparasitology Laboratory of the Department of Veterinary Pathology of FCAV/UNESP Jaboticabal, according to the method described by Machado et al. (1997). The concentration of the *B. bovis* antigen was determined by the bicinchoninic acid assay (BCA), using the Pierce™ Micro BCA™ Protein Assay Kit (Thermo Scientific, Waltham, Massachusetts, US), with the samples diluted to a concentration of 5 μg/mL. As controls for the qPCR and ELISA testing, serum from experimentally infected cattle with high titers of antibodies against *B. bovis* was used for positive control, while as negative control the serum samples were collected prior to infection and from calves before they suckled colostrum (Machado et al., 1997). In each ELISA run, two negative and two positive control samples were used. In summary, 100 μL of antigen diluted in carbonate-bicarbonate buffer 0.05 M, pH 9.6, with antigen concentrations of 5 μg/mL, was placed in wells of polystyrene ELISA microplates (96 wells, Thermo Fisher Scientific, Rochester, NY, US), which were sealed and incubated at 4 °C overnight. The plates were blocked with a 6% rabbit serum solution in PBS-Tween buffer (phosphate-buffered saline, pH 7.2 and PBS-Tween with 0.05% Tween 20) for one hour in a humid chamber at 37 °C. The plates were then washed three times with PBS-Tween buffer, after which 100 μL of bovine serum was added, diluted at 1:400 in PBS-Tween. The positive and negative control sera were also diluted at 1:400 in the same buffer. The plates were incubated at 37 °C in a humid chamber for 90 min and then washed three times with the same PBS-Tween solution. An aliquots of 100 μL of solution diluted to 1:10,000 of anti-IgG bovine conjugated with alkaline phosphatase (Sigma-Aldrich, St. Louis, MO, US) in PBS-Tween buffer was added to each well and the plates were incubated at 37 °C in the same conditions described above, for 90 min. The plates were again washed three times with PBS-Tween solution and 100 μL of the substrate for phosphatase and liquid p-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO, US) was added in each well. The plates were sealed and incubated at room temperature for 30 min. At the end of this period, the optical densities of the samples were read by a spectrophotometer (Dynex Technologies, MRX TC plus), with a 405 nm filter. The enzyme activity from each serum sample was calculated by determining the sample to positive serum ratio (S/P), considering positive and negative sera as reference, using the following equation: S/P = (mean sample

absorbance−mean absorbance of negative serum reference)/(mean absorbance of positive reference serum−mean absorbance of negative serum reference). S/P values were grouped into ELISA levels (EL), which ranged from 0 (lowest level) to 9 (highest level) (Machado et al., 1997). The maximum amplitude of the EL = 0 was determined by the mean of the absorbance values of the negative samples, plus two standard deviations from the corresponding mean. From this limit, the intervals between the other EL were increased by 35% each, as described by Machado et al. (1997), for the *Babesia* spp. system. The cutoff point was obtained by multiplying the maximum amplitude value of the EL zero by the coefficient 2.5. The serum samples were considered positive when the S/P values were greater than or equal to the cutoff point.

2.4. qPCR reactions

The CFX™ Real-Time PCR Detection System (BioRad, Hercules, CA, USA) was used in the qPCR assays to obtain the NC mt-cyB values, according to Buling et al. (2007). The primers used in the reactions were cbosg 1 (forward) 5′- TGTTCCTGGAAGCGTTGATTC-3′ and cbosg 2 (reverse) 5′-AGCGTGAAAATAACGCATTGC-3′, which flank a fragment of the mt-cyB gene of *B. bovis* and produce an amplicon with 88 base pairs (Salem et al., 1999; Buling et al., 2007). DNA samples extracted from *B. bovis* isolates (donated by the Animal Pathology Department, UNESP Jaboticabal, São Paulo, Brazil) were used to plot the calibration curve and as a positive control.

To construct the calibration curve, a DNA sample from the isolate of *B. bovis* was amplified using the specific primers for *B. bovis* (cbosg1 forward and cbosg2 reverse). The PCR products were purified using the PureLink™ PCR purification kit (Invitrogen), cloned into pGEMT Easy Vector Systems (Promega) according to the manufacturer's protocols. The recombinant clones were transformed in *E. coli* DH5α cells and the white colonies were selected and amplified to confirm the vector insert (Silva et al., 2014). The DNA was extracted using the PureLink® Quick Plasmid Miniprep Kit (Invitrogen), sequenced with an Applied Biosystems ABI Prism 3130 Avant® genetic analyzer and submitted to BLAST analysis to confirm the sequences obtained (Altschul et al., 1990).

To estimate the NC mt-cyB values, calibration curves were standardized based on the products cloned from the *B. bovis* isolate, which were quantified in a NanoDrop ND-1000 spectrophotometer (Thermo Scientific NanoDrop Products, Wilmington, Delaware, US) and based on the known value of each product they were serially diluted tenfold. The dilutions of the products of the amplicons from part of the cloned isolate were submitted to the qPCR tests together with the samples and controls to estimate the NC of the DNA from *B. bovis*. The reaction efficiencies (E) were calculated according to the formula of Pfaffl (2001) and Vandesompele et al. (2002): $E = 10^{(-1/Slope)}$, where Slope is the derivative (tangent line) of the calibration curve. The number of copies (NC) was computed by the formula described by Ke et al. (2006): NC (μl) = (6.022×10^{23} (copies/mol) × concentration (g/mol))/molecular mass (g/μl). The NC values for the dilutions from the calibration curves were utilized to calculate the NC values of the *B. bovis* DNA in each sample using the software that comes with the CFX96 system (BioRad).

The PCR mixture contained 0.3 μL of 10 μM of each primer (cbosg forward and reverse), 5 μL of SsoFast™ EvaGreen® Supermix (BioRad), 4.4 μL of ultrapure water (Invitrogen, Carlsbad, CA), and 2.0 μL of template DNA in a total volume of 12 μL. The qPCR protocol started at 95 °C for 2 min, followed by 45 cycles of 95 °C for 5 s and 57 °C for 30 s (annealing/extension). Following the last cycle, the melting curve was generated by heating from 65 °C to 95 °C at a rate of 0.5 °C/s. These conditions were according to the recommendations of the manufacturer of the reagent SsoFast™ EvaGreen® Supermix (BioRad), with some modifications. To prevent contamination, barrier pipettes were used. All the samples were analyzed in duplicate, as were the positive and negative controls (with an addition of water instead of DNA in the negative control).

2.5. Statistical analysis

The data from the S/P and NC were transformed into log10 (n + 1) for the purpose of approximating the normal distribution. After transformation, the data were analyzed using the mixed models method. A mixed model with repeated measures from the same animal, including the effect of the collection, quantification method (S/P and NC) and their interaction as fixed effects was used to analyze S/P and NC values. The structure of the (co)variance matrix (symmetric) was specified according to the following model:

$$\begin{bmatrix} \sigma^2_N & \sigma_{NN_{12}} & \sigma_{NA_{11}} & \sigma_{NA_{12}} \\ \sigma_{NN_{12}} & \sigma^2_N & \sigma_{NA_{12}} & \sigma_{NA_{11}} \\ \sigma_{NA_{11}} & \sigma_{NA_{12}} & \sigma^2_A & \sigma_{AA_{12}} \\ \sigma_{NA_{12}} & \sigma_{NA_{11}} & \sigma_{AA_{12}} & \sigma^2_A \end{bmatrix}$$

where σ^2_K = variance component of NC (K = N) or S/P (K = A) common to both collections; and $\sigma_{KL_{ij}}$ = covariance component between K and L (*K,L = N,A)* in collection *i* and *j* (*i*, = 1*j* = 1,2) (Littell et al., 2006).

For each trait, the repeatability was estimated from the (co)variance matrix, as the covariance of the two collections of the same trait divided by the variance of the trait. The repeatability for each trait indicates how much of variation in a trait can be attributed to the inner effect of the animal, and can be interpreted as the correlation between the measures for the same individual in the two collections. Likewise, correlations were estimated between the values from two variables taken from the same animal, in the same or different collections. The MIXED routine from the SAS statistical package was employed for the analyses and the option LIN(q) from the command line REPEATED was used to specify the (co)variance matrix (SAS, 2002; Littell et al., 2006).

3. Results

3.1. ELISA test and qPCR assay

The ELISA results obtained demonstrated high sensitivity in detecting IgG antibodies against *B. bovis* in the serum samples. The mean absorbance values of the positive and negative sera were 0.857 ± 0.152 and 0.090 ± 0.004, respectively. The ELISA scale was defined according to Table 1. Starting at EL 4, the animals were considered positive. The mean absorbance observed for the positive controls was within EL 9. All the serum samples from the two collections were positive for *B. bovis* (EL ≥ 4). The means ± standard errors for S/P values obtained in the ELISA were significantly differ-

Table 1
ELISA level (EL) scale according to the antibody titers (S/P) value ranges. (The serum samples were considered positive when the S/P values were greater than or equal to EL 4).

EL	S/P Ranges
1	0.000–0.098
2	0.099–0.133
3	0.134–0.181
4	0.182–0.245
5	0.246–0.332
6	0.333–0.449
7	0.450–0.607
8	0.608–0.820
9	<0.821

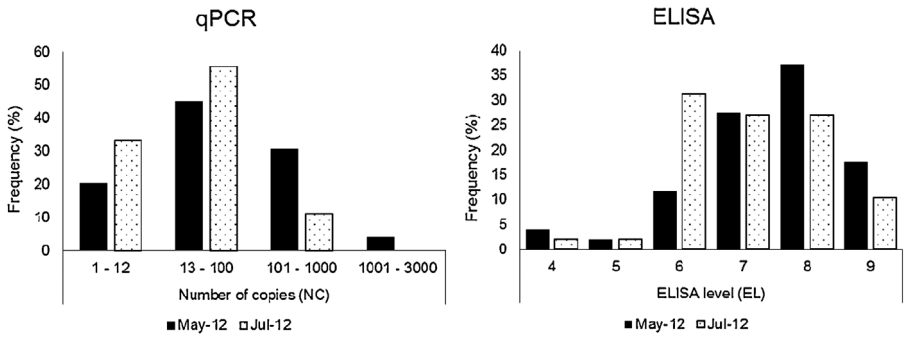

Fig. 1. Histogram of frequencies of the number of copies in the qPCR (NC) and ELISA level number (EL) for blood samples collected from Angus cattle on two occasions: May and July 2012.

ent between the two collections ($p < 0.05$), with 0.24 ± 0.01 in the first and 0.22 ± 0.01 in the second.

The qPCR tests allowed detecting the fragment of the mt-cyB gene of *B. bovis* in the blood samples from all the cattle. The negative controls did not show amplification and the mean ± standard deviation of the quantitative cycle (*Cq*) of the controls was 20.4 ± 0.15, thus indicating little variation among the tests. The means of the reaction efficiency (E) were within the 95–105%, range accepted for an efficient PCR reaction.

The means and standard errors of the transformed NC values for the mt-cyB gene of *B. bovis* for the first and second collection were 1.69 ± 0.08 and 1.33 ± 0.08, respectively. Fig. 1 presents the EL and NC frequencies for the two collections (seasons of the year). In Fig. 1 it's possible to note that the qPCR of the samples from the second collection, the frequencies of the NC values greater than 100 (non-transformed data) declined. Likewise, with respect to the EL, in the first collection (May 2012) levels 7 and 8 predominated, while in the second (July 2012), EL 6 increased, at the expense of levels 7 and 8 in relation to the first collection. EL 9 also declined in relation to the first collection.

For both collections (autumn and winter), the correlations estimated between NC and S/P were low, 0.10 and 0.12 respectively. Fig. 2 presents the distributions of the EL and NC values considering all the samples evaluated in both collections. It can be seen that except for EL 4, which showed low NC values, the values were practically independent. Only one animal in the autumn collection and three in the winter presented EL 4. Starting at EL 5, the same individuals with low NC values presented high EL value. The highest NC value found for a sample fell in the EL 6 range. Besides this, the animals with S/P values corresponding to EL 9 presented variations of NC for the autumn and winter collections of 1.12–2.75 and 0.82–2.13, respectively.

The repeatability values for S/P and NC in the two collections were 0.42 and 0.06 respectively. The very low repeatability value

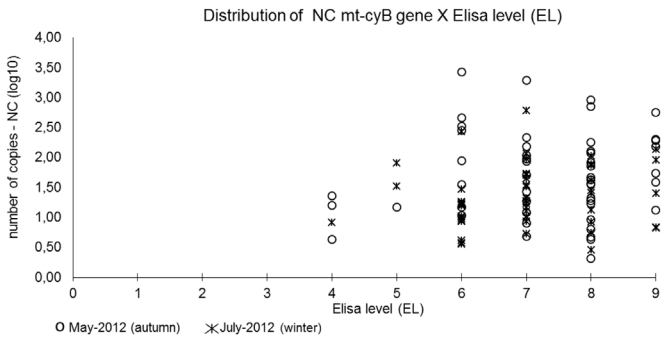

Fig. 2. Distribution of the log10-transformed number of copies (NC) determined by qPCR according to ELISA levels (EL) in blood samples from Angus cattle.

for NC and moderate value for S/P demonstrate that: (i) the animals with a higher number of antibodies against *B. bovis* in the first collection continued to have a higher number in the second; and (ii) the number of copies of the mt-cyB gene detected by the qPCR in an animal in the first collection had little relationship with the number found in the second one.

4. Discussion

The present experiment involved measuring the levels of antibodies and number of copies of the mt-cyB gene of *B. bovis* in blood samples of Angus cattle bred in an area of the state of São Paulo that according to a recent study (Bilhassi et al., 2014) is stably endemic for babesiosis. The results of this experiment confirm those findings, and the fact that the animals reared in this region have developed resistance to the clinical form of the disease through repeated infections during the year. The ELISA results indicated that 100% of animals were positive for *B. bovis*, with antibody titers both in the first and second collections. Various studies carried out in Brazil have found similar results to ours here. Juliano et al. (2007) found prevalence rates higher than 92% on two occasions, in studying the levels of antibodies in Curraleira cattle bred in a cerrado (savanna) area of northeastern Brazil. Guedes Junior et al. (2008) found 98.8% prevalence in zebu and crossbred cattle in the northern region, and Trindade et al. (2010) observed prevalence of 91.7% in zebu calves in the state of Tocantins in the midwestern region of Brazil.

Our results differ from those reported in southern Brazil by Osaki et al. (2002), who observed the prevalence of 64.2% among Nelore cattle, and Barros et al. (2005), who found 63.7% in cattle reared in the semi-arid region of northeastern Brazil. The South American continent is very heterogeneous regarding types of cattle production systems, and factors such as endemicity, susceptibility of host to tick, tick density and climate conditions can determine unique qualitative and quantitative characteristics for transmission of babesiosis (Nari, 1995). Since Brazil is the largest country in South America, the occurrence of these variations is understandable. Besides the high frequency of infection by *B. bovis* detected in this study, there were significant differences in the EL and S/P values between the two collections. The month of May marks the end of autumn in southeastern Brazil, and this season has been identified as having peak infestation by cattle ticks (Oliveira and Alencar, 1989; Andrade et al., 1998; Silva et al., 2007). This fact can explain, at least partly, the higher levels of antibodies observed in the first collection.

The qPCR technique proved to be highly sensitive and specific in detecting DNA of *B. bovis,* comparable to the results reported by Buling et al. (2007). All the animals were positive for *B. bovis* in both collections. The high sensitivity of qPCR to assess the level of

infection by *B. bovis* has also been reported by Bilhassi et al. (2014) and Giglioti et al. (2016).

The association of the molecular and serological techniques has been used to improve babesiosis monitoring and diagnostic services. Various research groups have observed that ELISA can be a more sensitive technique than molecular tests (Terkawi et al., 2011, 2012; Machado et al., 2012; Ibrahim et al., 2013). Ferreri et al. (2008) investigated the diagnosis of *B. bovis* in water buffaloes in Argentina using the nested-PCR and ELISA techniques. They found that the molecular technique was more sensitive. Different results were found by Rosales et al. (2013) in diagnosing *B. caballi* in Venezuelan horses by means of qPCR and ELISA. They did not manage to detect the hemoparasites by the molecular technique while ELISA identified infection prevalence of 23.2%. The authors attributed this discrepancy to problems inherent to the principles of each technique since qPCR detects DNA from the parasite while ELISA detects antibodies against the parasite. Our results corroborate those of Ramos et al. (2011), who compared molecular and serological techniques in Brazil to monitor two regions, one with endemic stability (Juíz de Fora, MG, Brazil) and another with instability (Bagé, RS, Brazil) for babesiosis. In the stable endemic area, the prevalences of infection by *B. bovis* measured by the qPCR, PCR, and ELISA techniques were 95.91, 82.65 and 92.85%, respectively. In the unstable endemic area, they only detected *B. bovis* by qPCR, and the infection rate was only 10%. One of the aspects that should be observed when using PCR is the nature of the gene used to amplify the parasite's DNA. Salem et al. (1999) developed a PCR test and assessed its sensitivity and specificity in detecting *B. bigemina* and *B. bovis* by comparing episomal and ribosomal sequences of DNA from these parasites. With the episomal technique they detected 85% prevalence of *B. bovis,* while with the ribosomal, technique the detection rate was only 65%. This information reinforces our findings, since we used primers that flank the region of the mt-cytB gene, specific for *B. bovis*, and that according to Buling et al. (2007) increase the test's sensitivity more than 100 times.

The main goal of this experiment was to check the repeatability of the qPCR and ELISA methods, in animals chronically infected by *B. bovis*, as well as the occurrence of associations between these two tests. We did not find in the literature any studies of this nature to be used for comparison. Our results indicate that both tests are highly sensitive in detection, be it of antibodies against *B. bovis* or DNA from this parasite. With respect to the fluctuation in the quantity of DNA from the hemoparasite in the animals' blood, it is hard to determine the cause. Considering the repeatability values found for the NC metric, we can infer that a large part of this variation can be attributed to environmental factors that act on the animal at the collection time, and only a small part can be attributed to the animals' ability to maintain low infection levels, as also verified by Giglioti et al. (2016). Although tick infestation can vary between collections, previous studies have shown no association between tick count and NC (Giglioti et al., 2016) and indicate this cannot be reported as the cause of the variation. The greater repeatability of the serological tests in relation to quantification of DNA of parasites in the bloodstream shows the existence, at least in peripheral blood, of a greater individual variation in the NC than in the level of antibodies. The immune response of animals frequently exposed to inoculations of the antigen is sufficient to keep the hemoparasites at low levels, but without eliminating them completely. As demonstrated by the low correlations between S/P and NC, the variation in the NC value is independent of the level of antibodies.

In conclusion, we can state that although the test used to estimate the NC in this experiment was able to detect 100% of *B. bovis* infection, the repeatability estimates showed that, in the conditions of this study, this test does not discriminate animals for resistance to babesiosis. On the other hand, higher repeatability of S/P indicates that animals with higher titers in the first sampling also exhibit higher titers in the second collection. However, the correlations between the S/P and NC were low in both collections, indicating that higher level of antibodies does not imply better ability to maintain low levels of infection. Therefore, it is possible that although detectable by the technique, the variations in the NC of this group of animals are not significant enough either to produce clinical signs in the animals or to trigger a momentaneous immune response. New studies needed to be carried out to try to identify quantitative traits that can be used in studies of selection for resistance to babesiosis.

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