


Rickettsial pathogens circulating in urban districts of Rio de Janeiro, without report of human Brazilian Spotted Fever

Circulação de patógenos riquetsiais em bairros urbanos do Rio de Janeiro sem relatos humanos da Febre Maculosa Brasileira

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Abstract

Spotted fever group rickettsioses are emerging diseases. In some of these diseases, domestic dogs act as sentinels. Canine serological studies have demonstrated that rickettsial dispersion is concentrated in rural areas, seroprevalence being higher where human rickettsioses are endemic. In Rio de Janeiro, the Atlantic forest vegetation has been devastated by urbanization. In this context, we aimed to detect *Rickettsia* spp. in urban areas of the West Zone of Rio de Janeiro. Sera from 130 dogs were tested by Indirect Immunofluorescence Assay, and ticks collected from these dogs were tested by polymerase chain reaction. We found the rate of serological reactions against *R. rickettsii* and *R. parkeri* in our study area to exceed those of rural and non-endemic areas, highlighting the importance of dogs as urban sentinels. The possibility of contact with opossums and capybaras increased the chances of exposure to *Rickettsia* spp., reinforcing the hypothetical link between the landscape and the rickettsial wild cycle. *Rhipicephalus sanguineus sensu lato* was the tick most frequently observed. PCR-positive samples showed similarity with *R. rickettsii* and *R. felis*, an emerging pathogen rarely reported from ticks. We observed that rickettsiae circulate in urban places and ticks from indoor environments, which may be involved in bacterial epidemiology.

Keywords: Rickettsioses, emerging infectious diseases, rainforest biome, *Rhipicephalus sanguineus*, Indirect Immunofluorescence Assay, polymerase chain reaction.

Resumo

Riquetsioses do Grupo da Febre Maculosa são doenças emergentes. Em algumas destas doenças, os cães domésticos agem como sentinelas. Estudos sorológicos caninos têm demonstrado que a dispersão de patógenos riquetsiais está concentrada em áreas rurais, sendo a soroprevalência maior onde as riquetsioses humanas são endêmicas. Na cidade do Rio de Janeiro, a vegetação de Mata Atlântica vem sendo devastada pela urbanização. Nesse contexto, objetivou-se detectar a presença de *Rickettsia* spp. em áreas urbanas da Zona Oeste do Rio de Janeiro. Amostras de soro obtidas de 130 cães foram testadas, utilizando-se a Imunofluorescência Indireta. Carrapatos coletados desses cães foram testados, utilizando-se a reação em cadeia da polimerase. Observou-se que as taxas de reações sorológicas contra *R. rickettsii* e *R. parkeri* nessa área de estudo excederam a prevalência das áreas rurais e não endêmicas, destacando-se a importância dos cães como sentinelas urbanos das riquetsioses. A possibilidade de contato com capivaras e gambás favoreceu a exposição à *Rickettsia* spp., reforçando a hipótese de ligação entre a paisagem local e o ciclo silvestre de transmissão riquetsial. O carrapato *Rhipicephalus sanguineus*

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sensu lato foi encontrado com maior frequência. Amostras com positividade pela PCR mostraram similaridade com *R. rickettsii* e *R. felis*, um patógeno emergente raramente descrito em carrapatos. Observou-se circulação riquetsial em áreas urbanas e em carrapatos obtidos do ambiente doméstico, os quais podem estar envolvidos na epidemiologia dessas bactérias.

Palavras-chave: Riquetsioses, doenças infecciosas emergentes, bioma de Mata Atlântica, *Rhipicephalus sanguineus*, Imunofluorescência Indireta, reação em cadeia da polimerase.

Introduction

Spotted fever group (SFG) rickettsioses are emerging and reemerging tick-borne zoonotic diseases (Fang et al., 2017). *Rickettsia rickettsii* is known as the etiological agent of Brazilian Spotted Fever (BSF), a multi-systemic acute disease with a high lethality rate endemic to Brazil's southeastern region (Oliveira et al., 2016). *Rickettsia rickettsii* transmission vectors are mostly ticks of the genus *Amblyomma*, such as *A. sculptum* (previously *A. cajennense*) and *A. aureolatum*, which can parasitize humans and are prevalent in rural areas (Labruna et al., 2001; Parola et al., 2013; Szabó et al., 2013). *Rhipicephalus sanguineus*, which is present throughout Brazilian biomes, has been associated with *R. rickettsii* human epidemics in the USA and Mexico (Demma et al., 2005; Tinoco-Gracia et al., 2018). In Brazil, its vectorial competence has not yet been definitively proven, although supporting evidence exists (Cunha et al., 2009).

Dogs that live close to both humans and the ticks that act as vectors of BSF are useful sentinels of the disease (Cardoso et al., 2006; Cunha et al., 2014; Poubel et al., 2018). Therefore, to improve the monitoring and to combat of rickettsioses, it is helpful to seek evidence of prior exposure in such dogs to SFG agents by using Indirect Immunofluorescence Assay (IFA) (Campos et al., 2016). Serological studies in dogs show that rickettsial dispersions in Rio de Janeiro State are concentrated in rural areas of the southern or northwestern regions, where human BSF has been reported (Gazeta et al., 2009; Cunha et al., 2014; Poubel et al., 2018). Few studies have been conducted in other regions of Rio de Janeiro, especially the metropolitan area (Cordeiro et al., 2015).

Lowlands and rocky hills, partly covered by an Atlantic forest vegetation, shape the landscape surrounding the municipality of Rio de Janeiro, divided into 33 districts (IBGE, 2012). This biome has been heavily impacted by colonization and land occupation in recent times (Herzog & Finotti, 2013). After 1960, expansion and urbanization programs led to the opening of a highway system in the West Zone of Rio de Janeiro municipality, located between the Pedra Branca massif and the Jacarepaguá watershed. Residential and commercial construction subsequently spread rapidly along with the green spaces (Rabha, 2010). Given this recent urbanization profile, this study aimed to assess *Rickettsia* spp. among dogs and the ticks they carry in districts without notification of human BSF and located close to the Pedra Branca massif, which houses one of the world's largest urban forests.

Materials and Methods

Ethical statement

This study was approved by the Ethics Committee on Animal Research of the Universidade Federal Fluminense (CEUA 633/2015). At the time of sampling, the dog owners provided signed informed consent authorizing the collection of animal data and biological samples.

Study areas and collection of samples

This study was conducted in two districts of the West Zone of the municipality of Rio de Janeiro, located near the Pedra Branca State Park, including the regions of Vargem (Vargem Grande and Vargem Pequena) and Guaratiba. Pedra Branca State Park, located in the massif of the same name, was established in 1974, covers an area of 12,500 hectares in the city of Rio de Janeiro, and is considered the most significant urban forest in Brazil (Herzog & Finotti, 2013). Harboring fauna and flora species native to the Atlantic forest biome, as well as waterfalls, river springs, and water reservoirs, this park encompasses a famous forest formation in an urban area (Costa et al., 2009; Herzog & Finotti, 2013; Iwama et al., 2014).

The households selected for participation in this study maintained contact with flowing water bodies, riparian vegetation, and forested areas. The dog owners reported horses, capybaras, and opossums identified in the vicinity of the dwellings. We visited each home one time and sampled every dog we encountered regardless of age and sex in a cross-sectional study between June 2014 and February 2015.

The collected blood was stored in anticoagulant-free tubes and later examined using IFA. At the time of sampling, all dogs were inspected for the presence of ticks collected via handpicking and stored in sterile 1.5 mL tubes with 70% ethanol at room temperature. The number of ticks collected per animal was not constant. Ticks were identified according to taxonomic keys (Barros-Battesti et al., 2006; Martins et al., 2010). The specimens initially identified as *A. cajennense* were later classified as *A. sculptum*, according to the reassessment and reinstatement of *A. cajennense* (Nava et al., 2014). After identification, ticks were ground under liquid nitrogen with a sterile pestle in preparation for DNA extraction and polymerase chain reaction (PCR) assays.

Indirect Immunofluorescence Assay (IFA)

Serum was extracted from the blood by centrifugation and examined using IFA on slides prepared with *R. rickettsii* str. Taiaçu and *R. parkeri* str. At24 antigens (Pinter & Labruna, 2006; Silveira et al., 2007). Sera were diluted to 1:64 in phosphate buffered saline (PBS), and IFA was conducted as described by Horta et al. (2004). Orifices showing generally uniform and coccoid, bacillary, or coccobacillary-shaped fluorescent spots at 1:64 titer were considered positive. Samples that have been previously demonstrated to be reactive and non-reactive were used as controls on each slide (Campos et al., 2017). Positive sera were diluted until negative results were obtained to determine the detection limit of each tested sample. The map of frequency of seroreactive dogs was produced using geo-referenced data regarding the positivity of dogs overlapping the vector layers of the neighborhood limits and the layer of classes of use and occupation of the city of Rio de Janeiro.

DNA extraction from ticks and molecular analysis

Lysis buffer, proteinase K (100 µg/mL) and sodium dodecyl sulfate (SDS) 10% were added to each ground-up tick to resuspend the material. The Phenol-Chloroform technique was used to proceed with DNA extraction, as described by Regnery et al. (1991). Extracted DNA was quantified (DeNovix® 260nm Nanospectrophotometer; DeNovix Inc., Wilmington, USA) and standardized for a concentration of 20 ng/µL. The adult ticks were processed individually, and nymphs were processed individually or in pools, ranging from 2 to 4 nymphs in each pool, according to their size and host. A total of 325 samples were obtained.

The presence of rickettsial DNA was tested for by conventional PCR, targeting the citrate synthase (*gltA*) gene following the procedures of Regnery et al. (1991), with modifications proposed by Campos et al. (2020). Amplifications were carried out using 10 pmol of each primer (5' TTGGGRCCTGCTCACGG 3' and 5' ATTGCAAAAAGTACAGTGAACA 3'), 1.5 mM of magnesium chloride, 0.2 mM of dNTPs, 1 U of Taq DNA polymerase, and 100 ng of genomic DNA. Reactions were subjected to an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 20 s, 58°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. For each reaction, a positive control (purified DNA of *R. parkeri* str. At24 extracted from cell cultures) and a negative control (nuclease-free water) were used.

Amplicons were visualized following electrophoresis in 1.5% agarose gel stained with ethidium bromide under ultraviolet (UV) light. Positive samples were excised from the gel and purified using a commercial kit (illustra GFX PCR DNA and Gel Band Purification Kit; GE Healthcare Life Sciences, São Paulo, Brazil) to prepare them for sequencing on a capillary-type Sanger platform (PDTIS/FIOCRUZ – RPT01A) at the Oswaldo Cruz Foundation.

Partial nucleotide sequences were trimmed, assembled into a contig, and compared with available sequences from the GenBank™ database, using the basic local alignment search tool (BLAST) to assess sequences similarities. Closely related sequences from the *Rickettsia* genus and outgroup sequences from the *Bartonella* and *Pseudomonas* genera were used in multiple alignments using GENEIOUS software version 7.1.9 (Biomatters Ltd., Auckland, New Zealand) with MUSCLE algorithm. Phylogram analysis was performed using MEGA X software version 10.0.5, with Maximum Likelihood method, Kimura 2-parameter substitution model, and Gamma distribution rates. The evolutionary model was defined by the lowest Bayesian Information Criterion (BIC) scores. A bootstrap confidence test was performed with 1000 replicates.

Statistical analysis

Considering previous studies on the frequency of anti-*Rickettsia* spp. antibodies in dogs from areas of low endemicity for BSF, the present study used an estimated prevalence of 8-10% (Milagres et al., 2010; Campos et al., 2017) to calculate the sample. A sample size of 130 dogs was obtained, according to the mathematical formula $n = p / (1-p) (1.96 / 0.05)$, with a 95% significance level (Pereira, 1995). Subsequently, the percentage of dogs with

positive serological reactions was calculated; variables were stored in an electronic worksheet and analyzed using Fisher's exact test and prevalence ratio (BioEstat, version 5.0, Instituto Mamirauá, Belém, Pará, Brazil) with a significance of 95%.

Results and Discussion

Blood samples were collected from 130 dogs; samples were classified as having originated from Vargem (n = 61) and the region of Guaratiba (n = 69). Considering that SFG rickettsiae produce a strong cross-reaction outcome and that this study used two rickettsial antigens and tested dogs in a non-endemic area, our positive predictive value had a low score and did not allow us to elucidate the identification of the species involved. However, we did reveal that there is a circulation of SFG pathogens in the study area. Thus, all samples with a minimum of 1:64 titration were considered reactive, resulting in 52.3% (68/130) of reactive dogs in the anti-*R. rickettsii* or anti-*R. parkeri* IFA assays, or both. For the *R. rickettsii* antigen, the titrations found were 1:64 (11; 8.5%), 1:128 (4; 3.1%), 1:256 (8; 6.1%), 1:512 (16 dogs, 12.3%), and 1:1024 (7; 5.4%). For the *R. parkeri* antigen, titers were 1:64 (14; 10.8%), 1:128 (17; 13.1%), 1:256 (1; 0.8%) and 1:512 (2; 1.5%). The percentage of low titers (1:64 and 1:128) was more common for *R. parkeri* than for *R. rickettsii* ($p < 0.05$).

Recent studies regarding the circulation of SFG rickettsiae in the state of Rio de Janeiro have demonstrated important differences among the evaluated dog populations in different regions. In areas where BSF is non-endemic, prevalence tends to be lower than in our research area. Cordeiro et al. (2015) and Campos et al. (2017) found 24.0% and 9.7% of dogs tested to display anti-*R. rickettsii* antibodies, respectively. Conversely, seropositivity rates increase among dogs from regions where BSF is endemic. For example, Gazeta et al. (2009) found a 58.7% reactivity rate against *R. rickettsii* in dogs from rural areas of southern Rio de Janeiro, and Poubel et al. (2018) found a 67.6% reactivity rate against *R. rickettsii* in urban dogs of northwestern Rio de Janeiro. Although both our study and that of Poubel et al. (2018) were carried out in urban regions, these studies differ epidemiologically. The latter was performed during a human outbreak of BSF, accounting for the markedly high presence of seroreactive dogs.

Of the dogs living in Vargem, 70.5% (43/61) showed serological reactivity, while 36.2% (25/69) of animals from Guaratiba displayed positive serology (table 1). The percentage of positive serological reactions was significantly higher in dogs from the region of Vargem ($p = 0.0001$). Dogs living in this region were almost two times more likely to be exposed to rickettsial agents than in the other region. The study areas displayed distinct occupational profiles, as shown in figure 1. Despite protection by law, Pedra Branca State Park has suffered intense anthropic pressure, especially since 1990, which has led to an increase in the resident population and in the vulnerability of its slopes due to erosion, land use, and loss of vegetation cover (Costa et al., 2009). By the year 2000, Pedra Branca State Park had lost about 10% of its forested area than other large parks in Rio de Janeiro city. This includes Gericinó-Mendanha Park, with a 3% reduction, and Tijuca Forest National Park, with no reductions during the same period (Rio de Janeiro, 2000). According to Iwama et al. (2014), mixed land use (rural and urban) predominates in Vargem, especially in proximity to preserved state-owned lands. The real estate is largely reflective of the medium to high income of residents. In contrast, the region of Guaratiba is characterized by irregular tenure, deforestation owing to banana cultivation, and large urban residential complexes. Figure 1 helped to observe that although both Vargem and Guaratiba have been afflicted by anthropic pressure, the region of Vargem maintains more areas bordering native ecosystems, allowing greater interaction with the rickettsial cycles of neighboring wildlife.

Contrary to studies of Cunha et al. (2014) and Campos et al. (2017), our study found no association ($p = 0.4826$) between dogs with access to outdoor environments and exposure to SFG rickettsiae (table 1). On the other hand, the percentage of positive IFA results was significantly higher ($p = 0.0367$) in dogs that had contact with capybaras and opossums (Table 1). We noted that many of the dogs sampled lived in farms or houses with large backyards. This potentially makes them more susceptible to exposure to SFG bacteria via ticks that typically parasitize stray dogs or that are found in environments with water bodies and wild opossums and capybaras.

As previously stated, recording the presence near dwellings of capybaras and opossums that may have had contact with the sampled dogs relied on the dog owners' reporting. Unfortunately, epidemiological data that depends exclusively on confidence in questionnaire responses may be inaccurate or misinterpreted. Due to the environmental profile of residence locations, more dogs may have been in contact with opossums and capybaras than was recorded. During sampling, we observed (in a subjective and unmeasured way) how easy it was for dogs to interact with wildlife, especially since many wild and synanthropic animals are nocturnal or crepuscular.

Table 1. Percentage, p-values, prevalence ratio and confidence interval in dogs sampled in the West Zone of the municipality of Rio de Janeiro and reagents by indirect immunofluorescence assay for the detection of anti-*Rickettsia rickettsii* and/or anti-*Rickettsia parkeri* antibodies, according to age, sex, residence place, contact with outdoor environments, horses and wild animals (opossums and capybaras).

Variable	Sampled dogs	Reactive dogs (%)	p-value ¹	PR	Confidence Interval
Residence place					
Vargem	61	43 (70.5)	0.0001	1.95	1.37-2.77
Guaratiba	69	25 (36.2)			
Contact with outdoor environments					
Yes	66	37 (56.1)	0.4826	1.16	0.83-1.61
No	64	31 (48.4)			
Contact with opossums and capybaras					
Yes	30	21 (70.0)	0.0367	1.49	1.09-2.04
No	100	47 (47.0)			
Contact with horses					
Yes	61	55 (90.2)	<0.0001	4.79	2.91-7.86
No	69	13 (18.8)			
Age					
≤1year	14	10 (71.4)	0.1622	1.43	0.98-2.08
>1year	116	58 (50.0)			
Sex					
Male	64	37 (57.8)	0.2253	1.23	0.88-1.71
Female	66	31 (47.0)			
Total	130	68 (52.3)	-	-	-

PR: Prevalence Ratio; ¹Values lower than 0.05 showed significance in Fisher's exact test.

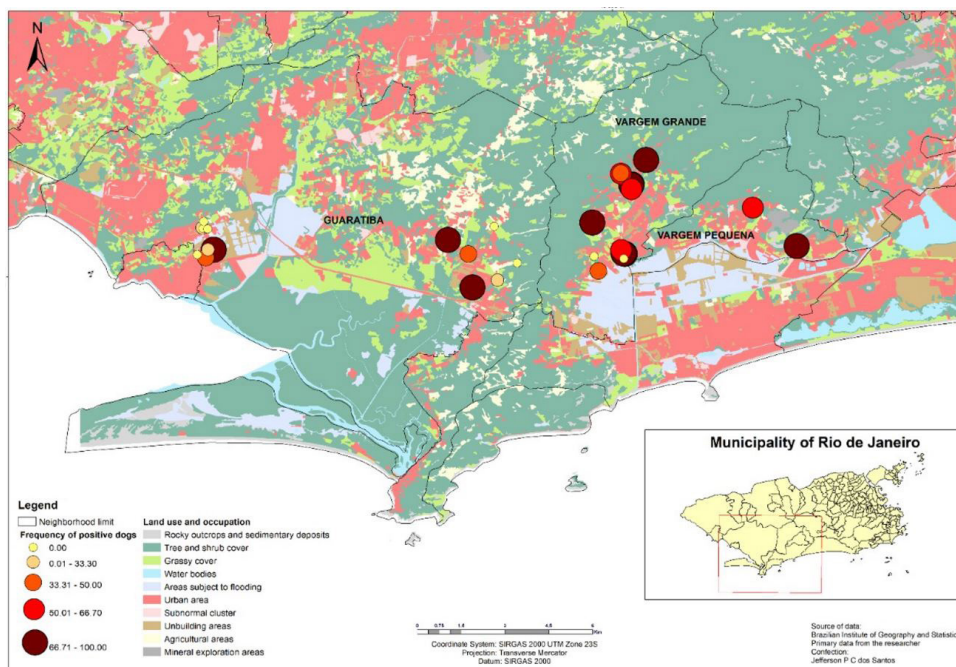


Figure 1. Distribution map of frequency of seroreactive dogs in districts of the West Zone of the municipality of Rio de Janeiro, Brazil.

The proximity to water bodies may have contributed to a high-risk environment around the sampled dwellings since capybaras have the habit of frequenting such environments, and capybara groups were often seen at these locations. Thus, even if dogs were unable to leave their backyards, many of these environments could still allow ticks to pass from wild animals to the dogs. This underscores the relationship between the local landscape and the propagation of BSF in human-populated areas, especially for the region of Vargem.

There was a significant increase ($p < 0.0001$) in the percentage of serological reactions among dogs with proximity to horses (Table 1). We cannot fail to mention here the importance of horses as primary hosts of *A. sculptum* and this species as an essential vector of BSF (Labruna et al., 2001; Szabó et al., 2013). Gazeta et al. (2009) suggested that dogs and horses are crucial for the maintenance of the *Rickettsia* spp. life cycle and for the occurrence of human epizootic events.

In accordance with a prior study on BSF outbreaks in urban areas (Poubel et al., 2018), sex and age were not relevant in anti-*R. rickettsii* seroreactivity in our study (Table 1). Other studies have shown that the frequency of IFA reactivity was higher among adult dogs than puppies (Cordeiro et al., 2015; Campos et al., 2017). This observation can be attributed to the time necessary for seroconversion, and the dogs' increasing overall opportunities for exposure to ticks over time.

Of the total of 353 ticks that were collected from 69 dogs, we identified two species: 95.5% of ticks were *R. sanguineus sensu lato* (37 nymphs and 300 adults), and 4.5% were *Amblyomma sculptum* (7 nymphs and 9 adults). *Rhipicephalus sanguineus s. l.* was observed most frequently in this study, consistent with other reports that have characterized this tick species as the most prevalent one in urban areas of Brazil (Ribeiro et al., 1997; Dantas-Torres et al., 2006; Gazeta et al., 2009; Szabó et al., 2013; Cordeiro et al., 2015). The presence of *A. sculptum* in the evaluated dogs can be explained by the low specificity of this species (especially in the immature stages) (Szabó et al., 2013; Oliveira et al., 2016).

In the present study, no *A. ovale* ticks were found. Although this tick has an extensive distribution in Brazilian biomes, a variety of canids and felids can act as its primary host. Therefore, the presence of other suitable hosts may have contributed to the absence of this species in the sampled domestic dogs. It should be noted that the distribution data for *A. ovale* in the Brazilian Atlantic Forest biome suggests this species is more prevalent in the states of São Paulo, Bahia, and Paraná (Vieira et al., 2013; Nieri-Bastos et al., 2018; Fournier et al., 2020). Furthermore, in the state of Rio de Janeiro, studies have shown that the frequency of parasitism by *A. ovale* in dogs tends to be low or even zero (Santos et al., 2013; Cordeiro et al., 2015; Campos et al., 2020).

Regarding the *gltA* gene, DNA of *Rickettsia* spp. was found in 0.89% (3/337) of the collected ticks, which were all *R. sanguineus s. l.* adults. The nucleotide sequence we obtained from the region of Guaratiba (MF095735) showed 97.22% similarity with the *R. rickettsii* DNA detected in human outbreaks in Colombia (accession MG206089) and Mexico (accession KU587806). The other two positive PCR samples — one from Guaratiba (KY966038) and one from Vargem (KY625211) — were close (97.38%–98.02%) to *R. felis* DNA obtained from flea pools (accession JN366415), sourced from common household insect pests (accession GQ329873), and *Ctenocephalides felis* and *C. canis*. These were found in different regions of Brazil, including Ceará (accession KT153040), Minas Gerais (accession KC158583), and Rio de Janeiro (accession JN375500). These last two sequences we obtained from Guaratiba and Vargem showed respectively 97.04% and 97.51% similarity with *R. felis* obtained from *R. sanguineus s. l.* that was collected from dogs in Peru (accession KY887024) (Temoche et al., 2018). As in our study, these authors highlighted that although positive results were obtained for the *gltA* gene, no PCR product amplification was achieved for the *ompA* gene.

Phylogenetic reconstruction (Figure 2) illustrates a consistent group formed by the previously described *R. rickettsii* sequences and the Guaratiba (MF095735) sequence. Another consistent group was formed by *R. felis* sequences, the Guaratiba (KY966038) sequence, and the Vargem (KY625211) sequence. These results are in agreement with the similarities found through the previously described BLAST search. In addition, *R. rickettsii* and *R. felis* were structured as distant groups, as seen in the phylogenetic study by Bitencourth et al. (2019), which used a concatenated strategy with *gltA*, *htrA*, *ompA*, and *ompB* genes. Due to the intrinsic limitations of molecular identification using the *gltA* gene solely, the sequences of the present study were named as *Rickettsia* sp. only.

The brown dog tick *R. sanguineus s. l.* is recognized as the most frequent ectoparasite of dogs, infesting both privately owned and stray dogs (Ribeiro et al., 1997). *Rhipicephalus sanguineus s. l.* also often parasitizes humans (Demma et al., 2005; Dantas-Torres et al., 2006) and has been associated to *R. rickettsii* human epidemics, either by the contact of the person with tick-infested dogs, a history of tick bites or even by the presence of ticks on the human body (Demma et al., 2005; Tinoco-Gracia et al., 2018). Added to this, natural infections of *R. sanguineus*

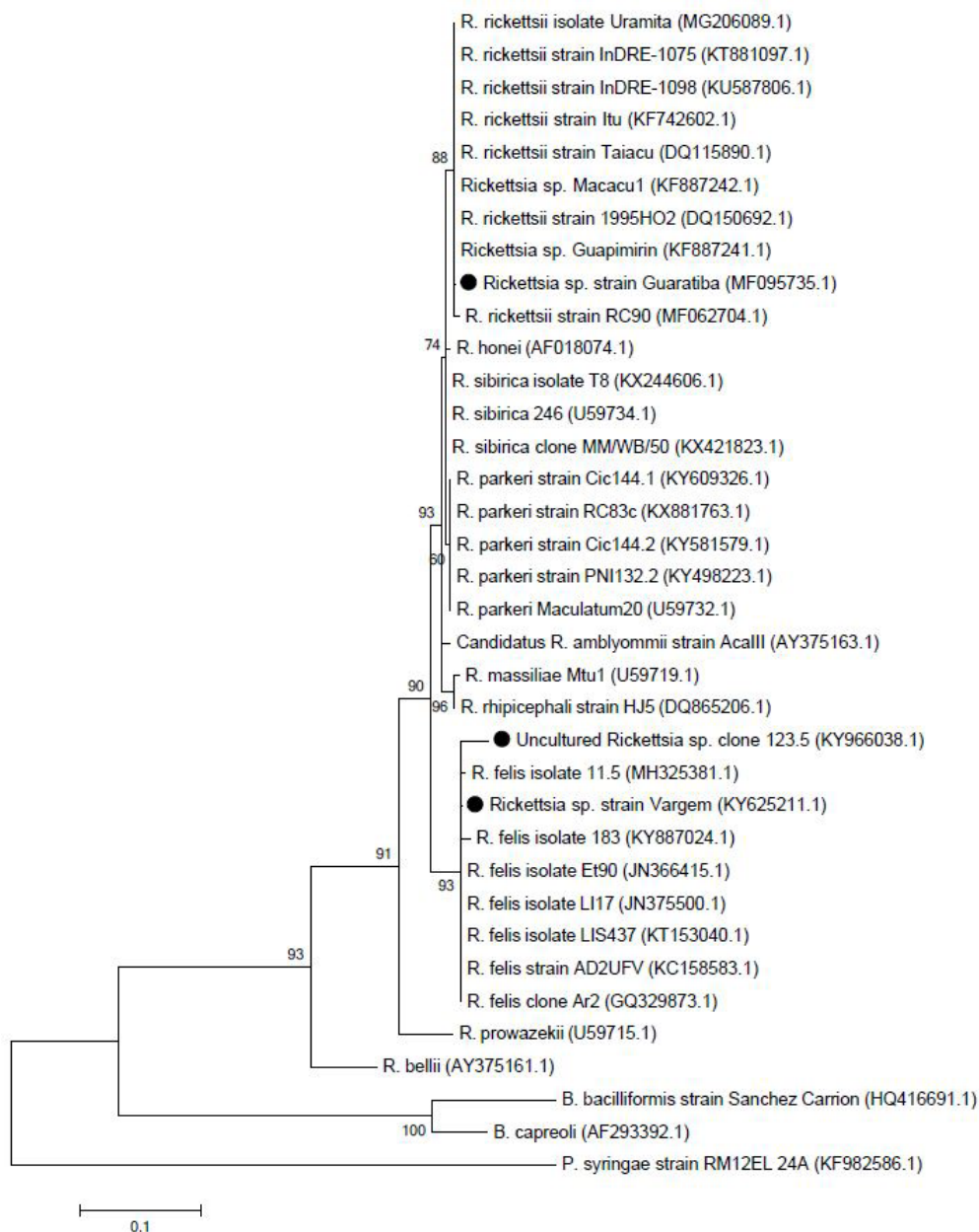


Figure 2. Phylogram generated using partial *gltA* sequences from *Rickettsia* species and outgroup sequences from *Pseudomonas syringae*, *Bartonella bacilliformis*, and *B. capreoli*. Note: The name and description for each sequence is followed by the corresponding GenBank accession number. The values on branch nodes indicate bootstrap confidence values. The dark circles indicate the *gltA* sequences identified in the present study.

s. l. by *R. rickettsii* have previously been demonstrated in Rio de Janeiro state (Cunha et al., 2009; Gehrke et al., 2009). BSF has also caused deaths in workers in an Rio de Janeiro animal shelter where the only ticks collected were *R. sanguineus s. l.*, and 97% of the dogs had anti-*R. rickettsii* antibodies (Costa et al., unpublished data). Thus, our study adds to evidence of the importance of this tick in the participation of the epidemiological cycle of BSF and other rickettsiosis.

Rickettsia felis is an emerging agent that can cause fever, rash, and headache in humans (Abarca et al., 2013). It is a pathogen primarily associated with fleas of the genus *Ctenocephalides*. Because fleas are common ectoparasites of dogs, we consider the possibility that *R. sanguineus* ticks may concomitantly feed on dogs harboring *R. felis*-infected fleas and thereby become infected themselves. This horizontal transmission mechanism, which might have been the source of infection in our study, has been previously proposed, but has not been fully elucidated (Szabó et al.,

2013). However, there are few reports of *R. sanguineus s. l.* ticks infected by *R. felis*, it has been previously confirmed in Chile, Peru and even in Brazil (Cardoso et al., 2006; Abarca et al., 2013; Tanikawa et al., 2013; Temoche et al., 2018).

Conclusions

Rickettsial pathogens circulate in dogs from recently anthropized places inside the Rio de Janeiro city, and in ticks frequently found in indoor environments (*R. sanguineus s. l.*). This condition might constitute a new rickettsioses profile distinct from the rural one, where BSF traditionally occurs. Dogs are useful sentinels of rickettsioses in the urban environment. The dogs that lived in the region of Vargem (less anthropic pressure and more natural landscape) were more likely to be exposed to rickettsial agents. Characteristics of the dog's residence, such as the presence of flowing water bodies, preserved lands, and proximity to the wild cycle of rickettsiae were important factors for exposure to rickettsial antigens. Our study suggests the participation of *R. sanguineus s. l.* in the rickettsial cycle, and points to the potential for the dissemination among dogs of the rickettsioses caused by *R. felis*, an emerging disease potentially pathogenic to human beings.

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