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Original Article

Molecular epidemiology of *Babesia vogeli* in dogs from the southeastern region of Rio de Janeiro, Brazil

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ABSTRACT

Hemoparasitic diseases are prominent in domestic animals, particularly in Brazil, a tropical country with a wide range of vectors. This study investigated the epidemiology of *Babesia vogeli* in the whole blood of dogs from the southeastern region of Rio de Janeiro, Brazil. Whole blood samples from 390 dogs were screened for the presence of *B. vogeli* DNA by qPCR using the *heat shock protein 70 kDa* (*hsp70*) gene of *B. vogeli*. Characteristics related to the host and its environment were collected using a questionnaire. Bivariate analysis was used to evaluate each factor individually. A phi correlation test was used to verify collinearity. The variables with $p < .1$ and a low or moderate correlation with the other variables were selected for the multivariate analysis. Multiple models were created, and the best logistic regression model was chosen using the Akaike Information Criterion (AIC). The final model was used to determine which variables were closely related to *B. vogeli* infections in dogs. Of the 390 dog blood samples, 15.66% were positive for *B. vogeli*. The variables cat contact, age, shelter, street or woods access, tick infestation and fur lengthwere included in the final model. Per the logistic regression analysis, three variables explained *B. vogeli* detection in dogs: age (odds ratio [OR] = 2.12; *p*-value < .05; confidence interval [CI]: 1.13–3.96), tick infestation (OR = 2.08; *p*-value < .05; CI: 1.10–3.93) and shelter (OR = 2.22; p-value < .05; CI: 1.16–4.26). These variables were determined to be associated with *B. vogeli* detection in domiciled dogs in the southeastern region of Rio de Janeiro, Brazil. These data indicate that the age of the animal, the presence of ticks and the lack of shelter directly affect the epidemiology of *B. vogeli*.

1. Introduction

Babesia vogeli is the primary etiological agent of canine babesiosis in Brazil. The *Rhipicephalus sanguineus* sensu lato tick is the biological vector of this protozoan in the country (Passos et al., 2005). Clinical signs of babesiosis are unspecific, but anorexia, pyrexia, petechiae, epistaxis and splenomegaly are often reported (Solano-Gallego et al., 2016). Laboratory findings include anemia, hemoglobinuria and thrombocytopenia (Martin et al., 2006). The routine diagnosis of canine babesiosis is based on clinical signs, hematological profiling and the presence of the parasite in blood smears (Vidotto and Trapp, 2004).

Therefore, due to the unspecific clinical signs, as well as the high frequency of false negatives on blood smears examined by light microscopy, other laboratory techniques, such as molecular methods and immunodiagnostic tests, are necessary to confirm the infection and proceed with treatment (Solano-Gallego et al., 2016). Thus, chronic or less virulent cases may not be detected by examination of the smear due to its low parasitemia (Solano-Gallego et al., 2016). In addition, examining blood smears can be tedious and requires the experience and time of a laboratory technician.

Confirming a babesiosis diagnosis is not common in routine veterinary practice. The infection caused by *B. vogeli* is often mistaken for the infection of other hemoparasites. Maciera et al. (2005) reported that many clinicians in Brazil, including those in Rio de Janeiro, use the presence of only thrombocytopenia to make a presumptive diagnosis of *Ehrlichia canis* infection in dogs. The use of doxycycline is often adopted to eliminate blood parasites, especially *E. canis*. Although antibiotics are not the therapy of choice for piroplasmids, Vercammen et al. (1996) reported that doxycycline may reduce the severity of clinical signs in canine babesiosis. However, this treatment does not eliminate *Babesia* spp., and the disease may resurface. Brandão et al. (2003) reported that the use of imidocarb dipropionate in two doses of 7 mg/kg administered

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over 14 days seemed to be effective in eliminating large *Babesia* spp. parasites. Therefore, babesiosis occurrence is often under diagnosed, and epidemiological studies are needed to determine the frequency of parasitized animals in a particular area, as well as the environmental variables and other host-related factors leading to *B. vogeli* infections in dogs (Maia et al., 2007).

In Brazil, epidemiological research on the frequency, as well as other factors related to canine babesiosis, has been conducted in both endemic and non-endemic areas. Many of these studies determined the presence of antibodies against *Babesia* spp. by indirect immunofluorescence antibody (IFA) assays (Mrljak et al., 2017). However, cross-sectional epidemiological studies based on serological techniques can lead to temporal bias, since the presence of circulating antibodies does not necessarily indicate a current infection. Moreover, there is a trend for the serological tests to overestimate the protozoan frequency as circulating antibodies may remain for long periods following treatment or resolution of clinical signs (Solano-Gallego et al., 2016).

A variety of molecular tools, including loop-mediated isothermal amplification assay (LAMP), real-time quantitative polymerase chain reaction (qPCR), and high resolution melting analysis (HRM), are used to directly detect hemoparasite DNA from clinical or environmental samples, providing relatively low-cost, rapid molecular tests of high yield (Lymbery and Thompson, 2012). Quantitative PCR allows visualization of the reaction process cycle by cycle, shortening the time to obtain results and avoiding contamination post-amplification. Therefore, quantitative PCR was chosen to estimate *B. vogeli* detection in this study.

Many epidemiological studies perform only bivariate analysis to determine infection-related factors (Nalubamba et al., 2011; Bashir et al., 2009). Thus, only single epidemiological variables are evaluated, making the statistical analysis incomplete, as other factors are not considered. Multiple logistic regression analysis is used to learn which other factors influence the epidemiology of canine babesiosis in a region (Medronho, 2009). In this study, multiple variables are analyzed together, providing a more comprehensive result.

This study used qPCR based on the *hsp70* gene to determine the frequency of *B. vogeli* and developed an epidemiological model using multiple logistic regression analysis to determine the factors associated with the presence of *B. vogeli* DNA in dogs from the southeast region of Rio de Janeiro, Brazil.

2. Materials and methods

2.1. Description of the studied area

This study was conducted in rural and urban areas of the Itaguai Microregion located in southeast of the State of Rio de Janeiro (Fig. 1), which encompasses the municipalities of Mangaratiba (22° 57′ 35″S; 44° 02′ 26″W; altitude: 18 m), Seropedica (22° 44′ 38″ S; 43° 42′ 27” W; altitude: 26 m) and Itaguai (22° 51′ 08″ S; 43° 46′ 31″ W; altitude: 13 m).

The area has a tropical climate (Aw) characterized by a dry season, summer rains and a well-defined winter season according to Köppen-Geiger classification (Kottek et al., 2006). This climate presents an average temperature during the coldest month of the year > 18 °C and an average maximum temperature above 25 °C.

The population of Itaguai has 100,362 inhabitants followed by Seropedica with 76,045 and Mangaratiba with 34,966. The region has a total of 211,373 inhabitants (IBGE, 2010).

2.2. Sampling

The absence of frequency data on *B. vogeli* in dog whole blood samples through molecular techniques led to an expected prevalence of 50%, which represents the maximum number for the sample size, an error of 5% and a precision of 5% (Medronho, 2009). The sample number was set per the equation described by Sampaio (2002): $n = nx1.962P_{exp}(1\text{-}P_{exp})/d^2$, where n = sample size; P_{exp} = expected prevalence; and d^2 = absolute accuracy desired. Per this equation, 384 dog blood samples were needed in the study region. Dog blood samples were collected by cephalic venipuncture in sterile vacuum tubes containing EDTA (ethylenediaminetetraacetic acid). Samples were randomly collected in a volume of 2 to 5 mL from 390 domestic dogs from the rural and urban areas of southeast Rio de Janeiro, Brazil.

During the visits, the dogs were systematically examined by manual inspection for tick infestation for 20 min. The specimens found were collected and properly identified according to a taxonomic key described by Barros-Battesti et al. (2006). Ticks were collected and stored in polypropylene tubes containing isopropyl alcohol.

2.3. Standard control

The blood used as a positive control was obtained from a dog presenting clinical signs of babesiosis, with intraerythrocytic inclusions being observed in cytological examination of a blood smear, and by conventional PCR using 18S rDNA as molecular marker and (Carret et al., 1999) and the *hsp70* gene of *B. vogeli*, as described by Paulino et al. (2018).

The fragment was purified with CleanSweep (Applied Biosystems®) and sequenced. The 18S rDNA sequence showed 100% identity with *B. vogeli* and was deposited into GenBank under the accession number MF459002.

The negative standard control was obtained from a naïve dog, which has never been exposed to ticks. Next, the blood was extracted and tested using the molecular marker 18S rDNA as a target to detect *B. vogeli* by conventional PCR, as described by Carret et al. (1999), and using the *hsp70* gene of *B. vogeli* by conventional PCR, as described by Paulino et al. (2018). The results were negative in all of the molecular assays. Nuclease-free water (Ambion®, Thermo Scientific, Wilmington, DE, USA) was also used as a negative control.

To avoid contaminating the reactions, the DNA extraction step, preparation of the PCR reagents mixture, the addition of DNA samples and electrophoresis were carried out in different environments, respecting a unidirectional flow.

2.4. Extraction of total DNA

DNA extraction was performed using a commercial kit (Qiagen DNeasy-tissues-Blood, Valencia, CA, USA) as per the manufacturer's recommendations. After extraction, the total DNA was quantified in a spectrophotometer (Nanodrop ND-2000 ®, Thermo Scientific, Wilmington, DE, USA) and standardized at a concentration of 100 ng/μL. The samples were frozen in triplicate at −20 °C until the molecular analyses.

2.5. Detection by qPCR

All samples were tested by qPCR based on the *hsp70* gene using the primers *BvqF* (5'-GCTGGTGACACCCACCTT-3′) and *BvqR* (5'-CAACAA GCGTGCCCTCC-3′) and the hydrolysis probe Bvq (5'-*NED*-CCTCCTCG TTGAGCACT-*MGB*-3′) as described by Paulino et al. (2018).Reactions were performed in a final volume of 12 μL on StepOnePlus (Applied Biosystems®, ThermoFisher Scientific, Wilmington, DE, USA) equipment, containing 1× TaqMan® Universal PCR Master Mix (2×), 800 nM of each primer, 250 nM of probe and 300 ng of total DNA. The thermocycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s and 60 °C for 1 min. All reactions were performed in duplicate. Samples with amplification < 40 quantification cycles (Cq) were considered positive.

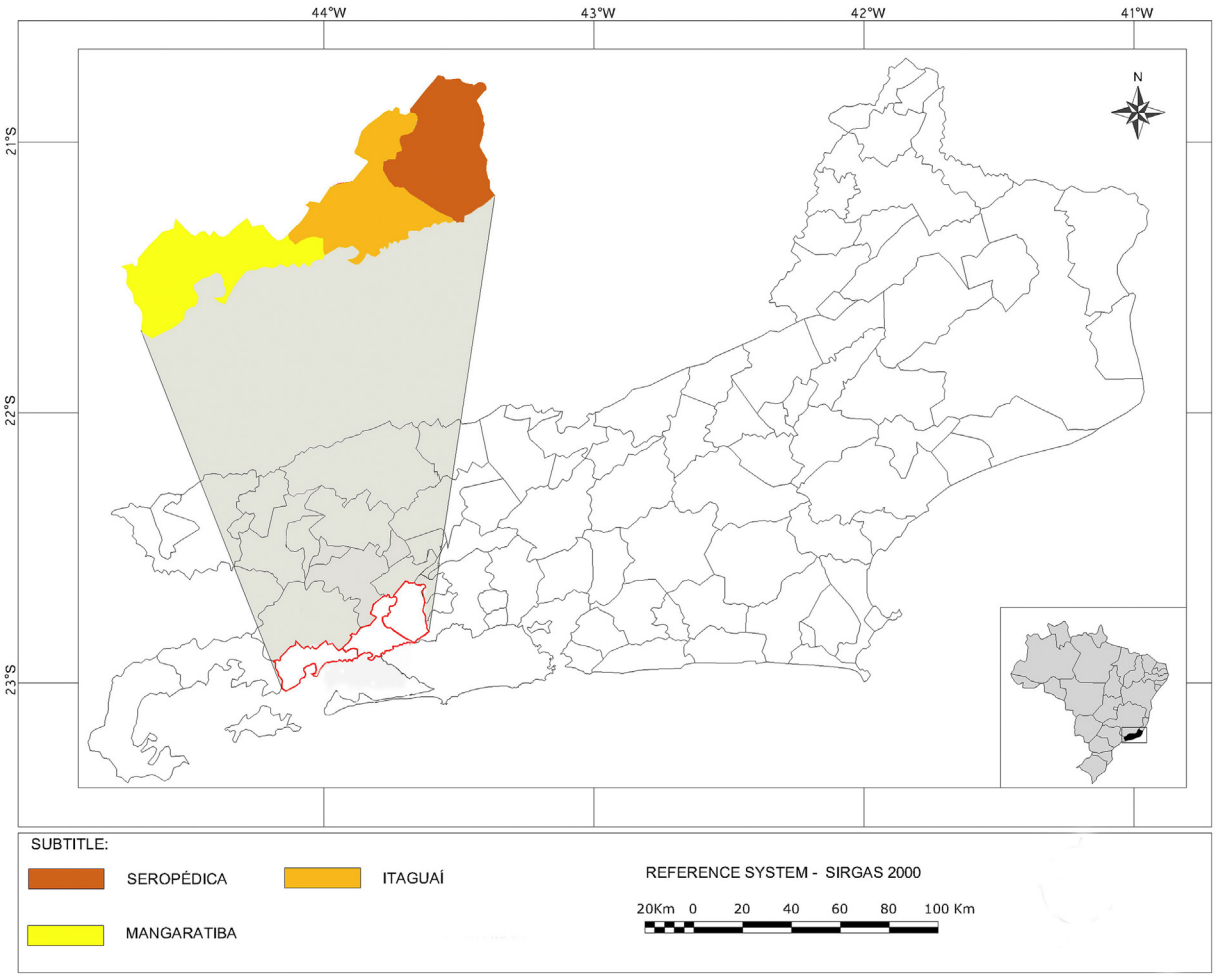


Fig. 1. Microregion of Itaguai, State of Rio de Janeiro, Brazil. Location of Seropedica, Itaguai and Mangaratiba municipalities where blood samples were collected from dogs. Scale bar = 20 Km.

2.6. Detection of PCR inhibitors

All DNA samples from the canine whole blood were tested by conventional PCR targeting the *Beta Actin* gene (ACTB) following the conditions described by Brinkhof et al. (2006). This reaction evaluated the efficiency of the DNA extraction process and identified the presence of inhibitors. An inhibition test was also performed by adding a standard control with 100 copies of a fragment of the *hsp70* gene to each DNA extract to verify the presence of PCR inhibitors.

2.7. Epidemiological questionnaire

A semi-structured questionnaire based on the questionnaire described by Santos et al. (2013) was given to the dog owner to obtain information on each animal, highlighting such aspects as residence area (urban or rural area, based on the urban perimeter stipulated by the municipality's official information); gender (female or male); age (< 5 or ≥ 5 years); breed (breed or mixed breed animals); fur (short or medium/long); indoor-only (yes or no); shelter access (yes or no); outdoor access (neighborhood streets or woods); contact with cats (yes or no); ectoparasiticide treatment (yes or no); environmental hygiene conditions (satisfactory or unsatisfactory, based on the cleanliness of the home/shelter); veterinary care (yes or no)andtick infestation (presence or absence of ticks at the time of blood sample collection).

2.8. Statistical analysis

To evaluate the possible factors associated with positive test results in the dogs, the variables from the epidemiological questionnaire were independently analyzed as a function of *B. vogeli* detection frequency using the chi-square test at a 5% significance level in the BioEstat program 5.0 (Ayres et al., 2007). Variables with a significant difference ($p < .1$) from the chi-square test were selected. Correlation matrices were made using the phi correlation test (φ) to verify collinearity and eliminate the correlated variables. The values of φ range between -1 and $+1$. This approach was also employed in the BioEstat 5.0 program (Ayres et al., 2007).

To evaluate the risk factors associated with *B. vogeli* in dogs, a logistic regression method was performed using R version 2.11.1 software (R Development Core Team, 2010). Selecting the appropriate statistical model is crucial for data analysis (Bozdogan, 1987). The most parsimonious model accurately explains the variable response behavior, that is, the model that requires the fewest possible parameters to be estimated. Therefore, a criterion was chosen to classify the candidate models and find the models that exhibited optimal values of the chosen criterion. The criterion chosen to evaluate the best model was the Akaike Information Criterion (AIC).

2.9. Ethical considerations

These procedures were approved by the Ethics Committee on Animal Use of Federal Rural University of Rio de Janeiro, number

Table 1
Descriptive and bivariate analysis of the extrinsic characteristics related to dogs infected with *Babesia vogeli*, detected by qPCR using *hsp70* gene in the southeast of Rio de Janeiro State, Brazil.

Extrinsic characteristics	Descriptive analysis				Bivariate analysis		
Of dogs	N	N +	N -	%	CI 95%	χ^2	P
Residence area							
Rural/Peri urban	184	30	154	16.30%	0.76–2.61	1.39	0.23
Urban	206	25	181	12.14%			
Cities							
Mangaratiba	90	9	83	10.00%			
Seropedica	150	25	125	16.67%	0.31–1.56	2.23	0.32
Itaguai	150	21	127	14.00%			
Live only inside the house							
Yes	50	6	44	12.00%	0.48–3.73	0.21	0.64
No	340	49	291	14.41%			
Contact with cats							
Yes	79	18	61	22.78%	1.09–4.23	6.16	0.01
No	311	37	274	11.90%			
Acess							
Streets	191	17	174	8.90%	1.26–4.74	8.36	0.004
Woods	199	38	161	19.10%			
Tick infestation							
Yes	179	38	141	21.23%	1.61–6.04	13.87	0.0002
No	211	17	194	8.06%			
Shelter							
Yes	256	24	232	9.38%	1.56–5.44	13.74	0.0002
No	134	31	103	23.13%			
Environmental hygiene conditions							
Unsatisfactory	187	38	149	20.32%	1.46–5.48	11.46	0.007
Satisfactory	203	17	186	8.37%			
Veterinary Assistance							
Yes	177	15	162	8.47%	1.28–5.04	8.47	0.004
No	213	40	173	18.78%			
Ectoparasitecide treatment							
Yes	261	35	226	13.41%	0.44–1.62	0.31	0.57
No	129	20	109	15.50%			
TOTAL	390	55	335	14.10%			

N: number of dogs tested by qPCR; χ2: Chi-square test; P: p-value; CI: Confidence interval.

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3. Results

For the *hsp70* gene, 14.10% of the animals (n = 55/390) were positive by PCR for the 118-bp fragment of *B. vogeli* in the region. Of these animals, 16.67% (n = 25/150) belonged to the Seropedica municipality, while 14.00% (n = 21/150) belonged to Itaguai, and 10.00% (n = 9/90) to Mangaratiba (Table 1).

The following extrinsic factors were associated with the presence of *B. vogeli* DNA by bivariate analysis: presence of ticks ($p < .0002$), absence of shelter ($p < .0002$), access to neighborhood streets or woods ($p < .004$), poor environmental hygiene ($p < .007$), lack of veterinary care ($p < .004$) and direct contact with cats ($p < .01$) (Table 1).

Age of dog and fur length both correlated with *B. vogeli* infections in the bivariate analysis ($p < .05$) (Table 2). Animals with short fur had a p-value of < 0.1 in the chi-square test ($p > .05$). The other host-related intrinsic variables showed no association ($p > .1$) with *B. vogeli* based on qPCR and bivariate analysis; thus, they were not included in the final logistic regression model.

The phi(φ) test was performed, but the factors showed no strong collinearity. The φ values ranged from 0.01 to 0.51, and it was considered weak collinearity between the variables. Therefore, all of the variables were entered into the multivariate analysis.

Table 2
Descriptive and bivariate analysis of the intrinsic characteristics of dogs infected with *Babesia vogeli*, detected through the qPCR using the *hsp70* gene in the southeast of Rio de Janeiro State, Brazil.

Intrinsic characteristics	Descriptive Analysis				Bivariate Analysis		
Of dogs	N	N +	N-	(%)	CI 95%	χ^2	P
Gender							
Female	186	25	161	13.44%	0.48–1.65	0.13	0.72
Male	204	30	174	14.71%			
Breed							
Mixed breed	264	41	223	15.53%	0.74–3.04	1.37	0.25
Breed	126	14	112	11.11%			
Age							
< 5 years	127	27	100	21.26%	1.21–4.20	7.39	0.005
> 5 years	263	28	235	10.65%			
Fur							
Small	191	34	157	17.80%	0.29–1.01	4.22	0.056
Medium/long	199	21	178	10.55%			
TOTAL	390	55	335	14.10%			

N: number of dogs tested by qPCR; χ2: Chi-square test; P: *p*-value; CI: Confidence interval.

After the logistic regression model analysis, the final model was selected by the lowest AIC value (295.33). In this analysis, the AIC values ranged from 295.33 to 303.19. Tick infestation was the factor that most influenced *B. vogeli* infection in dogs. Dogs with ticks were 2.22 times more likely to be infected with *B. vogeli* (OR = 2.22; $p < .05$; CI: 1.23–3.93) than dogs without a tick infestation at the time of sample collection (Table 3). In this study, *R. sanguineus* sensu lato and *Amblyomma ovale* were the only tick species found infesting the dogs. Age was also included in the final logistic regression model, and dogs under five years of age were 2.12 times more likely to be infected by the hemoparasite than those over five years of age (OR = 2.12; $p < .05$; CI: 1.13–3.96) (Table 3). The shelter variable was correlated with *B. vogeli*, and dogs without shelter access were 2.08 times more likely to be infected with *B. vogeli* (OR = 2.08; p < .05; CI: 1.16–4.26) (Table 3). The fur length, cat contact and environmental access variables were included in the model because they presented lower *p*-values than the one estimated as a cut-off line in this study (Table 1); however, these factors were not associated with *B. vogeli* infection.

4. Discussion

Several epidemiological studies have demonstrated distinct results between molecular and serological techniques, and data obtained by PCR has shown greater accuracy (Kubelová et al., 2013; Mrljak et al., 2017). Molecular biology techniques are important in detecting DNA from many pathogens in vectors and hosts, especially hemoparasites. PCR may aid in the ability to diagnose babesiosis, and it has provided taxonomic classification information for these and other infectious agents. In addition, PCR can detect the pathogen DNA in suspected vectors, thereby assisting in the identification of species that may be responsible for transmission of those agents, and can detect new species variants.

Real-time PCR (qPCR) allows monitoring the reaction from start to finish. Additionally, quantification is performed at the beginning of the qPCR assay, when a fluorescent signal is emitted in proportion to the PCR product accumulation during the reaction, enabling quantification of the gene copies. Real-time PCR has several advantages. First, it does not require handling the PCR products after the reaction to analyze the results (Heid et al., 1996). Second, it is highly sensitive and can amplify small quantities of DNA (Bustin et al., 2009). Finally, it has a lower coefficient of variation than those methods that are based on measuring

Table 3
Multivariate analysis of factors associated with the infection of dogs by *Babesia vogeli* in the southeast of Rio de Janeiro State, Brazil.

Associated	Descriptive Analysis				Multivariate Analysis				
Variables	N	N +	N-	(%)	C (ß)	SE	P	OR	CI 95%
Age									
< 5 years	127	27	100	21.26%	0.63	0.31	0.05	2.12	1.13–3.96
> 5 years	263	28	235	10.65%	*	*	*	*	*
Tick infestation									
Yes	179	38	141	21.23%	0.84	0.32	0.05	2.22	1.23–3.93
No	211	17	194	8.06%	*	*	*	*	*
Shelter									
Yes	256	24	232	9.38%	*	*	*	*	*
No	134	31	103	23.13%	0.83	0.32	0.05	2.08	1.16–4.26

N: number of animals; (%): Percentage of positives; C (ß); Coefficient of the model; SE: Standard error; P: *p*-value; OR: Odds Ratio; CI: Confidence Interval. * Category of Reference.

DNA bands by electrophoresis or probe hybridization (Bustin et al., 2009).

Vilela et al. (2013), using a real-time PCR system based on an intercalating DNA dye, found that 11.9% of dogs in the municipality of Seropedica were positive for *Babesia* spp. This percentage is less than the 16.66% (n = 25/150) observed for the municipality of Seropedica in this study. This difference may be due to the qPCR sensitivity used by Vilela et al. (2013). The detection limit of the qPCR technique applied in the study was not available, which may be lower than the method used in this study. Furthermore, using fluorescent markers for the hydrolysis probe system allows greater specificity in amplifying the product as it is necessary for annealing followed by breaking the probe to release the fluorophore (Heid et al., 1996).

The indirect immunofluorescence antibody (IFA) technique has been widely indicated as a reference serological test for babesiosis for > 30 years (Irwin, 2009). However, in studies conducted by Furuta et al. (2009), a comparison was made between indirect enzyme-linked immunosorbent assay (ELISA) and IFA tests, where a higher incidence of babesiosis was observed in the ELISA test (67%) than in the IFA test (59%). Serology is considered a method with high sensitivity, although the specificity is considered moderate/low for detection of antibodies against *Babesia* spp. in dogs. This fact justifies the large amount of cross-reactions that occur between species of *Babesia* spp. and other hemoparasites (Zanette et al., 2013). In addition, false negatives can occur in hyperacute infections, since antibodies only manifest with 8 to 10 days or more following infection (Boozer &Macintire, 2003). Another relevant point is that dogs treated with imidocarb dipropionate may be seropositive for long periods. Thus, it is impossible to differentiate between acute and chronic infections by serology (Irwin, 2009).

In Brazil, *B. vogeli* is endemic to urban areas where there is a high prevalence of dogs infested with *R. sanguineus* sensu lato ticks (Passos et al., 2005). However, in rural and peri-urban areas, dogs are bred in restricted spaces on the property, which mimics the situation in urban areas (Dantas-Torres et al., 2006). This breeding may be why no significant difference was seen between the rural and urban areas. No association was seen between the presence of *B. vogeli* DNA and the dogs' municipalities of origin, indicating that the infection rate throughout the study area was relatively homogenous. Brazil's tropical climate favors the life cycle and movement of *R. sanguineus* sensu lato almost all year round (Labruna and Pereira, 2001), helping to maintain the endemic level of *B. vogeli*. The environmental characteristics observed throughout the study areas were similar, which may have favored the *B. vogeli* infection in the dogs.

No association was found between either gender or breed with *B. vogeli* detection. The results found in this study agreed with studies conducted by Maia et al. (2007) and Costa-Júnior et al. (2009), where the authors also reported no association between these variables and *B. vogeli* detection. However, Dell Porto (1986) observed a significantly higher prevalence of *B. vogeli* in females and attributed this finding to puerperium. Post-whelping, female dogs are sedentary, which facilitates parasitism by the vector.

This study found that animalsunder 5 years of age were more likely to test positive for*B. vogeli* DNA (OR = 2.12; $p < .05$; CI = 1.13–3.96). The immune system in young animals is immature, meaning they may not yet have the full capacity to produce antibodies to fight pathogens (Bashir et al., 2009). Rotondano et al. (2015) observed that newly weaned young dogs are more susceptible to disease due to the stress of adapting to feeding and to the environment. These data were similar to those of other epidemiological studies conducted in different countries such as Italy (Solano-Gallego et al., 2008), Pakistan (Bashir et al., 2009), Zambia (Nalubamba et al., 2011) and Brazil (Rotondano et al., 2015). Although young animals are susceptible to infection, geriatric animals also have a greater chance of being infected, since the immune system of these animals becomes inherently more fragile, also placing them at risk (Bashir et al., 2009).

The presence of ticks was correlated with *B. vogeli* infection (OR = 2.22; $p < .05$; CI = 1.23–3.93*). R. sanguineus* sensu lato is the species that transmits *B. vogeli* to dogs in Brazil (Passos et al., 2005). Many reports highlight the presence of this tick species in urban and rural areas (Labruna and Pereira, 2001; Dantas-Torres et al., 2006). Due to its wide distribution in Brazil, the presence of *R. sanguineus* sensu lato in dogs was the factor that was most associated with *B. vogeli* infection in this study. Although *R. sanguineus* sensu lato possesses high host specificity, in conditions where the preferred host is scarce, the vector can parasitize other species,such as cats, humans, and rodents (Mendes-de-Almeida et al., 2011; Dantas-Torres et al., 2006; Winkel et al., 2014). Therefore, it is possible that the vector may spread the pathogen through cats that have contact with dogs, thus increasing the chances of infection. However, further work should be carried out to delineate the role of cats as *E. canis* reservoirs. In this study, the chi-square value was significant ($p < .01$). Therefore, this factor was included in the model but was not significant in the multivariate analysis.

The absence of shelter was considered a risk factor for *B. vogeli* infection in dogs (OR = 2.29; $p < .05$; CI = 1.23–4.26). Animals deprived of shelter are exposed to varied environments due to their constant movement and have access to different surroundings (Santos et al., 2013). Additionally, these animals may visit other places, such as abandoned houses or civil constructions, which offer many hiding spots for ticks. Therefore, the probability of a tick infestation is high, making these animals susceptible to *B. vogeli* infection. Animals that roam loose or are work animals often belong to people of low economic means who cannot afford veterinary care (Maia et al., 2007). There may be an indirect relationship between the lack of veterinary care and the presence of *B. vogeli* DNA in dogs since these animals often live in hazardous conditions and do not receive proper nutrition or ectoparasiticide treatment. This variable was significant in the chi-square test. This correlation between veterinary care and *B. vogeli* infection in dogs was also observed by Araujo et al. (2015).

5. Conclusions

The epidemiological analysis of *B. vogeli* in dogs in the southeastern region of Rio de Janeiro by multiple logistic regression analysis revealed that dogs who were younger than five years old, raised without access to shelter and infested with ticks were more likely to be infected with *B. vogeli*.

Ethical statement

The authors Patrícia G. Paulino, Marcus S. Pires, Claudia B. da Silva, Maristela Peckle, Renata L. da Costa, Gabriela L. V. Vitari, Ana Paula M. de Abreu, Carlos L. Massard, Huarrisson A. confirm that all the research meets the ethical guidelines, including adherence to the legal requirements of the study country. The procedures were approved by the Ethics Committee on Animal Use of the Federal Rural University of Rio de Janeiro, number 3915240616.

Additionally, we state that all the authors and the institutions Federal Rural University of Rio de Janeiro (UFRRJ) approved this research.

Best regards.

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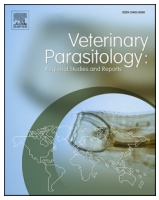
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Erratum regarding missing Declaration of Competing Interest statements in previously published articles

Declaration of Competing Interest statements were not included in the published version of the following articles that appeared in previous issues of *Veterinary Parasitology: Regional Studies and Reports*.

The appropriate Declaration of Competing Interest statements, provided by the Authors, are included below.

1. "Molecular detection of Cryptosporidium species in street-sampled dog faeces in Ibadan, Nigeria" [*Veterinary Parasitology: Regional Studies and Reports*, December 2018; Volume 14: 225]. DOI: https://doi.org/10.1016/j.vprsr.2018.08.005.
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2. "Respiratory disease associated with migrating Ascaris larvae in a beef calf" [*Veterinary Parasitology: Regional Studies and Reports*, May 2018; Volume 12: 157]. DOI: https://doi.org/10.1016/j.vprsr.2017.12.005.
   Declaration of Competing Interest: No conflicts of interest to declare.
3. "Serological survey of cattle toxoplasmosis in Medea, Algeria" [*Veterinary Parasitology: Regional Studies and Reports*, May 2018; Volume 12: 175]. DOI: https://doi.org/10.1016/j.vprsr.2018.02.009.
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4. "Paropisthorchis caninus Stephens, 1912: Synopsis of identity and pathological findings due to spontaneous infection in the liver of a street dog in Assam, India" [*Veterinary Parasitology: Regional Studies and Reports*, January 2019; Volume 15: 253]. DOI: https://doi.org/10.1016/j.vprsr.2018.11.005.
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5. "Effects of long-term indiscriminate use of macrocyclic lactones in cattle: Parasite resistance, clinical helminthosis, and production losses" [*Veterinary Parasitology: Regional Studies and Reports*, April 2020; Volume 20: 100381]. DOI: https://doi.org/10.1016/j.vprsr.2020.100381
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6. "Genetic characterisation of *Fasciola gigantica* from Ghana" [*Veterinary Parasitology: Regional Studies and Reports*, December 2018; Volume 14: 237]. DOI: https://doi.org/10.1016/j.vprsr.2018.09.009
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7. "Polyparasitism and zoonotic parasites in dogs from a rural area of the Argentine Chaco" [*Veterinary Parasitology: Regional Studies and Reports*, April 2019; Volume 16: 100287]. DOI: https://doi.org/10.1016/j.vprsr.2019.100287
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8. "Molecular epidemiology of Babesia vogeli in dogs from the southeastern region of Rio de Janeiro, Brazil" [*Veterinary Parasitology: Regional Studies and Reports*, August 2018; Volume 13: 204]. DOI: https://doi.org/10.1016/j.vprsr.2018.06.004
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9. "First report of Libyostrongylus douglassii (Strongylida: Trichostrongylidae) in ostriches (*Struthio camelus*) from Mexico" [*Veterinary Parasitology: Regional Studies and Reports*, May 2018; Volume 12: 165]. DOI: https://doi.org/10.1016/j.vprsr.2018.01.007
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