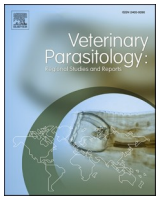
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Original Article



Sharing of living area and exposure of domestic and wild canids to ticks, *Rickettsia parkeri* and *Rangelia vitalii* in the Atlantic Forest of southern Brazil

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ABSTRACT

Spotted fever caused by the bacterium *Rickettsia parkeri*, and canine rangeliosis caused by the protozoan *Rangelia vitalii*, are emerging or re-emerging tick-borne diseases in Brazil, where the main tick vectors are *Amblyomma ovale* and *Amblyomma aureolatum,* respectively. In the present study, we evaluated exposure to *R. parkeri* and *R. vitalii* infection among domestic (*Canis lupus familiaris*) and wild (*Cerdocyon thous*) canids sharing living areas within an Atlantic Forest area of southern Brazil. Four different domestic dogs and three different wild canids were captured. Most of the ticks collected from either domestic or wild canids were identified as *A. ovale* and *A. aureolatum.* DNA of *R. parkeri* was amplified from 14% of the *A. ovale* specimens. Two domestic dogs and two wild canids were seroreactive to *R. parkeri* antigens with high endpoint titers (>1024). *Rangelia vitalii* DNA was detected in two wild canids; one of them was resampled 93 days later, again infected by *R. vitalii.* We report exposure/infection of domestic dogs and wild canids to *R. vitalii* and/or *R. parkeri*-infected ticks in an Atlantic Forest area shared by both canid species, indicating that they also shared the same populations of the tick vectors, *A. aureolatum* and *A. ovale*. While *A. ovale, A. aureolatum*, *R. parkeri, R. vitalii* and *C. thous,* are all native to the Atlantic Forest, further studies are needed to evaluate the impact of an exotic canine species – *C. lupus familiaris* – on the enzootic cycles of *R. vitalii* and *R. parkeri,* as well as the possible role of domestic dogs in emergence and re-emergence of *R. parkeri*-spotted fever in humans and canine rangeliosis, respectively.

1. Introduction

South America has experienced the emergence and reemergence of vector-borne diseases, many of them associated with domestic dogs and with zoonotic potential (Maggi and Krämer, 2019). Among these diseases are spotted fever caused by the bacterium *Rickettsia parkeri*, which was recognized only during this century (Silva-Ramos et al., 2021), and canine rangeliosis caused by the protozoan *Rangelia vitalii*, which was neglected for almost 100 years (Soares et al., 2011).

Rickettsia parkeri was first reported infecting *Amblyomma maculatum* ticks in the United States during the first half of the previous century (Parker et al., 1939), but its demonstration to cause human spotted fever was reported only in 2004 in that same country (Paddock et al., 2004). Recently, different strains of *R. parkeri* have been reported infecting different tick species of the genus *Amblyomma* in South America (Nieri-Bastos et al., 2018). At least two of these strains, *R. parkeri* sensu stricto (associated with *Amblyomma triste* and *Amblyomma tigrinum*), and *Rickettsia parkeri* strain Atlantic rainforest (associated primarily with *Amblyomma ovale*), have been shown to cause human spotted fever illness in South America, including Brazil (Silva-Ramos et al., 2021). Although domestic dogs and wild canids can act as major hosts for the adult stage of *A. triste, A. tigrinum* and *A. ovale* (Nava et al., 2017), there has never been evidence that they get sick from *R. parkeri* infection (Ebani et al., 2023). Because dogs develop a strong humoral response,

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they can be used as sentinels for *R. parkeri*-spotted fever (Barbieri et al., 2014; Krawczak et al., 2023).

In the beginning of the previous century, the piroplasmid *R. vitalii* was described as the etiological agent of a severe and potentially fatal disease affecting domestic dogs in rural areas of Brazil (Carini and Maciel, 1914). Although the validity of the taxon *R. vitalii* was considered doubtful until the end of the 20th century, Soares et al. (2011) confirmed it as a valid piroplasmid agent through phylogenetic analyses. In Brazil, *R. vitalii* is distributed in the Southeastern and Southern regions (Soares et al., 2011; Lemos et al., 2017; Silva et al., 2019), within the distribution area of its primary vector, *Amblyomma aureolatum,* in which *R. vitalii* perpetuates through transstadial and transovarial passages (Soares et al., 2018). Besides the domestic dog, *R. vitalii* has been reported infecting several wild canid species [e.g., the crab-eating fox, *Cerdocyon thous*; the pampas fox, *Lycalopex gymnocercus* (Quadros et al., 2015; De Lorenzo et al., 2021)], natural hosts of *A. aureolatum* (Nava et al., 2017).

The state of Santa Catarina is in the south of Brazil, within the Atlantic Forest biome domain. Previous studies in Santa Catarina have reported exposure to *R. parkeri* among household dogs (Barbieri et al., 2014), and infection by *R. vitalii* in dogs seen at veterinary hospitals (Rodrigues et al., 2022). In this same state, *R. vitalii* infection has been reported in wild canids (the pampas fox, *L. gymnocercus*) that were debilitated and rescued to veterinary hospitals (Silva et al., 2018). In the present study, we sampled free-ranging domestic dogs and wild canids within an Atlantic Forest fragment of Santa Catarina. Our aim was to evaluate exposure to *R. parkeri* and *R. vitalii* infection among domestic and wild canids sharing living areas within the Atlantic Forest, where they were likely exposed to the same tick populations.

2. Materials and methods

2.1. Study site

The Serra do Itajaí National Park is a remaining fragment of Dense Ombrophylous Forest of 57,000 ha covering nine municipalities in the Itajaí Açu River Valley region, within the Atlantic Forest biome in Santa Catarina state, southern Brazil. Domestic and wild canids were sampled in the Park, within the locality Nova Rússia in the municipality of Blumenau. The region has a high density of dogs and cats due to the practice of abandonment, and is a 'hot spot' of tick-borne spotted fever caused by *R. parkeri* strain Atlantic rainforest affecting humans in southern Brazil (Barbieri et al., 2014; Krawczak et al., 2016a).

2.2. Collection of animals and biological samples

Between November 2012 and May 2013, five field campaigns were carried out to capture domestic and wild canids using self-made cage type traps (1.20 m × 0.8 m × 0.8 m) and self-made Tomahawk-like traps (0.45 m × 1.75 m × 0.15 m) arranged in places of sightings or records in camera traps or finding traces such as footprints, feces, and hair of canids. Traps were baited with pieces of raw meat (chicken), dog food (Pedigree® Dry Dog Food Small Dog Grilled Steak and Vegetable Flavor, Brazil) and wet cat food (Whiskas® Poultry Feasts in Jelly, Brazil), and checked every 12 h in a sampling effort of 125 traps/night (5 traps/night x 5 nights/campaign x 5 campaigns).

Each captured animal was sedated with 10 mg/kg Tiletamine and Zolazepam (Zoletil 50, Virbac, São Paulo, Brazil) intramuscularly through the projection of a 3 mL anesthetic dart (Dist-inject®, Broomfield, USA) using a handmade blowpipe; 23 G hypodermic needles (0.6 × 25 mm) were used. After sedation, the animals were weighed using a scale (Pesola® Spring Scale, 20KG/40LB - D = 200G/8OZS, Schindellegi, Switzerland) and an electronic subcutaneous transponder (Animaltag®, São Carlos, Brazil) was implanted in the intra scapular region. Wild canids weighing >3.7 kg and with complete tooth replacement were considered adults (Cheida et al., 2006).

From each canid, 10 mL of blood was collected through the jugular vein. Five mL was immediately stored in an EDTA tube and the other 5 mL was stored in a dry tube, from which the serum was further separated from the clot by centrifugation at 4000*g* for 10 min. Aliquots of EDTA-blood and serum were frozen at −20 °C until laboratory tests. The animals were examined for the presence of attached ticks through looking at and brushing the fur. When ticks were found, they were manually collected and conserved in absolute alcohol. Ticks were morphologically identified to species following Barros-Battesti et al. (2006), Martins et al. (2010) and Kohls (1960).

2.3. Molecular analyses for Rickettsia spp.

In the laboratory, the ticks were left for at least 1 h on a sterile paper towel to allow the ethanol to evaporate. Thereafter, the ticks were individually submitted to DNA extraction using the guanidine isothiocyanate-phenol technique, as previously described (Sangioni et al., 2005). For every 10 individual ticks, a blank tube was included in the DNA extraction. Samples were tested individually by polymerase chain reaction (PCR) using primers CS-78 and CS-323 targeting a 401 base pairs (bp) fragment of the rickettsial gene *gltA*, as previously described (Labruna et al., 2004). In each set of reactions, negative control tubes containing water and a positive control tube containing DNA of *R. parkeri* strain NOD were included. Samples that yielded visible amplicons of the expected size by the *gltA*-PCR were further tested by a second PCR assay using primers Rr190.70p and Rr190.602n targeting a 532 bp fragment of the rickettsial gene *ompA*, as previously described (Regnery et al., 1991). PCR products were electrophoresed through a 1.5% agarose gel, stained with ethidium bromide, and examined by UV transillumination. All *gltA*- and *ompA*-PCR amplicons of the expected size were purified with ExoSap (USB, Cleveland, OH, USA) and sequenced in a Sanger automatic sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California) according to the manufacturer's protocol. Generated sequences were submitted to BLAST analysis (Altschul et al., 1990) to determine the closest identities in GenBank.

2.4. Molecular analyses for Rangelia vitalii

Canine clots were submitted to DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Successful DNA extraction was verified by amplifying a fragment of the vertebrate mitochondrion cytochrome *b* gene (*cyt b*), as previously described (Steuber et al., 2005). Canid DNA samples were tested by *R. vitalii*-specific taqman real-time PCR assay, as previously described (Soares et al., 2018). Positive samples by this assay were tested by a conventional PCR using primers BAB143–167 and BAB694–667, targeting a ≈ 540-bp fragment of the piroplasmid 18S rRNA gene, as previously described (Soares et al., 2011). Amplicons of the 18S rRNA gene-expected size were submitted to DNA-sequencing and the sequences to BLAST analysis as described above.

2.5. Serological analyses

Wild and domestic canid blood serum samples were tested by an indirect immunofluorescence assay (IFA) using a *R. parkeri* strain At24 crude antigen and a fluorescein isothiocyanate-labeled rabbit anti-dog IgG (Sigma, St Louis, MO, USA), as previously described (Labruna et al., 2007). Canid sera were serially diluted in two-fold increments with PBS starting from 1:64 dilution. For each serum sample, the endpoint titer reacting with *R. parkeri* was determined. In each slide, a canine serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested at the 1:64 dilution.

2.6. Hematological exams

Wild canid EDTA-blood samples were immediately stored in a refrigerated cool box and were analyzed within six hours of collection in an automated hematology analyzer (Sysmex pocH-100i, Sysmex America, Lincolnshire, IL, USA) to count red blood cells, total leukocytes, lymphocytes, eosinophils, platelets, dosage of hemoglobin, hematocrit, determination of erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume.

3. Results

Four different domestic dogs (all mongrels, designated as D-dog 1, D-dog 2, D-dog 3, D-dog 4) and three different wild canids (crab-eating fox, *Cerdocyon thous*, designated as *C. thous* 1, *C. thous* 2, *C. thous* 3) were captured in the traps. The individual *C. thous* 3 was recaptured and resampled 93 days later, totalizing four sampled *C. thous*. All canids were adults, except for *C. thous* #3, which at the first capture weighed 2 kg and had milk canine teeth. At recapture, *C. thous* 3 weighed 4 kg and had completely replaced its teeth.

All captured canids were infested by ticks (Table 1). A total of 107 tick specimens were collected, being 49 from domestic dogs (mean: 12.3 ticks/infested dog) and 58 ticks from wild canids (mean: 14.5 ticks/*C. thous*). Ticks were identified into four species: *A. ovale* (64% of all ticks), *A. aureolatum* (31%), *Amblyomma brasiliense* (3%), and *Haemaphysalis juxtakochi* (2%). The former three tick species were found on either domestic dogs or *C. thous,* while *H. juxtakochi* was found only on a single *C. thous* (Table 1).

A sample of 49 adult tick specimens (21 *A. ovale* and 28 *A. aureolatum*) were processed by molecular analyses. Rickettsial DNA was amplified from 3 *A. ovale* (14% of the *A. ovale* specimens), one collected from a domestic dog and two collected from two *C. thous* (Table 1). Both the *gltA* and the *ompA* amplicons amplified from the three ticks yielded partial sequences (350 bp for *gltA,* 490 for *ompA*) that were 100% identical to corresponding *gltA* and *ompA*, respectively, sequences of *R. parkeri* strain Atlantic rainforest available in GenBank (MF536974 and KJ855085, respectively).

Two out of the four domestic dogs were serologically reactive to *R. parkeri,* with endpoint titers of 4096 (D-dog 1) and 1024 (D-dog 3). Among the four samples from wild canids, only *C. thous* 1 and *C. thous* 2 were reactive to *R. parkeri,* both with endpoint titers of 2048.

PCR assay targeting piroplasmid DNA yielded amplicons from three wild canid blood clot samples, which corresponded to *C. thous* 2 and the two samples from *C. thous* 3, which were collected 93 days apart (Table 1). These three samples generated DNA sequences (495 bp) that were 100% identical to a partial sequence of the 18S rRNA gene of *R. vitalii* from GenBank (KF218606).

The hematological parameters were determined for *C. thous* 1 and the two samples of *C. thous* 3; i.e., logistic reasons precluded testing *C. thous* 2 sample. In the three tested samples, the parameters such as hematocrit, erythrocytes and hemoglobin were below reference values for *C. thous* in captivity or free-living (Table 2). Platelet counts of the two samples of *C. thous* 3 were below 100 (x10^3/μL).

Table 2
Hematological values of wild canids (*Cerdocyon thous*) in the Serra do Itajaí National Park, Santa Catarina state, southern Brazil.

Parameter (unit)	Wild canids			Reference values [a]	
	C. thous 1	*C. thous* 3	*C. thous* 3 [b]	Captivity	Free-living
Leukocytes (x10^3/μL)	8.9	9.6	11.7	11.05 (±2.87)	11.5 (±2.88)
Lymphocytes (x10^3/μL)	1	1.7	1.3	1.7 (±0.67)	1.74 (±0.6)
Neutrophils (x10^3/μL)	7.4	7.4	9.7	8.1 (±2.4)	7.1 (±2.5)
Eosinophils (x10^3/μL)	0.5	0.5	0.8	0.77 (±0.57)	1.87 (±0.57)
Erythrocytes (x10^6/μL)	2.6	2.91	3.94	5.54 (±1.23)	4.55 (±0.84)
Hemoglobin (g/dL)	10.4	8.5	10.7	14.82 (±1.86)	14.99 (±1.26)
Hematocrit (%)	34.4	28	35.7	44 (±5.5)	45.5 (±4.08)
MCV	94.7	96.2	90.6	82.01 (±13.93)	91.23 (±14.96)
MCH	28.9	29.8	27.2	27.42 (±5.62)	31.1 (±4.32)
MCHC (g/dL)	30.5	30.7	30.2	34.95 (±3.08)	33.18 (±1.29)
Platelets (x10^3/μL)	102	38	79	–	–

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.
[a] Reference values for *C. thous* according to Santos (1999).
[b] Recapture of *C. thous* 3, 93 days after the first capture.

Table 1
Ticks collected from domestic dogs (D-dog) and wild canids (*Cerdocyon thous*), results of molecular analyses of the ticks (PCR targeting DNA of *Rickettsia* spp.) and canid blood samples (PCR targeting *Rangelia vitalii* DNA), and serological analysis of canid sera for the presence of anti-*Rickettsia parkeri* antibodies (serum was reactive if endpoint titer was ≥64).

Canids	Number of ticks [a]				Number PCR-positive ticks for *Rickettsia*/ Number tested ticks		*R. vitalii* in blood by PCR	Seroreactivity to *R. parkeri* (endpoint titer)
	Aova (adults)	Aaur (adults)	Abra (nymphs)	Hjux (larvae)	Aova	Aaur		
D-dog 1	8	1			0/3	0/1	No	Yes (4096)
D-dog 2	12	1			1/4	0/1	No	No (<64)
D-dog 3	22	4			0/6	0/4	No	Yes (1024)
D-dog 4			1				No	No (<64)
Total D-dogs	42	6	1	0	1/13 (8%)	0/6 (0%)	None	2 samples
C. thous 1	15	19			1/3	0/15	No	Yes (2048)
C. thous 2	11	5	1		1/4	0/5	Yes	Yes (2048)
C. thous 3		1				0/1	Yes	No (<64)
C. thous 3 [b]	1	2	1	2	0/1	0/1	Yes	No (<64)
Total *C. thous*	27	27	2	2	2/8 (25%)	0/22 (0%)	3 samples	2 samples
Total all hosts	69	33	3	2	3/21 (14%) [c]	0/28 (0%)	3 samples	4 samples

[a] Aova: *Amblyomma ovale*; Aaur: *Amblyomma aureolatum*; Abra: *Amblyomma brasiliense*; Hjux: *Haemaphysalis juxtakochi.*
[b] Recapture of *C. thous* 3, 93 days after the first capture.
[c] The three PCR-positive ticks yielded DNA sequences that were 100% identical to *R. parkeri* strain Atlantic rainforest.

4. Discussion

In the present study, we demonstrated that free-ranging domestic dogs and wild canids (*C. thous*), sharing the same Atlantic Forest fragment, also shared infestations by the same tick species, namely *A. ovale, A. aureolatum* and *A. brasiliense.* Some of the *A. ovale* specimens, collected either from domestic or from wild hosts, were found infected by *R. parkeri* strain Atlantic rainforest, with an overall infection rate of 14%. This rate is similar to the 13–15% infection rates previously reported for *A. ovale* collected from domestic dogs in other regions of Brazil (Szabó et al., 2013; Oliveira et al., 2019). Because *A. ovale* is a proven vector of *R. parkeri* strain Atlantic rainforest (Krawczak et al., 2016b; Sevá et al., 2019), the presence of infected ticks in the study area supports our findings of two domestic dogs and two wild canids with high antibody endpoint titers ($\geq$ 1024) to *R. parkeri* antigens; i.e., these animals are likely to have been previously exposed to *R. parkeri*-infected ticks. All together, these results illustrate an enzootic cycle of *R. parkeri* strain Atlantic rainforest, in which the tick vector (*A. ovale*) is found on one of its natural hosts (*C. thous*) in an Atlantic Forest fragment, where intruding domestic dogs might acquire infestations by the tick vector, and consequently, become seropositive to *R. parkeri.* In addition, domestic dogs within this scenario could bring *R. parkeri*-infected ticks into the home environment, increasing the risk of spotted fever in humans.

While *A. aureolatum* was found infesting both domestic and wild canids, most of the specimens (33 specimens; 85%) was collected from *C. thous*; only 6 *A. aureolatum* specimens were collected from domestic dogs. This disproportional numbers, coupled to the fact that *A. aureolatum* is the natural vector of *R. vitalii,* might explain our findings of *R. vitalii* only in *C. thous*; i.e., higher the abundance of *A. aureolatum* on a host, higher the chances of being infested by a *R. vitalii*-infected tick. Indeed, these results corroborate previous studies that proposed an enzootic cycle of *R. vitalii,* in which the agent circulates between its natural hosts (wild canids; e.g., *C. thous*) and the tick vector (*A. aureolatum*); however, domestic dogs are potentially exposed to *R. vitalii* when intruding these environments, where they are likely to become infested by *A. aureolatum* (Soares et al., 2014; De Lorenzo et al., 2021).

Although *R. vitalii* is undoubtedly highly pathogenic to domestic dogs (França et al., 2014), it has been proposed that it is not pathogenic or is much less pathogenic to one of its main natural hosts, the crab-eating fox *C. thous* (Soares et al., 2014; De Lorenzo et al., 2021). These two pathogenic profiles could be related to a short-duration schizogony phase of *R. vitalii* in *C. thous*, in contrast to a more pathogenic schizogony phase in domestic dogs (De Lorenzo et al., 2021). In the present study, *C. thous* individuals did not show any clinical abnormality when they were captured (data not shown); even then, *C. thous* #1 and #3 showed some hematological values below the reference values. Because *C. thous* #1 was not infected by *R. vitalii,* and *C. thous* #3 was infected, it is hard to link the low hematocrit, erythrocytes, and hemoglobin of *C. thous* #3 to its active infection by *R. vitalii,* since these values were like those found for *C. thous* #1. On the other hand, it is likely that the lowest platelet count of *C. thous* #3 at its first capture event (38×10^3/μL) was linked to *R. vitalii* infection, since severe thrombocytopenia has been reported as one of the most pronounced hematological alterations in *R. vitalii*-infected dogs (França et al., 2010; Paim et al., 2012).

Interestingly, *C. thous* #3 was also infected by *R. vitalii* when sampled 93 days after its first sampling. Because this animal had canine milk teeth when first sampled, it was only a few months old (Brady, 1978). Thus, it is possible that it was acutely infected by *R. vitalii* at first capture and had remained infected till its second capture 93 days later, when its counts of erythrocytes, hemoglobin, hematocrit and platelets were slightly higher. This statement is corroborated by data from *R. vitalii*-experimental infected dogs, which remained infected for 5–7 months after being infested with infected ticks (Soares, 2014).

5. Conclusion

We report exposure/infection of domestic dogs and wild canids (*C. thous*) to *R. vitalii* and/or *R. parkeri*-infected ticks in an Atlantic Forest area shared by both canid species. While previous studies have reported *R. vitalii* infection and canine exposure to *R. parkeri* in households close to the present study area (Barbieri et al., 2014; Rodrigues et al., 2022), here we demonstrated living-area sharing of the two canid species in the Atlantic Forest, indicating that they also shared the same populations of the tick vectors of *R. vitalii* (*A. aureolatum*) and *R. parkeri* (*A. ovale*). While these two tick species and tick-borne pathogens, as well as *C. thous,* are native to the Atlantic Forest, further studies are needed to evaluate the impact of the presence of an exotic species (domestic dogs) in the enzootic cycle of *R. vitalii* and *R. parkeri,* and their possible consequences to the emergence and re-emergence of *R. parkeri*-spotted fever in humans and canine rangeliosis, respectively.

Ethical statement

This study has been approved by the Institutional Animal Care and Use Committee of the Regional University of Blumenau - FURB (protocol No. 117/12). Field captures of wild animals were authorized by the Brazilian Ministry of the Environment (permit SISBIO Nos. 36038-1).

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Declaration of Competing Interest

The authors declare no conflict of interest.

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