Ticks and Tick-borne Diseases 12 (2021) 101785

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Spatial distribution and molecular epidemiology of *Babesia vogeli* in household dogs from municipalities with different altitude gradients in the state of Rio de Janeiro, Brazil

Tays Araujo Camilo [a], Leo Paulis Mendonça [a], Débora Martins dos Santos [a], Lúcia Herran Ramirez [a], Nathalia Alves Senne [a], Patrícia Gonzaga Paulino [a], Priscila Almeida Oliveira [c], Maristela Peckle Peixoto [b], Carlos Luiz Massard [b], Isabele da Costa Angelo [a], Huarrisson Azevedo Santos [a,*]

[a] *Department of Epidemiology and Public Health, Veterinary Institute, Federal Rural University of Rio de Janeiro, Br 465, km 7, Seropedica, Rio de Janeiro 23897-000, Brazil*
[b] *Department of Animal Parasitology, Veterinary Institute, Federal Rural University of Rio de Janeiro, Br 465, km 7, Seropedica, Rio de Janeiro 23897-000, Brazil*
[c] *Department of Surveying and Cartographic Engineering, Engineering Institute, Federal Rural University of Rio de Janeiro, Br 465, km 7, Seropedica, Rio de Janeiro 23897-000, Brazil*

ARTICLE INFO

Keywords:
Canine babesiosis
Geotechnology
Associated factors
18S rRNA sequence
Phylogenetic analysis
Phylogeographic analysis

ABSTRACT

We performed a cross-sectional epidemiological study with 456 household dogs from urban and rural areas in two different regions situated at different altitudes in the state of Rio de Janeiro. The PCR technique using *18S rRNA* as target revealed prevalence of 7.9% of dogs positive for piroplasmids. These samples were sequenced, and all the sequences were 99.9% to 100% similar to *Babesia vogeli* sequences from other countries. The spatial distribution of positive cases was analysed using kernel interpolation in the QGIS software, and the spatial correlation indicators among positive dogs, altitude, and presence of ticks were obtained by calculating the local Moran index using the GeoDa software. The spatial correlation between positive cases and altitude was clear based on both visual and statistical observations. Logistic regression applying the Wald method with a cutoff point of 0.1 revealed that dogs from a region with altitude <600 m had a 2.29-fold chance of *B. vogeli* infection (OR = 2.29; p-value = 0.04; CI: 1.03-5.07), while the rainy season was 2.45 times more associated with *B. vogeli* infection (OR = 2.45; p-value = 0.01; CI: 1.20-5.01), and dogs infested with *Rhipicephalus sanguineus* sensu lato had a 2.47 times higher chance of being infected (OR = 2.47; p-value = 0.02; CI: 1.13-5.38). Entropy analysis of the alignment between *B. vogeli* 18S rRNA (> 1.600 bp) sequences revealed that the most variable region corresponds to the hypervariable V4 region. Genetic homogeneity was observed among the *B. vogeli* 18S rRNA sequences, with distance values ranging from 0 to 0.007 and a mean value of 0.001. The evolutionary distance (0.003) was greater between the sequences from the municipalities of Barra do Pirai (low altitude) and Teresopolis (high altitude). This study expands the molecular epidemiologic knowledge of *B. vogeli* and shows points of variability in the *B. vogeli* 18S rRNA. The results indicate the potential use of spatial analysis tools to improve screening for positive cases, enabling more in-depth studies to strengthen understanding of tick infection prevention in dogs.

1. Introduction

Haemoprotozoan infections of the order Piroplasmorida that occur in dogs include organisms from the families Theileriidae and Babesiidae. *Theileria equi* and *Theileria* sp. have been previously detected in dog blood samples (Criado-Fornelio et al., 2003; Inácio et al., 2019; Matjila et al., 2008). Infections by the genera *Babesia* and *Rangelia* from the family Babesiidae may occur in dogs. Infections by *Rangelia vitalii*

* Corresponding author at: Department of Epidemiology and Public Health, Veterinary Institute, Federal Rural University of Rio de Janeiro, BR 465, Km 7, Rio de Janeiro, Seropédica 23890-000, Brazil
E-mail address: huarrisson@yahoo.com.br (H.A. Santos).

https://doi.org/10.1016/j.ttbdis.2021.101785
Received 16 September 2020; Received in revised form 17 May 2021; Accepted 21 June 2021
Available online 12 July 2021
1877-959X/© 2021 Elsevier GmbH. All rights reserved.

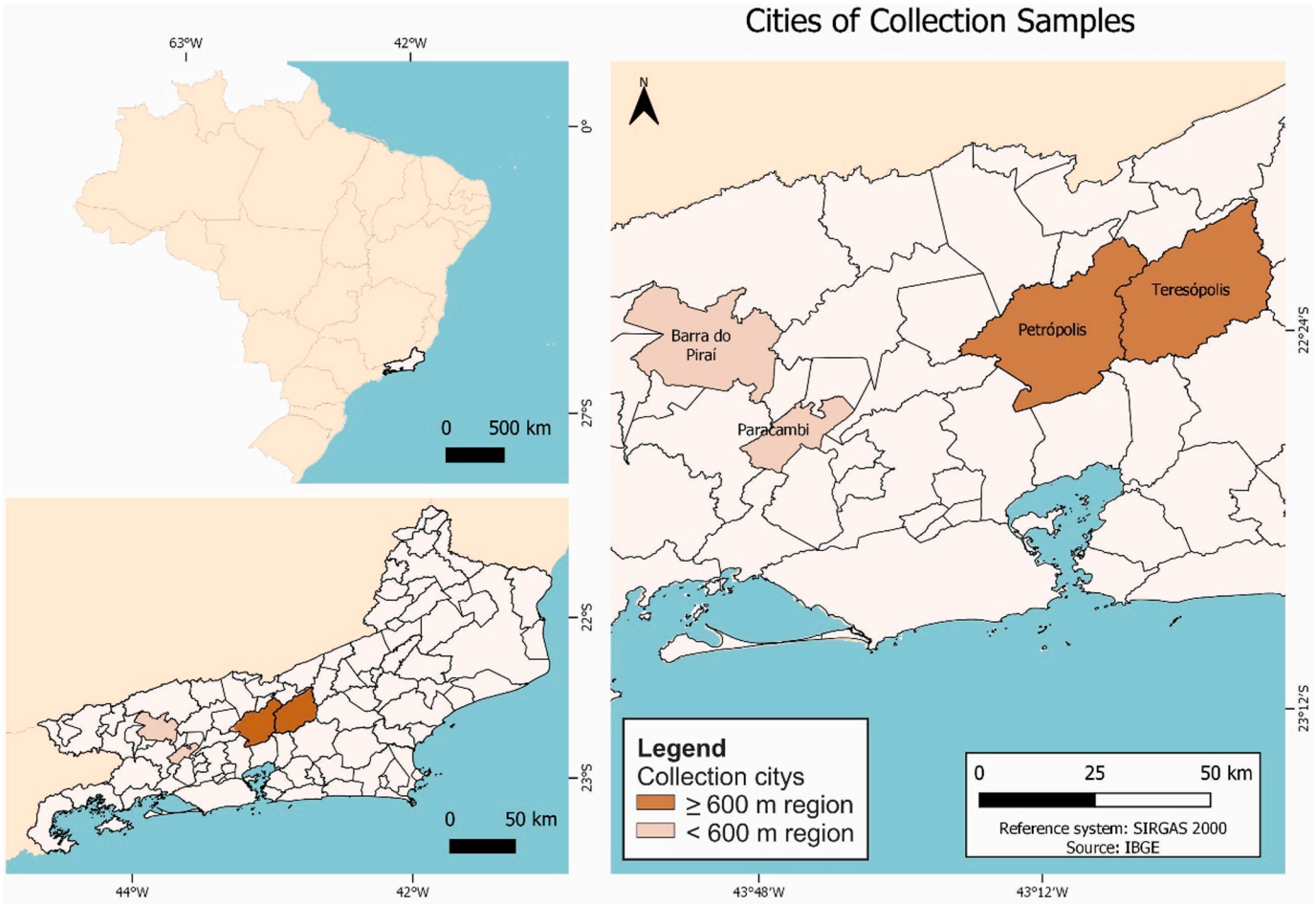

Fig. 1. Thematic map of the four municipalities used for sample collection in the state of Rio de Janeiro, Brazil. Barra do Piraí and Paracambi are below altitude of 600 m while Petrópolis and Teresópolis are above that altitude.

(França et al., 2014; Silva et al., 2019), *Babesia gibsoni* (Trapp et al., 2006), and *Babesia vogeli* (Paulino et al., 2018) have also been reported in Brazil. *Babesia vogeli* is considered the most prevalent canine piroplasmid in Brazil (Araujo et al., 2015; Barbosa et al., 2020; Passos et al., 2005; Paulino et al., 2018).

Among the existing diagnostic methods, polymerase chain reaction (PCR) is often used because of its high sensitivity and specificity to detect pathogens at the beginning of infection (Solano-Gallego et al., 2016). This and other molecular techniques allow identifying parasite species that occur in restricted areas (Barbosa et al., 2020; Inácio et al., 2019). They also enable statistical analysis to identify potential associations between infections that occur in dogs and other factors. As a result, molecular studies aligned with epidemiological data can identify environmental variables that can contribute to *B. vogeli* infections in dogs (Araujo et al., 2015; Paulino et al., 2018). Moreover, PCR followed by sequencing enables accurate measurements of prevalence in epidemiological studies.

Factors including breed (Araujo et al., 2015; Barbosa et al., 2020), sex (Dell'Porto et al., 1990), age (Paulino et al., 2018; Rotondano et al., 2015), absence of a shelter for dogs (Mrljak et al., 2017; Paulino et al., 2018), and the presence of a vector (Passos et al., 2005; Paulino et al., 2018) have been associated with *B. vogeli* infection. Nevertheless, no previous study performed in the state of Rio de Janeiro has considered the impact of altitude and seasonality on *B. vogeli* transmission in dogs. The use of georeferencing interpolations enables the clear visualisation of agents' geographic distribution patterns and their association with abiotic factors (Mrljak et al., 2017; Zemtsova et al., 2016; Zhang et al., 2019).

The study described here aimed to understand the intrinsic and extrinsic variables that can contribute to piroplasmid infections in domestic dogs from two different altitude gradients in Rio de Janeiro, as well as to identify the distribution of agents by using spatial geographic tools.

2. Materials and methods

2.1. Geographic area

The study was conducted in four municipalities in the state of Rio de Janeiro, two at low altitude and two at high altitude. Those at low altitude were Paracambi (latitude: -22.6114, longitude: -43.7095), with average altitude of 100 m and Barra do Pirai (latitude: -22.4705, longitude: -43.8268), with average altitude of 363 m inside the Paraiba Valley. Both areas have Aw climate according to Köppen-Geiger classification. The other two were Petropolis (latitude: -22.5046, longitude: -43.1823) and Teresopolis (latitude: -22.4123, longitude: -42.9664), with average altitudes of 838 m and 869 m, respectively. These municipalities are located at the mountain regions of the state and have climates classified as Cwb.

The geographic positions of the collection points were obtained via the Global Positioning System (GPS) and were processed in decimal degrees in the QGIS software to prepare the thematic maps (Fig. 1).

2.2. Sampling

The sample collections were performed monthly over 12 months

(November 2018 to October 2019) in rural and urban areas of the municipalities of Paracambi, Barra do Pirai, Petropolis and Teresopolis as shown in the collection organogram (Supplementary data 1). The formula $n = 1.96^2\, p\,(1-p)/d^2$ was used to calculate the sample size with a confidence level of 95%, desired absolute precision (d^2) of 5%, and expected prevalence (p) of 50% (Thrusfield, 2007). The collection of samples was randomised and expected to include 384 dogs. A total of 456 whole blood samples were randomly collected from dogs in the studied areas. All the target animals were household dogs, and their owners agreed to participate in the research as volunteers by signing the informed consent form.

2.3. Semi-structured questionnaire

A semi-structured questionnaire (Santos et al., 2013) was used in this study. One dog was randomly chosen from each property without considering previous health conditions. The exploratory variables included municipality (Barra do Pirai, Paracambi, Petropolis, or Teresopolis), altitude (< 600 m or ≥ 600 m), area (urban or rural), seasonality (dry or rainy period), and temperature (< 25°C or ≥ 25°C), with location of data collection determined by GPS by consulting the site https://weather.com/. Other variables were the number of animals in the household (one or more), the presence of other domestic animals (no or yes), the presence of wild animals nearby (no or yes), the presence of vegetation in the property (this included any kind of vegetation, like a grass yard, large garden, or trees if the dog was in contact with them) (no or yes), presence of shelter (no or yes), access to roam outside the property (no or yes), presence of ticks (no or yes), presence of *R. sanguineus* s.l infestation (no or yes), presence of *A. sculptum* infestation (no or yes), veterinary assistance (no or yes), hygienic conditions (satisfactory or unsatisfactory), sex (female or male), age (> 5 or ≤ 5 years old), previous tick-borne diseases (no or yes), breed (mixed or pure), coat (short or long), body score (subnormal or regular) according to Laflamme (1997), animal size (<15 kg or ≥ 15 kg), castration (no or yes), and health status (asymptomatic or symptomatic). Each animal was evaluated for all physiological parameters on the collection day.

2.4. Blood and tick collection

Whole blood samples from each dog were obtained using venous puncture of the cephalic or jugular vein (2 mL) and stored in a tube containing EDTA as anticoagulant. The samples were placed in chests with ice until they arrived at the laboratory. Whole blood was aliquoted in RNase- and DNase-free microtubes at a volume of 200 μL and stored at -20°C. One aliquot of each sample was used for DNA extraction.

Ticks (larvae, nymphs and adults) were collected from the dogs and the environment. The specimens were placed in glass tubes closed with cotton and transported in an ice chest. The taxonomic identification of ticks was performed using the dichotomous keys of Aragão and Fonseca (1961) and Barros-Battesti et al. (2006). The *A. sculptum* nymphs were identified using the taxonomic key described by Martins et al. (2010). The larvae of *Amblyomma* sp. were identified only at the genus level.

2.5. DNA extraction and sample controls

DNA extraction from whole blood was performed with the PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's recommendations. All samples were quantified with a NanoDrop™ 2000 (Thermo Fisher Scientific) and aliquoted with a final concentration of 60 ng/μL. The positive and negative standard controls chosen according to a previously published study (Paulino et al., 2018). Ultrapure DNase- and RNase-free water was also used as a negative amplification control in the PCR.

2.6. Molecular detection of piroplasmid haemoparasites

The DNA samples extracted from blood were subjected to nested PCR (nPCR) as described by Jefferies et al. (2007) and modified by Inácio et al. (2019), targeting the 18S rRNA sequence for the haemoparasite detection of the order Piroplasmorida. The first and second PCR steps amplified products of 930 bp and 800 bp, respectively. The final volume of both reactions was 25 μL, containing PCR 1x buffer, 2.5 mM of $MgCl_2$, 0.4 mM of dNTPs, 0.5 μM of primers, 1.0 U of Taq polymerase, and 100 ng of total DNA. The second step was modified to include 2.5 mM $MgCl_2$ and 2 μL of the amplified product of the first step. The thermocycling conditions of the first nPCR step were 94°C for 30 s initially, followed by 45 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, before finishing at 72°C for 3 min. All the conditions were similar in the second step, except for the annealing temperature, which was 62°C for 30 s. The reactions were performed in a ProFlex thermal cycler (Applied Biosystems, Thermo Fisher Scientific). The PCR products were detected by agarose gel electrophoresis (2%) and ethidium bromide (0.4 mg/mL). The positive products were inspected under ultraviolet light with an E-Gel Imager Camera (Life Technologies – Thermo Fisher Scientific).

2.7. Detection limits

The detection limit was determined for both nPCR steps. According to the manufacturer's recommendations, the products of the first and second steps were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Next, each product was quantified using the fluorometric Qubit™ dsDNA BR Assay (Invitrogen™). The concentration obtained is suitable for estimating the number of copies. The following equation was used to determine the number of copies of each dilution point: Number of copies = $(6.02 \times 10^{23}$ (copies per mole) x DNA concentration (g) / (PCR amplicon size (base pairs) x 660 (g / mol / bp). In the sequence, serial decimal dilutions of 10^6 to 10^0 were used for nPCR reactions.

2.8. Purification of PCR products and sequencing

The positive nPCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer's recommendations, and sequenced using Sanger methods in an ABI 3730 DNA Analyser (Applied Biosystems/Perkin Elmer, CA, USA). The sequencing reactions were performed with the second primer pairs for nPCR.

2.9. 18S rRNA sequence and phylogenetic analysis

The obtained chromatogram was trimmed, and contigs were formed using sense and antisense sequences. The representative sequences obtained (n=33) were deposited in the GenBank under accession numbers MN823187 to MN823219. Sequence similarity was evaluated via an initial search using the BLAST tool, available at https://blast.ncbi.nlm.nih.gov/Blast.cgi. The evolutive distance was assessed using a distance matrix and calculated using the software MEGA X (Kumar et al., 2018). Sequences showing less than 100% identity were inserted in the phylogenetic analysis. The dataset was composed of 18S rRNA sequences of the Piroplasmorida order organisms, such as *Theileria* spp., *R. vitalii,* and *Cytauxzoon felis*. The sequence alignment was performed using the ClustalW algorithm of MEGA X (Kumar et al., 2018). Phylogenetic rebuilding was performed using the maximum likelihood method. The best model of nucleotide substitution was selected using jModelTest v2.1.9 (Posada and Crandall, 1998). The distance between pairs was calculated using the GTR + G + I nucleotide substitution model (Tamura et al., 2013). A bootstrap test with 1000 pseudo-replicates was used to analyse the groups. Phylogenetic analysis was performed using MEGA X (Kumar et al., 2018). The sequence "EU052685", codifying the species *Cardiosporidium cionae,* was used as

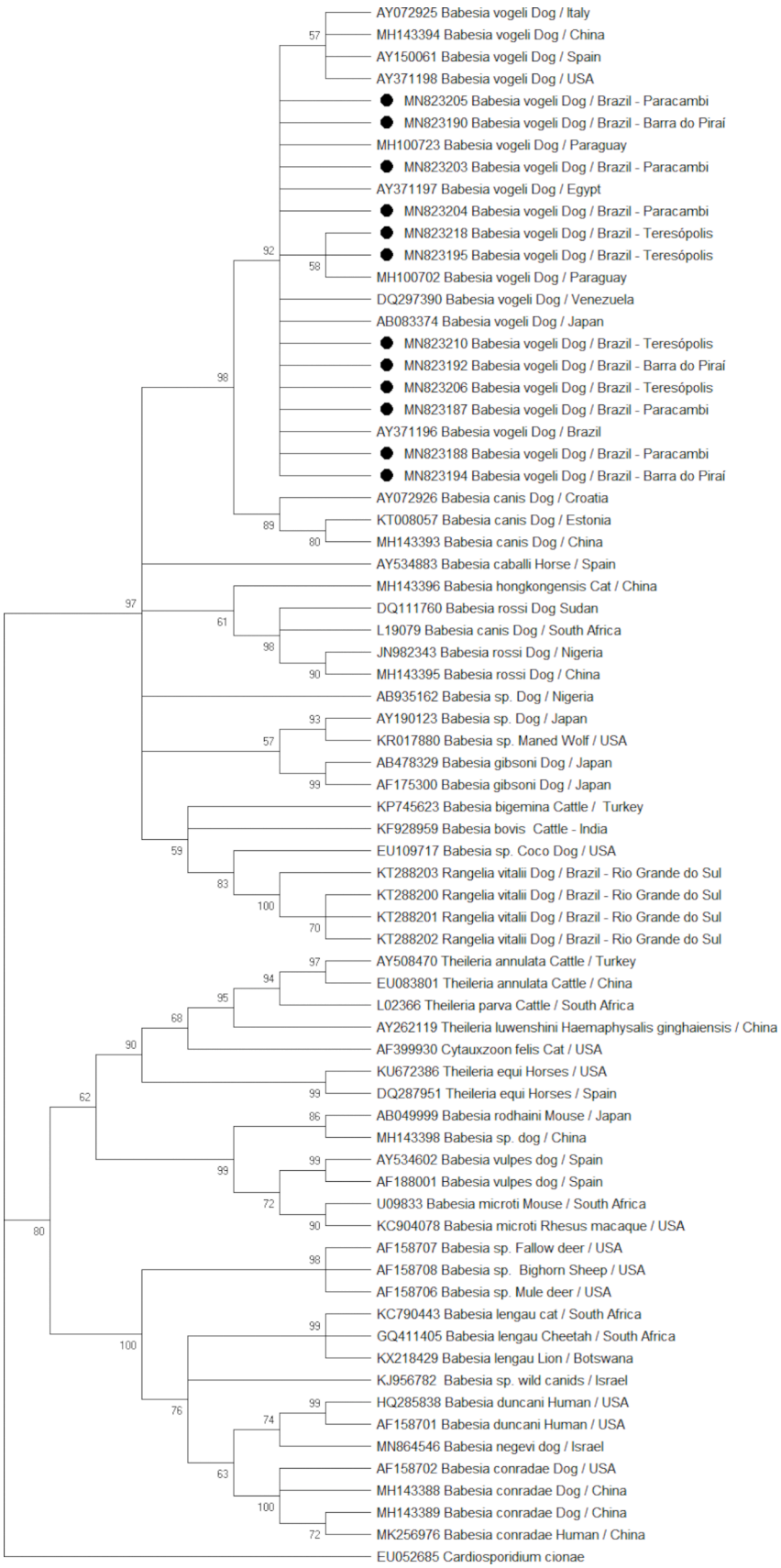

Fig. 2. Phylogenetic tree estimated by the maximum likelihood method from partial 18S rRNA sequences of *Babesia vogeli* and other species of the genera *Babesia* spp., *Rangelia* sp., *Theileria* sp., and *Cytauxzoon felis*. The numbers on the branches indicate the bootstrap value out of 1000 replicates. The sequence EU052685 of *Cardiosporidium cionae* was used as outgroup.

Table 1
Prevalence of *Babesia vogeli* in dogs and frequency of dogs infested by ticks in the municipalities of Paracambi, Barra do Pirai (low altitude) and Teresopolis, Petropolis (high altitude) in the state of Rio de Janeiro.

Municipality	Altitude	Number of Dogs	Prevalence *Babesia vogeli* (%)	Frequency of dogs infested by ticks		
				Rhipicephalus sanguineus s.l	*Amblyomma sculptum*	*Mixed infestation*
Barra do Pirai	<600 m	127	10.24	17.32% (22/127)	3.15% (4/127)	1.57% (2/127)
Paracambi	<600 m	122	11.47	17.21% (21/122)	4.92% (6/122)	1.64% (2/122)
Petropolis	>600 m	103	3.88	5.83% (6/103)	0.97% (1/103)	0
Teresopolis	>600 m	104	4.80	13.46% (14/104)	2.88% (3/104)	0

an outgroup.

The ClustalW algorithm was used to perform the alignment to determine the most variable region of *B. vogeli* 18S rRNA sequences available from the GenBank. Only sequences more extensive than 1600 bp were used. The most variable sites were determined by analysing the alignment's entropy using the BioEdit software version 7.0.9.0 (Hall, 1999). A second database focusing on the V4 region of 18S rRNA sequences available in the GenBank was used for genetic heterogeneity analysis. A total of 96 *B. vogeli* sequences were recovered in the GenBank database. The sequence alignment was performed using the ClustalW algorithm of MEGA X (Kumar et al., 2018). Phylogenetic analysis was performed as previously described.

2.10. Statistical analysis

A database containing exploratory variables was constructed to investigate the association with the dependent variable on positive *B. vogeli* detection and to verify the factors associated with positive results. Statistical analyses were performed using R in the RStudio environment (R Development Core Team, 2011). Each of the variables was independently evaluated. The chi-square test was used to assess significance at a threshold of 5% (Thrusfield, 2007). All qualitative variables were organised in dichotomous form. The logistic regression model was built by applying the Wald method with a 0.1 cutoff point. The model included variables with p-values of < 0.25. The collinearity was verified, according to Jessen and Menard (1996), assuming a tolerance value of $>$ 0.1 and a variance inflation factor (VIF) < 10. The accuracy of the final regression model was established using the Hosmer-Lemeshow test (p-value > 0.05).

2.11. Spatial analysis

Geographic information obtained by GPS was extracted from the semi-structured epidemiological questionnaires applied and tabulated in a CSV format file. Thematic maps were built using the Rio de Janeiro shapefile, available from the Brazilian Institute of Geography and Statistics (IBGE, 2019). The map comparisons and interpolation points were performed with the QGIS software version 3.16.0 (2019), following the Americas Geocentric Reference System (SIRGAS 2000, n.d.) previously defined by the IBGE. The value applied in the altitude map was graduated using the natural break classifier, according to Jenks (1997). The distribution of positive cases was determined using kernel interpolation (Worton, 1989). The existence of spatial autocorrelation between the prevalence of positive cases versus altitude and the positive cases versus presence of ticks was verified using the local indicators of spatial association (LISA) according to the local Moran index (Anselin, 1995), ranging from -1 to +1, and the pseudo-significance value (performed with 999 permutations at a 5% significance level) was executed using the GeoDa™ software 1.14 (2019).

2.12. Ethical considerations

The Animal Use Ethics Committee of Federal Rural University of Rio de Janeiro (CEUA/UFRRJ) approved the study design under number 3915240616.

3. Results

An overall piroplasmid prevalence of 7.9% (n = 36/456) was observed in the household dogs in the four municipalities of the state of Rio de Janeiro using PCR targeting of the 18S rRNA sequences. All 36 PCR products were sequenced. However, only 33 sequences were confirmed to be *B. vogeli* species (Fig. 2). The other three sequences were not considered for analysis due to the low quality of their chromatograms. The municipalities with the highest piroplasmid prevalence in dogs were Paracambi with 11.5% (n = 14/122) followed by Barra do Pirai with 10.2% (n = 13/127), which were the municipalities located in low altitude regions (< 600 m) (Table 1). In high-altitude municipalities (>600 m), the piroplasmid prevalences were 4.8% (n = 5/104) in Teresopolis and 3.9% (n = 4/103) in Petropolis (Table 1). *Rhipicephalus sanguineus* s.l. and *A. sculptum* were the species of ticks identified in household dogs in all four municipalities. *Rhipicephalus sanguineus* s.l. was identified in 13.8% (n = 63/456) of dogs, *A. sculptum* in 3.1% (n = 14/456) and mixed infestations were observed in 0.9% (n = 2/456) of dogs. The frequency of tick infestation in each municipality is shown in Table 1.

The 18S rRNA sequences showed a percentage of identity that ranged from 99.9 to 100% with *B. vogeli* isolates available in the GenBank (MN823187 to MN823219). Additionally, phylogenetic analysis showed that the sequences obtained in this study were grouped in the *B. vogeli* clade.

The univariate analysis showed that the environmental variables altitude and seasonality were associated with positive dogs. Low altitude (p = 0.007), rainy season (p = 0.003), animals without shelter (p = 0.014), the presence of ticks (p = 0.015), and tick species *R. sanguineus* s.l. (p = 0.003) displayed a significant association with dogs' positive for *B. vogeli*. The variables municipality, zone, temperature, number of dogs in the household, presence of other domestic or wild animals, vegetation in the household, access to different environments, *A. sculptum* presence, veterinary assistance, and hygiene conditions were not statistically significant. They were not considered to be associated with the dogs that were positive for *B. vogeli* (Table 2).

The dogs' intrinsic factors, including sex, age, breed, coat, body score, animal size, castration, and health status, were not associated with *B. vogeli* DNA presence in the animals (Table 3).

The following variables were subjected to logistic regression analysis: altitude, seasonality, presence of ticks, animal's sanitary condition, health status, presence of *R. sanguineus* s.l., body score, animal size, and presence of shelter. In the final model, the variables altitude, seasonality, and presence of *R. sanguineus* s.l. ticks more efficiently explained 72.6% of the positive *B. vogeli* cases (Table 4).

The p-value of the final model confirmed these factors' influence on the independent variable (*B. vogeli* DNA presence). The Hosmer-Lemeshow test results showed that the model had high accuracy of 91% ($X^2 = 0.55$, d.f.3, p-value 0.91). Household dogs in low altitude regions were 2.29 times more likely to be infected by *B. vogeli* (OR = 2.29, 95%, CI: 1.03-5.07). This result indicated that is more likely for a dog to be infected by these haemoparasites in Barra do Pirai and Paracambi than Teresopolis and Petropolis. In the rainy season, dogs were 2.45 times more likely to be infected with *B. vogeli* than in the dry season (OR= 2.45, 95%, CI: 1.20-5.01), which indicates that abiotic/climatic

Table 2
Descriptive and univariate analysis of environmental factors performed by Chi-square or Fisher's exact test in dogs naturally infected with *Babesia vogeli* in the municipalities of Paracambi, Barra do Pirai (low altitude) and Teresopolis, Petropolis (high altitude) in the State of Rio de Janeiro.

Environmental factors	Descriptive analysis				Univariate analysis			
	N	N+	N-	%	CI 95%	χ^2	P	OR
Municipalities								
Barra do Pirai	127	13	114	10.24%	-	6.75	0.81	
Paracambi	122	14	108	11.47%				-
Petropolis	103	4	99	3.88%				
Teresopolis	104	5	99	4.80%				
Altitude								
≥ 600 m	207	9	198	43.5%	1.23-5.83	6.56	0.007	2.68
< 600 m	249	27	222	10.84%				
Zone								
Urban	244	19	225	7.79%	0.49-1.91	0.008	0.53	0.97
Rural	212	17	195	8.02%				
Season								
Winter	142	8	134	5.63%	-	2.66	0.45	-
Autumn	125	10	115	8.00%				
Spring	82	6	76	7.32%				
Summer	107	12	95	11.21%				
Seasonality								
Dry	283	14	269	4.95%	1.39-5.63	8.91	0.003	2.80
Rainy	173	22	151	12.72%				
Temperature								
< 25°C	186	14	172	7.53%	0.54-2.19	0.06	0.48	1.09
≥ 25°C	270	22	248	8.15%				
Numbers of animals inside the house								
One	185	14	171	7.57%	0.54-2.17	0.05	0.49	1.08
More	270	22	248	8.15%				
Presence of domestic animals								
No	199	18	181	9.04%	0.38-1.50	0.64	0.26	0.76
Yes	257	18	239	7.53%				
Presence of wild animals								
No	377	31	346	8.22%	0.50-3.52	0.32	0.38	1.33
Yes	79	5	74	6.33%				
Presence of vegetation in house								
No	138	11	127	7.97%	0.47-2.06	0.002	0.55	0.98
Yes	318	25	293	7.86%				
Presence of shelter								
No	180	21	159	11.66%	1.15-4.59	0.014	5.82	2.30
Yes	276	15	261	5.43%				
Acess of urban ambience								
No	259	20	239	7.72%	0.53-2.10	0.02	0.50	1.06
Yes	197	16	181	8.12%				
Acess of pasture								
No	297	25	272	9.19%	0.39-1.69	0.32	0.36	0.81
Yes	159	11	148	6.92%				
Acess of stream								
No	308	25	283	8.12%	0.43-1.90	0.06	0.48	0.91
Yes	148	11	137	7.43%				
Acess of woods								
No	268	21	247	7.83%	0.51-2.03	0.003	0.54	0.54
Yes	188	15	173	7.98%				
Presence of tick								
No	343	21	322	6.12%	1.16-4.73	5.98	0.01	2.35
Yes	113	15	98	13.27%				
***Rhipicephalus sanguineus* tick infestation**								
No	393	24	369	6.50%	1.57-7.01	10.83	0.003	3.39
Yes	63	51	12	80.95%				
***Amblyomma sculptum* tick infestation**								
No	442	35	407	7.92%	0.11-7.04	0.01	0.70	0.89
Yes	14	1	13	7.14%				
Veterinary assistence								
No	228	20	208	8.77%	0.40-1.56	0.48	0.30	0.78
Yes	228	16	212	7.02%				
Hygienic conditions								
Satisfactory	305	25	280	8.19%	0.42-1.86	0.10	0.46	0.89
Insatisfactory	150	11	139	7.33%				

N: number of dogs tested by qPCR; N+: Number of positive dogs; N-: Number of negative dogs; %: Positive dogs percentual χ2: Chi-square test; P: p-value; CI: Confidence interval; OR: Odds Ratio

factors can contribute to *B. vogeli* infection. Furthermore, dogs infested by *R. sanguineus* s.l. were 2.47 times more likely to be infected by *B. vogeli* (OR: 2.47, 95%, CI: 1.14-5.38), confirming ectoparasite existence as a risk factor for *B. vogeli* positivity in dogs (Table 4).

In this study, entropy analysis was used to locate the hypervariable regions in the 18S rRNA of *B. vogeli* (> 1600 bp). The results revealed that the most variable region corresponded to the hypervariable V4 area (Supplementary data 2). In the *B. vogeli* 18S rRNA sequence, this region

Table 3
Descriptive and univariate analysis of intrinsic characteristics of dogs performed by Chi-square or Fisher's exact test in dogs naturally infected with *Babesia vogeli* in the municipalities of Paracambi, Barra do Pirai (low altitude) and Teresopolis, Petropolis (high altitude) in the State of Rio de Janeiro.

Intrinsic characteristics	Descriptive analysis				Univariate analysis			
	N	N+	N-	%	CI 95%	χ^2	p-valor	OR
Sex								
Female	233	18	215	7.7%	0.48-1.88	0.02	0.51	0.95
Male	223	18	205	8%				
Idade								
> 5 years old	178	13	165	7.30%	0.56-2.32	0.14	0.43	1.14
≤ 5 years old	278	23	255	8.27%				
Previous tick-borne diseases								
No	433	35	398	8.08%	0.07-3.95	0.42	0.44	0.52
Yes	23	1	22	4.35%				
Breed								
Mixed Race	305	26	279	8.52%	0.62-2.80	0.50	0.30	1.31
Pure	151	10	141	6.62%				
Coat								
Short	263	23	240	8.74%	0.37-1.53	0.62	0.27	0.75
Long	193	13	180	6.73%				
Body score								
Subnormal	91	10	81	10.98%	0.75-3.47	1.50	0.16	1.61
Normal	365	26	339	7.12%				
Size of animal								
<15 kg	314	28	286	8.91%	0.27-1.37	1.45	0.15	0.61
≥ 15 kg	142	8	134	5.63%				
Castration								
No	343	28	315	8.16%	0.52-2.64	0.14	0.44	1.17
Yes	113	8	105	7.07%				
Health status								
Asymptomatic	328	22	306	6.10%	0.84-3.45	2.27	0.10	1.71
Symptomatic	128	14	114	10.9%				

N: number of dogs tested by qPCR; N+: Number of positive dogs; N-: Number of negative dogs; %: Positive dogs percentual χ2: Chi-square test; P: p-value; CI: Confidence interval; OR: Odds Ratio

Table 4
Multivariate logistic regression of factors associated with *Babesia vogeli* infection in household dogs from municipalities of Paracambi, Barra do Pirai (low altitude) and Teresopolis, Petropolis (high altitude) in the State of Rio de Janeiro.

Variable	B	S.E	Wald	Df	Sig	OR	CI (95%)
Altitude							
≥ 600 m or < 600 m	0.83	0.41	4.14	1	0.04	2.29	1.03-5.07
Seasonality							
Dry or rainy	0.90	0.36	6.06	1	0.01	2.45	1.20-5.01
***Rhipicephalus sanguineus* s.l. tick infestation**							
No or Yes	0.91	0.40	5.22	1	0.02	2.47	1.14-5.38

B: estimates of equation parameters; SE: standard error; Wald: Wald statistic; df: degrees of freedom; Sig: significance of Wald statistic; OR: Odds Ratio; CI: confidence interval 95% for OR.

is located between nucleotides 573 and 630. *Babesia vogeli* 18S rRNA sequences containing the hypervariable V4 region available in the GenBank were used for phylogenetic analysis (Supplementary data 3).

Genetic homogeneity was observed among 18S rRNA sequences by scanning the evolutionary distance matrix among the 33 sequences obtained in this study, with distance values ranging from 0 to 0.007 and a mean value of 0.001 (Supplementary data 3). The evolutionary distance of the 18S rRNA sequences of naturally infected dogs was higher in Teresopolis (mean value of 0.0045), followed by Barra do Pirai (0.0014) and Paracambi (0.0009). All sequences obtained from dogs in Petropolis were identical. The evolutionary distance (0.003) was greater between the sequences from Barra do Pirai (low altitude) and Teresopolis (high altitude). The sequences which were not 100% similar to *B. vogeli* clustered in a large clade, including other piroplasmids from South America, North America, Europe, Africa, and Asia (Fig. 2).

Phylogenetic analysis of *B. vogeli* 18S rRNA sequences demonstrated no segregation due to the geographic location of the sequences obtained in this study compared to isolates from other parts of the world (Supplementary data 4).

Regarding the spatial analysis, the kernel interpolation highlighted the concentration of positive cases in the low altitude cities (< 600 m) (Fig. 3 - A). The Local Spatial Autocorrelation Moran Index of positive cases and altitude resulted in a Moran's I value of -0.605 and assigned the presence of higher spatial association cases in low regions (> 600 m), with a pseudo-p-value of 0.001 (Fig. 3 – B). The spatial autocorrelation of positive cases with tick presence showed a Moran's I value of -0.309 with a p-value 0.26, demonstrating low association (Fig. 3 - C). However, the cluster map revealed a spatial association between ticks and low altitude (Moran's I value: 0.222 with p-value 0.046) (Fig. 3 - D).

4. Discussion

This study found that the prevalence of *B. vogeli* in dogs was 7.9% in some areas of the state of Rio de Janeiro, which is higher than the prevalence rates observed in other parts of South America, such as Paraguay (5.5%) (Inácio et al., 2019), Argentina (7.7%) (Mascarelli et al., 2015), and Colombia (5.4%) (Vargas-Hernández et al., 2012). In the current research, both statistics and spatial analysis indicated that low altitude regions (< 600 m) were associated with *B. vogeli* infection. A spatial association was observed between lower altitudes and the presence of ticks in positive cases. This link has been described in studies by Gilbert (2010) and Tsatsaris et al. (2016), who reported that lower altitude regions had more tick specimens than higher ones. Moreover, the hosts could be accompanied by more tick infestations within an environment with more ticks, as noted in this study.

Regarding the statistical association of positivity with seasonality, the rainy period was more associated with positive cases, corroborating the results reported by Costa-Júnior et al. (2009). They observed a decrease in *B. vogeli* prevalence in the Brazilian state of Minas Gerais during the dry period. The warmer climate (Zemtsova et al., 2016) and the rainy period at low altitudes provide favourable environments for tick proliferation (Gilbert, 2010) because both humidity and temperature contribute to tick reproduction, especially regarding the incubation

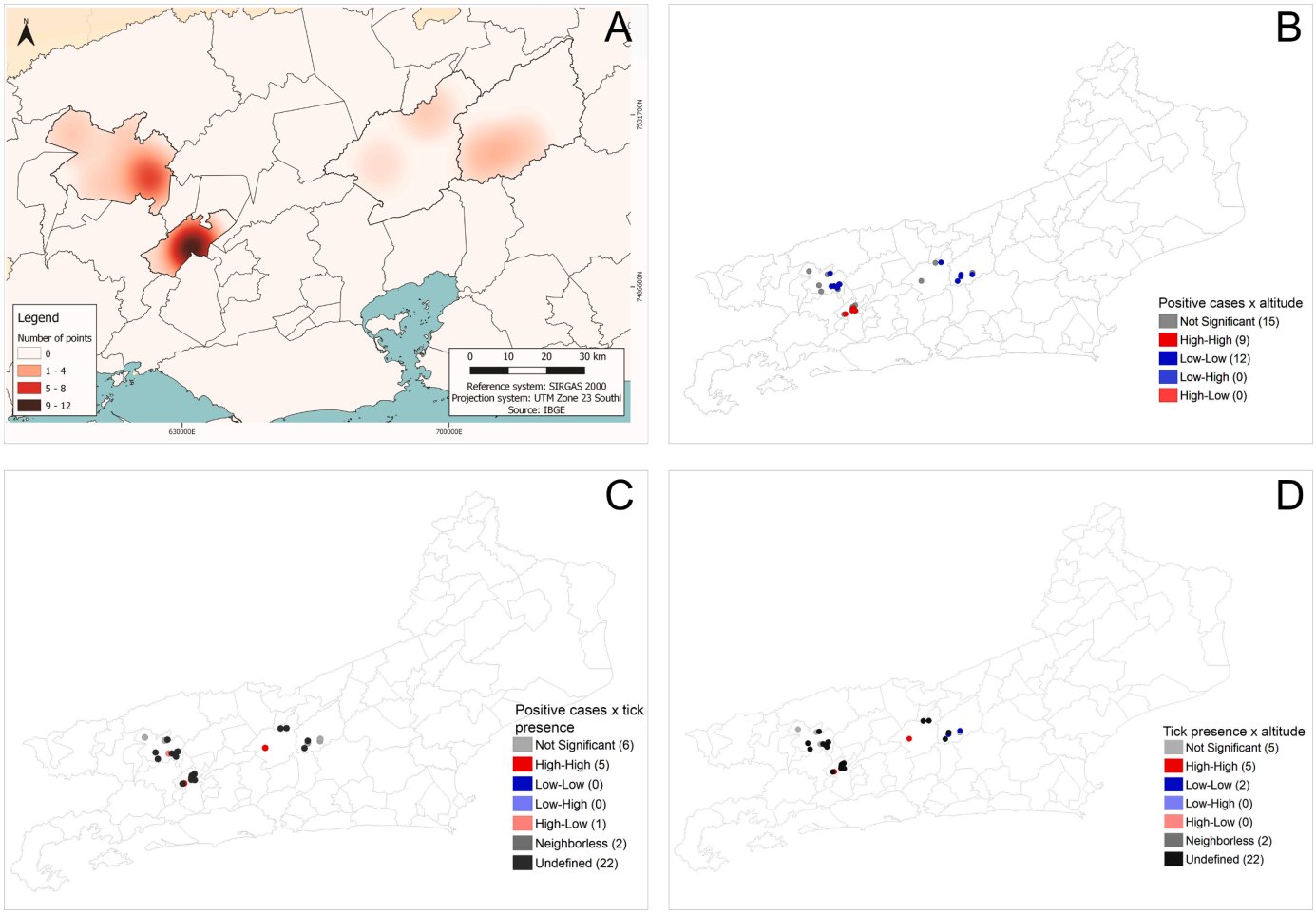

Fig. 3. A - Kernel maps show the concentration of positive *Babesia vogeli* cases in Rio de Janeiro State's low altitude region. B - Cluster map of spatial correlation of positive *Babesia vogeli* cases and altitude. The plot is divided into four quadrants according to the four scenarios that emerged from the LISA clustering. The red points show high spatial correlation with positive cases and altitude, and the blue points show lower correlation with both variables. The other colours did not show spatial correlation. C - Cluster map of spatial correlation of positive *Babesia vogeli* cases and the presence of ticks. The plot is divided into four quadrants according to the four scenarios that emerged from the LISA clustering. The red points show high spatial correlation with positive cases and the presence of ticks, and the rose point shows high spatial correlation with positive cases but lower with tick presence. The other colours did not exhibit spatial correlation. D - Cluster map of spatial correlation of positive cases of presence of tick and altitude. The plot is divided into four quadrants based on the four scenarios that emerged from the LISA clustering. The red points show high spatial correlation with ticks and altitude, and the blue points show low correlation with both variables. The other colours did not show spatial correlation.

of eggs in the soil (Sonenshine, 1993).

The absence of shelter was also a factor influencing tick infections, especially in relation to *R. sanguineus* s.l., based on the strong statistical association with *B. vogeli* in dogs in this study. This effect is related to the animal being exposed to more significant environmental adversities and not having a fixed place to rest. Previous studies have also found this association (Mrljak et al., 2017; Paulino et al., 2018; Santos et al., 2013).

Since abiotic factors influenced tick behaviour, the existence of more ticks increased the potential for infections. In this study, there was a statistical association with abiotic factors, such as altitude and rainy season, associated with *B. vogeli* positivity. This connection is in line with previous studies (Passos et al., 2005; Paulino et al., 2018), who verified that the transmission potential increases with the vector's presence. Paulino et al. (2018) found *A. sculptum* and *R. sanguineus* s.l. on the animals. *Amblyomma sculptum* has low parasitic specificity and can parasitise innumerable wild and domestic species. However, horses are its preferred domestic host (Barros-Battestti et al., 2006). *Amblyomma sculptum* specimens were only found parasitising dogs in a few households where horses were also present, which explains this tick species' low frequency in the current study. However, only *R. sanguineus* s.l. was epidemiologically associated (OR = 3.32; p 0.003; CI: 1.57. 7.01) with the presence of *B. vogeli* in dogs. The result mentioned above is also in agreement with the previous knowledge that *R. sanguineus* s.l. is considered the main vector of *B. vogeli* in Brazil (Passos et al., 2005).

In the logistic regression, the *B. vogeli* transmission factors were directly related to the vector's presence. Dogs from the low altitude region were 2.29 times more likely to be infected by the pathogenic agent during the rainy period, which increased the chance of infection occurrence 2.45 times. Both variables are directly related to the vector's occurrence, which also showed that dogs infested by *R. sanguineus* s.l. were 2.47 times more likely to be infected by *B. vogeli*.

Regarding the genetic variability, entropy analysis showed that the corresponding region V4 was more heterogeneous between *B. vogeli* 18S rRNA sequences. In this study, no phylogeographic variant was observed in *B. vogeli* 18S rRNA sequences. Furthermore, this homogeneity in *B. vogeli* has been perceived in other parts of the world in other studies (Barbosa et al., 2020; Costa-Júnior et al., 2009; Inácio et al., 2019; Passos et al., 2005). Moreover, molecular analysis of the 18S rRNA of *B. vogeli* facilitated the observation of a low rate of genetic divergence over the years and can be considered to be genetically stabilised.

Concerning the spatial analysis, the use of georeferencing enhances epidemiological study results, including for zoonotic (Bouchard et al.,

2018) and exclusively tick-borne diseases (Mrljak et al., 2017; Silveira et al., 2009; Torina et al., 2018). Nevertheless, the selection of the correct tool is essential. In this study, two geographically distant regions were targeted. In this case, single point maps can present overlapping points when there is a concentration of positive cases in a small area due to the distance between them. Zhang et al. (2019) observed this situation when analysing the distribution of tick species using only point maps, where some points were underneath others. If kernel interpolation had been applied, the distribution would be better observed, as we did in this study. Moreover, the geospatial tools allow the enlargement of ticks' ecological dynamics in different environments, as demonstrated by Tsatsaris et al. (2016). Furthermore, spatial analysis tools in tick disease studies, including *B. vogeli*, can enhance preventive programs with more engagement of the dynamics of diseases, vector, and environment.

On the other hand, Qviller et al. (2016), who also developed a spatial analysis of tick distribution, applied interpolation of the distribution using kernel mapping and could verify the tick distribution within each 20 m^2. Here, our kernel map categorisation allowed us to observe the concentration of nine to 12 cases in Paracambi, a region with altitudes below 200 m, as observed in the altitude map in Fig. 2. As the height increased, the concentration of cases decreased considerably.

Since the two municipalities at lower altitudes showed a higher concentration of positive *B. vogeli* cases than those at high altitudes, it was necessary to explore the spatial data obtained to confirm the relationship between both variables. Gilbelt (2010) reported a tendency for a higher number of tick specimens in hot and low-lying regions. This was also observed in the present study, where the low altitude areas had a higher number of tick specimens than the high areas (Table 2). Although high conservancy of *B. vogeli* 18S rRNA sequences was observed, the lack of phylogeographic variations could be due to the low number of sequences analysed. Spatial autocorrelation was determined using the local Moran index to understand the spatial distribution pattern of positive dogs for *B. vogeli* in relation to altitude and tick presence. A high spatial correlation between altitude and positive cases was observed, supporting the association discussed here. In the case of positive dogs without spatial correlation with ticks, this is most likely because many dog owners had used tick control methods. In this case, the *B. vogeli* infection may have been introduced by ticks before the interview day.

5. Conclusion

The occurrence of *B. vogeli* is linked to the presence of *R. sanguineus* s. l. and the environmental factors studied, including the rainy season and low altitude regions (below 600 m). These factors provide favourable conditions for the vector's proliferation and maintenance, consequently favouring infections by *B. vogeli* in household dogs in these regions. This study determined that the most variable area in the *B. vogeli* 18S rRNA sequences is the V4 region. Although the variable region V4 of the 18S rRNA sequences is useful for genetic variability studies, it is insufficient to analyse phylogeographic variations of *B. vogeli*. The search for molecular markers that are useful for this purpose should be performed. Several studies have already shown that environmental changes resulting from climate change can favour arthropod vectors' proliferation. Therefore, it is necessary to improve research into climate change's influence on the epidemiology of tick-borne diseases, particularly diseases that affect dogs.

Funding

This study received financial support from the National Council for Scientific and Technological Development (CNPq) and the 'Carlos Chagas Filho' Foundation for Research Support of the State of Rio de Janeiro (FAPERJ).

CRediT authorship contribution statement

Tays Araujo Camilo: Writing – original draft, Investigation, Software, Data curation. **Leo Paulis Mendonça:** Investigation, Data curation. **Débora Martins dos Santos:** Investigation. **Lúcia Herran Ramirez:** Formal analysis, Writing – review & editing. **Nathalia Alves Senne:** Writing – review & editing. **Patrícia Gonzaga Paulino:** Formal analysis, Writing – review & editing. **Priscila Almeida Oliveira:** Software, Formal analysis, Writing – review & editing. **Maristela Peckle Peixoto:** Software, Formal analysis, Writing – review & editing. **Carlos Luiz Massard:** Funding acquisition, Supervision. **Isabele da Costa Angelo:** Supervision, Writing – review & editing. **Huarrisson Azevedo Santos:** Funding acquisition, Supervision, Writing – review & editing, Project administration.

Declaration of Competing Interests

The authors declare no conflicts of interest concerning the research, authorship, or publication of this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2021.101785.

References

Anselin, L., 1995. Local Indicators of Spatial Association-LISA. Geogr. Anal. 27, 93–115. https://doi.org/10.1111/j.1538-4632.1995.tb00338.x.

Aragão, H., Fonseca, F.da, 1961. Notas de ixodologia: VIII. Lista e chave para os representantes da fauna ixodológica brasileira: notas de ixolodologia. Mem. Inst. Oswaldo Cruz. https://doi.org/10.1590/s0074-02761961000200001.

Araujo, A.C., Silveira, J.A.G., Azevedo, S.S., Nieri-Bastos, F.A., Ribeiro, M.F.B., Labruna, M.B., Horta, M.C., 2015. *Babesia canis vogeli* infection in dogs and ticks in the semiarid region of Pernambuco. Brazil. Pesqui. Vet. Bras. 35, 456–461. https://doi.org/10.1590/S0100-736x2015000500012.

Barbosa, C.O.S., Garcia, J.R., de Melo Nasser Fava, N., Pereira, D.A., da Cunha, M.J.R., Nachum-Biala, Y., Cury, M.C., Baneth, G., 2020. Babesiosis caused by *Babesia vogeli* in dogs from Uberlândia State of Minas Gerais. Brazil. Parasitol. Res. 119, 1173–1176. https://doi.org/10.1007/s00436-019-06515-3.

Barros-Battesti, D.M., Arzua, M., Bechara, G.H., 2006. Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies. São Paulo, Vox/ICTTD-3/Butantan, 223 p. https://repositorio.butantan.gov.br/handle/butantan/3153.

Bouchard, C., Aenishaenslin, C., Rees, E.E., Koffi, J.K., Pelcat, Y., Ripoche, M., Milord, F., Lindsay, L.R., Ogden, N.H., Leighton, P.A., 2018. Integrated social-behavioral and ecological risk map to prioritise local public health responses to Lyme disease. Environ. Health Perspect. 18, 126. https://doi.org/10.1289/EHP1943.

Costa-Júnior, L.M., Ribeiro, M.F.B., Rembeck, K., Rabelo, E.M.L., Zahler-Rinder, M., Hirzmann, J., Pfister, K., Passos, L.M.F., 2009. Canine babesiosis caused by *Babesia canis vogeli* in rural areas of the State of Minas Gerais, Brazil and factors associated with its seroprevalence. Res. Vet. Sci. 86, 257–260. https://doi.org/10.1016/j.rvsc.2008.07.002.

Criado-Fornelio, a., Martinez-Marcos, a., Buling-Saraña, a., Barba-Carretero, J.C., 2003. Molecular studies on *Babesia, Theileria* and *Hepatozoon* in southern Europe: Part I. Epizootiological aspects. Vet. Parasitol. 113, 189–201. https://doi.org/10.1016/S0304-4017(03)00078-5.

Dell'Porto, A., Oliveira, M.R.de, Miguel, O., 1990. *Babesia canis* em cães de rua da Cidade de São Paulo. I. Estudo comparativo de métodos de diagnóstico. Brazilian J. Vet. Res. Anim. Sci. 27, 41–45. https://doi.org/10.11606/issn.0000-0000.27141-45.

França, R.T., Da Silva, A.S., Loretti, A.P., Mazzanti, C.M., Lopes, S.T.A., 2014. Canine rangeliosis due to *Rangelia vitalii*: From first report in Brazil in 1910 to current day - A review. Ticks Tick. Borne. 5, 466–474. https://doi.org/10.1016/j.ttbdis.2014.04.005. Dis.

Gilbert, L., 2010. Altitudinal patterns of tick and host abundance: A potential role for climate change in regulating tick-borne diseases? Oecologia 162, 217–225. https://doi.org/10.1007/s00442-009-1430-x.

IBGE, 2019. Base Cartográfica Nacional IBGE – Escala 1250 mil. URL https://portaldemapas.ibge.gov.br/portal.php#mapa222602.

Inácio, E.L., Pérez-Macchi, S., Alabi, A., Bittencourt, P., Müller, A., 2019. Prevalence and molecular characterisation of piroplasmids in domestic dogs from Paraguay. Ticks Tick. Borne. Dis. 10, 321–327. https://doi.org/10.1016/j.ttbdis.2018.11.009.

Jefferies, R., Ryan, U.M., Irwin, P.J., 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. Vet. Parasitol. 144, 20–27. https://doi.org/10.1016/j.vetpar.2006.09.022.

Jessen, H.C., Menard, S., 1996. Applied Logistic Regression Analysis. Stat 45, 534. https://doi.org/10.2307/2988559.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Laflamme, D., 1997. Development and Validation of a Body Condition Score System for Dogs. Canine Pract. 2, 10–15.

Martins, T.F., Onofrio, V.C., Barros-Battesti, D.M., Labruna, M.B., 2010. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. Ticks Tick. Borne. Dis. 1, 75–99. https://doi.org/10.1016/j.ttbdis.2010.03.002.

Mascarelli, P.E., Elmore, S.A., Jenkins, E.J., Alisauskas, R.T., Walsh, M., Breitschwerdt, E. B., Maggi, R.G., 2015. Vector-borne pathogens in arctic foxes, *Vulpes lagopus*, from Canada. Res. Vet. Sci. 99, 59. https://doi.org/10.1016/j.rvsc.2014.12.011.

Matjila, P.T., Leisewitz, A.L., Oosthuizen, M.C., Jongejan, F., Penzhorn, B.L., 2008. Detection of a *Theileria* species in dogs in South Africa. Vet. Parasitol. 157, 34–40. https://doi.org/10.1016/j.vetpar.2008.06.025.

Mrljak, V., Kuleš, J., Mihaljević, Z., Torti, M., Gotić, J., Crnogaj, M., Zivičnjak, T., Mayer, I., Šmit, I., Bhide, M., Barić Rafaj, R., 2017. Prevalence and geographic distribution of vector-borne pathogens in apparently healthy dogs in Croatia. Vector-Borne Zoonotic Dis. 17, 398–408. https://doi.org/10.1089/vbz.2016.1990.

Passos, L.M.F., Geiger, S.M., Ribeiro, M.F.B., Pfister, K., Zahler-Rinder, M., 2005. First molecular detection of *Babesia vogeli* in dogs from Brazil. Vet. Parasitol. 127, 81–85. https://doi.org/10.1016/j.vetpar.2004.07.028.

Paulino, P.G., Pires, M.S., da Silva, C.B., Peckle, M., da Costa, R.L., Vitari, G.L.V., de Abreu, A.P.M., Massard, C.L., Santos, H.A., 2018. Molecular epidemiology of *Babesia vogeli* in dogs from the southeastern region of Rio de Janeiro, Brazil. Vet. Parasitol. Reg. Stud. Reports. 13, 160–165. https://doi.org/10.1016/j.vprsr.2018.06.004.

Posada, D., Crandall, K.A., 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14, 817–818. https://doi.org/10.1093/bioinformatics/14.9.817.

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Rotondano, T.E., de, F., Almeida, H.K.A., Krawczak, F., da, S., Santana, V.L., Vidal, I.F., Labruna, M.B., Azevedo, S.S.de, Almeida, A.M.P.de, Melo, M.A.de, 2015. Survey of *Ehrlichia canis, Babesia* spp. and *Hepatozoon* spp. in dogs from a semiarid region of Brazil. Rev. Bras. Parasitol. Veterinária. 24, 52–58. https://doi.org/10.1590/s1984-29612015011.

Santos, H.A., Thomé, S.M.G., Baldani, C.D., Silva, C.B., Peixoto, M.P., Pires, M.S., Vitari, G.L.V., Costa, R.L., Santos, T.M., Angelo, I.C., Santos, L.A., Faccini, J.L.H., Massard, CL, 2013. Molecular epidemiology of the emerging zoonosis agent *Anaplasma phagocytophilum* (Foggie, 1949) in dogs and ixodid ticks in Brazil. Parasites and Vectors. 6, 348. https://doi.org/10.1186/1756-3305-6-348.

Silva, B.R.F., Labruna, M.B., Marcili, A., Dos Santos, C.R., Bastos, B.B.B., Bordin, J.T., Moraes-Filho, J., 2019. *Rangelia vitalii* infection in a dog from São Paulo city, Brazil: Case report. Brazilian J. Vet. Res. Anim. Sci. 56, e150791 https://doi.org/10.11606/issn.1678-4456.bjvras.2019.150791.

Silveira, J.A.G., Passos, L.M.F., Ribeiro, M.F.B., 2009. Population dynamics of *Rhipicephalus sanguineus* (Latreille, 1806) in Belo Horizonte, Minas Gerais state, Brazil. Vet. Parasitol. 161, 270–275. https://doi.org/10.1016/j.vetpar.2009.01.028.

SIRGAS 2000, n.d. Sistema Referenceamento Geocéntrico para las Américas. URL http://www.sirgas.org/pt/sirgas-realizations/sirgas2000/.

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A., Miró, G., 2016. A review of canine babesiosis: The European perspective. Parasit Vectors 9, 336. https://doi.org/10.1186/s13071-016-1596-0.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197.

Thrusfield, M., 2007. Veterinary epidemiology, 3rd Edition. Blackwell Science Ltd, Oxford.

Torina, A., Blanda, V., Blanda, M., Auteri, M., Russa, F.La, Scimeca, S., D'Agostino, R., Disclafani, R., Villari, S., Currò, V., Caracappa, S., 2018. A geographical information system-based approach for integrated strategies of tick surveillance and control in the peri-urban natural reserve of Monte Pellegrino (Palermo, Southern Italy). Int. J. Environ. Res. Public Health. 15, 404. https://doi.org/10.3390/ijerph15030404.

Trapp, S.M., Dagnone, A.S., Vidotto, O., Freire, R.L., Amude, A.M., de Morais, H.S.A., 2006. Seroepidemiology of canine babesiosis and ehrlichiosis in a hospital population. Vet. Parasitol. 140, 223–230. https://doi.org/10.1016/j.vetpar.2006.03.030.

Tsatsaris, A., Chochlakis, D., Papadopoulos, B., Petsa, A., Georgalis, L., Angelakis, E., Ioannou, I., Tselentis, Y., Psaroulaki, A., 2016. Species composition, distribution, ecological preference and host association of ticks in Cyprus. Exp. Appl. Acarol. 70, 523–542. https://doi.org/10.1007/s10493-016-0091-9.

Vargas-Hernández, G., André, M.R., Faria, J.L.M., Munhoz, T.D., Hernandez-Rodriguez, M., Machado, R.Z., Tinucci-Costa, M., 2012. Molecular and serological detection of *Ehrlichia canis* and *Babesia vogeli* in dogs in Colombia. Vet. Parasitol. 186, 254–260. https://doi.org/10.1016/j.vetpar.2011.11.011.

Worton, B.J., 1989. Kernel Methods for Estimating the Utilisation Distribution in Home-Range Studies. Ecology 70, 164–168. https://doi.org/10.2307/1938423.

Zemtsova, G.E., Apanaskevich, D.A., Reeves, W.K., Hahn, M., Snellgrove, A., Levin, M.L., 2016. Phylogeography of *Rhipicephalus sanguineus* sensu lato and its relationships with climatic factors. Exp. Appl. Acarol. 69, 191–203. https://doi.org/10.1007/s10493-016-0035-4.

Zhang, G., Zheng, D., Tian, Y., Li, S., 2019. A dataset of distribution and diversity of ticks in China. Sci. Data. 6, 105. https://doi.org/10.1038/s41597-019-0115-5.