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ORIGINAL ARTICLE

Epidemiology of *Rickettsia* spp. in Atlantic rainforest areas of island and seashore mainland, southern Brazil

Louise Bach Kmetiuk[1] | Warley Vieira de Freitas Paula[2] | Gracielle Teles Pádua[2] | Ruana Renostro Delai[3] | Aaronson Ramathan Freitas[3] | João Henrique Farinhas[3] | Luiza Gabriella Ferreira de Paula[2] | Rogério Giuffrida[4] | Claudia Turra Pimpão[5] | Vamilton Álvares Santarém[4] | Andrea Pires dos Santos[6] | Fabiano Borges Figueiredo[1] | Felipe da Silva Krawczak[2] | Alexander Welker Biondo[2]

[1]Laboratory of Cell Biology, Instituto Carlos Chagas, Fundação Oswaldo Cruz, Curitiba, Brazil

[2]School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Brazil

[3]Department of Veterinary Medicine, Federal University of Paraná State, Curitiba, Brazil

[4]Laboratory of Veterinary Parasitology, Veterinary Teaching Hospital, University of Western São Paulo (UNOESTE), Presidente Prudente, Brazil

[5]Department of Animal Science, School of Life Sciences, Pontifical Catholic University of Paraná, Curitiba, Brazil

[6]Department of Comparative Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA

Correspondence
Alexander Welker Biondo, Department of Veterinary Medicine, Federal University of Paraná State, Funcionários Street, 1540, Curitiba, Paraná State, 80035–050, Brazil.
Email: abiondo@ufpr.br

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Abstract

Non-fatal cases of rickettsial infection with different clinical features than the classic BSF (Brazilian Spotted Fever) have been reported in seashore areas of Paraná state, southern Brazil. In addition, *Amblyomma ovale* tick infected by *Rickettsia parkeri* strain Atlantic rainforest has been also described in this area. Accordingly, the aim of this study was to investigate the occurrence of anti-*Rickettsia* spp. antibodies in human and dog populations, and *Rickettsia* spp. infection in ticks from oceanic islands and seashore mainland cities of southern Brazil. Serum samples were collected from 328 persons and their 282 dogs from three islands and two seashore mainland cities. A total of 211 ticks were collected from dogs, identified as *A. ovale* and *R. sanguineus*. In overall, 40 of 328 (12.2%) human samples were seropositive for *Rickettsia* spp., including 21 of 190 (11.1%) on islands and 19 of 138 (13.7%) on seashore mainland, and 62 of 282 (22.0%) dog samples, including 31 of 153 (20.3%) on islands and 31 of 129 (24.0%) in seashore mainland areas. In overall, nine of 82 (11.0%) ticks were positive to real-time PCR assay targeting a fragment of the rickettsial *gltA* gene, including two of 64 (3.1%) *Rickettsia sanguineus* and seven of 18 (38.9%) *A. ovale*, of which four were infected with the *R. parkeri* strain Atlantic rainforest. Despite no association between risk factors and *Rickettsia* spp. seropositivity was found in human beings, access to natural areas ($p = .011$) and tick infestation ($p = .004$) was significantly associated to dog seropositivity. The serological and molecular findings herein have confirmed previous tick and clinical case reports and enlarged the geographical occurrence of *A. ovale* infected by *R. parkeri* strain Atlantic rainforest in oceanic islands and seashore mainland cities of Paraná State, indicating a new likely transmission area of this new rickettsial infection in human beings and dogs of southern Brazil.

KEYWORDS
One Health, spotted fever, vector-borne diseases

1 | INTRODUCTION

The genus *Rickettsia* (family Rickettsiaceae; order Rickettsiales) has comprised gram-negative and obligate intracellular bacteria, including the spotted fever group (SFG) rickettsiae (Parola et al., 2013). The SFG are currently recognized as emerging and re-emerging tick-borne zoonotic pathogenic agents to humans and animals, such as *Rickettsia rickettsii* and *Rickettsia parkeri* (Parola et al., 2013; Parola & Raoult, 2001).

As the second most important rickettsial agent of Brazil (Faccini-Martínez, Krawczak, Oliveira, Labruna, & Angerami, 2021), *R. parkeri* has been primarily transmitted to human beings by Ixodid ticks, including *Amblyomma ovale* in the Atlantic rainforest biome (Krawczak, Agostinho, Polo, Moraes-Filho, & Labruna, 2016). Small rodents and wild birds have been recognized as primary hosts of *A. ovale* larval and nymphal (Labruna et al., 2005), and wild carnivores of *A. ovale* adults (Martins et al., 2015). *Amblyomma ovale* is also a anthropophilic tick throughout South America (Guglielmone et al., 2006), and human cases of *R. parkeri* strain Atlantic rainforest infection have been associated to mild febrile disease, characteristic inoculation eschar and nonspecific signs, such as headache, myalgia and regional lymphadenopathy, which generally do not require hospitalization (Faccini-Martínez, Vilges De Oliveira, Junior, & Labruna, 2018).

Seashore and islands of Paraná State, southern Brazil have been sustaining the largest continuous preserved area of Atlantic Forest biome in Brazil, which has been historically inhabited by traditional human communities. Recent studies have shown *R. parkeri* strain Atlantic rainforest infection in *A. ovale* at nearby Paranaguá microregion, within Atlantic Forest biome of Paraná State (Bitencourth, Amorim, de Oliveira, Voloch, & Gazêta, 2019), along with seven non-fatal cases notified in 2020 (Brazil, 2022). In addition, dogs living in *R. parkeri* strain Atlantic rainforest transmission areas of south-eastern Brazil have been reported to be infested by adults of *A. ovale* (Krawczak, Binder, et al., 2016), which may potentially increase the risk of human infection when bringing infected ticks to owner households (Szabó, Nieri-Bastos, et al., 2013). Finally, risk of SFG disease may occur when infected ticks bite human beings when walking on trails and entering forest areas (Faccini-Martínez et al., 2018).

Despite human and dog populations living on oceanic islands and seashore mainland cities in the state of Paraná, southern Brazil may be exposed to tick-borne rickettsiae, no study to date has concurrently assessed this potential life cycle of spotted fever in such owner-dog populations. Accordingly, the aim of the present study was to investigate anti-*Rickettsia* spp. antibodies in human and dog populations, and *Rickettsia* spp. infection in ticks from oceanic islands and seashore mainland cities of southern Brazil.

2 | MATERIALS AND METHODS

The study herein was a cross-sectional epidemiological study of human, dog and parasitizing ticks living on oceanic islands and seashore mainland cities of southern Brazil.

2.1 | Study areas

The study was conducted on three oceanic islands, Ilha do Mel Island (25°30′42″S, 48°20′20″ W), Superagui Island (25°20′47″S, 48°12′9″W) and Peças Island (25°20′22″S, 48°15′29″W), and two mainland seashore cities, Pontal do Paraná (25°40′26″S, 48°30′39″W) and Guaraqueçaba (25°18′25″S, 48°19′44″W), all within the Paraná State, southern Brazil. The three islands herein were environmentally preserved conservation units at the time, with the Ilha do Mel Island State Park and the Superagui National Park located within the largest continuous remnant of the Atlantic Forest in Brazil.

2.2 | Sampling

In total, five on-field expeditions were performed from July 2019 through February 2020 in the studied area. Owners and their dogs were sampled following signed consent and completion of an epidemiological questionnaire. Human serum samples were collected by cephalic puncture and dog serum samples by jugular puncture. All samples were placed in tubes without anti-coagulant and kept at room temperature (25°C) until visible clot retraction, centrifuged at 1500 rpm for 5 min, and serum separated and kept at −20°C until processing. After blood samplings, dogs were thoroughly examined for the presence of ticks in their entire body. All ticks collected from dogs were preserved in isopropyl alcohol and taken to laboratory for taxonomic identification.

2.3 | Serological testing

Human and dog serum samples were individually tested by indirect immunofluorescence assay (IFA) for four Brazilian *Rickettsia* isolates: *R. rickettsii* strain Taiaçu, *R. parkeri* strain Atlantic rainforest, *Rickettsia amblyommatis* strain Ac37 and *Rickettsia bellii* strain Mogi as previously described (Barbieri et al., 2014; Horta et al., 2004; Labruna et al., 2007). Individual sera were initially screened at a 1:64 dilution against each of the rickettsial antigens. A fluorescein isothiocyanate-labelled rabbit anti-human IgG dilution 1:500 (IgG, Sigma Diagnostics®, St. Louis, MO) was used as conjugate for the human samples, and fluorescein isothiocyanate-labelled rabbit anti-dog IgG dilution 1:500 (IgG, Sigma Diagnostics®, St. Louis, MO) as conjugate for dog samples. An endpoint titre at least fourfold higher for a *Rickettsia* species than that observed for other *Rickettsia* species was considered probably homologous to the first *Rickettsia* species or a very closely related species (Barbieri et al., 2014). In each slide, a non-reactive (negative control) and known reactive sera (positive control) were tested at the 1:64 dilution.

2.4 | Tick identification and molecular testing

Tick taxonomic identification was performed following standard morphological keys (Barros Battesti, Arzua, & Bechara, 2006; Clifford,

1961; Dantas-Torres, Fernandes Martins, Muñoz-Leal, Onofrio, & Barros-Battesti, 2019; Martins, Onofrio, Barros-Battesti, & Labruna, 2010). Taxonomic identification of *Amblyomma* larval and nymphs was based on Martins et al. (2010) and Clifford et al. (1961), and for adult ticks by Barros-Battesti et al. (2006).

Only adult ticks were processed individually for DNA extraction by the guanidine isothiocyanate phenol technique (Sangioni et al., 2005). Total DNA was extracted only from adult ticks due to transstadial perpetuation of rickettsiae infection in trioxene ticks, with adult ticks more likely to be positive for *Rickettsia* spp. than immature stages including larvae and nymphs (Gerardi et al., 2019; Krawczak, Agostinho, et al., 2016).

Tick DNA samples were tested by a Taqman real-time PCR assay targeting a 147-bp fragment of the rickettsial *gltA* gene (Guedes et al., 2006; Labruna et al., 2004). Positive samples by real-time PCR were subsequently tested by a conventional PCR using primers Rr190.70 and Rr190.602, targeting a 532-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*), present in SFG rickettsiae, as previously described (Regnery, Spruill, & Plikaytis, 1991). Samples that were negative to *ompA* gene PCR were submitted to a conventional protocol using primers CS-78/CS-323 that amplify a 401-bp fragment of the *gltA* gene (Labruna et al., 2004).

For each PCR run, a negative control (water) and positive control (*Rickettsia vini* DNA) were included. The *ompA* PCR products were sequenced; the sequences obtained were submitted to BLAST analyses (www.ncbi.nlm.nih.gov/blast) to infer the closest similarities available in GenBank.

All tick DNA samples that were shown to contain no rickettsial DNA by the real-time PCR described above were tested by the conventional PCR targeting the tick mitochondrial 16S rDNA gene (Mangold, Bargues, & Mas-Coma, 1998), in order to validate the DNA extraction protocol.

2.5 | Data collection and statistical analysis

Epidemiological analysis of human characteristics was based on a questionnaire that assessed potential associated risk factors to ticks and *Rickettsia* spp. and included the household geographical location, age, sex, education level, occupation, household income, family members at the household, time living in the area, animal ownership, frequency of contact with forest areas, previous tick bite and location of tick contact (household, after contact with forest area, other). Epidemiological analysis of dog characteristics was based on a questionnaire that assessed potential associated risk factors to ticks and *Rickettsia* spp. and included the sex, breed, animal origin, geographical location of household, access to natural areas, hunting habit, ectoparasite presence and control and tick sampling. The data were organized in spreadsheets, and the analytical process started with a descriptive exploration of the databases.

A dataset with an outcome stratified according to the sampling area (island vs. seashore mainland) was constructed. Potential associated risk factors to *Rickettsia* spp. seropositivity were evaluated by categorization of variables. Questions with significant impact related to missing information (>10% of the total) were removed from the analyses. After categorization, the variable was arranged in double-entry tables according to seropositivity for anti-*Rickettsia* antibodies and tested for associations using Pearson's chi-square test.

Fisher's exact test was used as an alternative when one or more cells in the table had expected values lower than 5. Variables with statistical significance equal to or less than 0.2 were included in the multivariate logistic regression model, applying the stepwise backward selection method as the basis for the best estimate of the Akaike information criteria.

Problems related to multicollinearity between predictor variables were evaluated by calculating the inflationary value of variance (VIF), being considered significant when VIF > 5.0. Odds ratio per interval was estimated in both univariate and multivariate models at 95% confidence. The seropositivity for *Rickettsia* spp. was compared between dogs and owners by the chi-square test with Yates correction. All analyses were assessed using R software, considering 5% level of significance.

3 | RESULTS

Serum samples were collected from 328 human beings, including 190 of 328 (57.9%) on islands and 138 of 328 (42.1%) on seashore mainland areas, and from 282 owned dogs, including 129 of 282 (45.7%) on islands and 153 of 282 (54.3%) on seashore mainland areas (Table 1).

A total of 40 out of 328 (12.2%) human samples were seropositive for *Rickettsia* spp., including 21 of 190 (11.1%) on islands and 19 of 138 (13.8%) on the seashore continent (Table 1). Antibodies against *Rickettsia* spp. were detected in 62 of 282 (22.0%) dog samples, including 31 of 129 (24.0%) on islands and 31 of 153 (20.2%) on seashore mainland areas (Table 1). In addition, the possible antigen involved in a homologous reaction (PAIHR) among human beings was found in two of 40 (5%) for *R. amblyommatis* and four of 40 (10%) *R. bellii*, and in 12 of 62 (19.3%) for *R. parkeri* and 35 of 62 (56.4%) for *R. bellii* dogs (Table 01). *Rickettsia* spp. seropositivity in dogs ($p = .001$) was statistically higher than that observed in human beings, both for islands ($p = .026$) and seashore mainland ($p = .046$).

A total of 211 ticks were collected from dogs, including six of 211 (2.8%) larvae of *Rhipicephalus* spp., six of 211 (2.8%) nymphs of *Rhipicephalus* spp., 96 of 211 (45.5%) males of *Rhipicephalus sanguineus* s.l, 72 of 211 (34.1%) females of *R. sanguineus* s.l, one of 211 (0.5%) nymphs of *A. ovale*, 14 of 211 (6.6%) males of *A. ovale* and 16 of 211 (7.6%) females of *A. ovale*. Real-time PCR targeting rickettsial DNA was performed on 82 tick specimens. Nine of 82 (11.0%) ticks were positive to real-time PCR assay targeting a fragment of the rickettsial *gltA* gene, including two of 64 (3.12%) *R. sanguineus* s.l and seven of 18 (38.9%) *A. ovale*. All nine positive samples (*A. ovale* and *R. sanguineus* s.l) in real-time PCR for *gltA* gene were further tested by conventional PCR assay, which amplified fragments of the rickettsial gene *ompA*, and only four of 9 (44.4%) *A. ovale* ticks were positive for this conventional PCR.

TABLE 1 Results of indirect immunofluorescence assay (IFA) for four *Rickettsia* species in human beings and dogs of oceanic islands and seashore mainland cities of southern Brazil

Study area	Samples (number of positive/number of tested)	Number of seroreactive human beings and/or dogs to each of the *Rickettsia* species/% seroreactivity for human beings or dogs (range of endpoint titre)				
		R rickettsii	*R. parkeri*	*R. bellii*	*R. amblyommatis*	No. samples with determined homologous reaction (PAIHR in parenthesis)[a]
Peças Island	Humans (7/28)	1/3.6 (64)	2/7.1 (64–256)	2/7.1 (64–128)	6/21.4 (64–128)	–
	Dogs (11/29)	3/10.3 (128)	4/13.8 (256–1024)	7/24.1 (128–2048)	4/13.8 (64–128)	4 (*R. parkeri*); 6 (*R. bellii*);
Ilha do Mel Island	Humans (14/113)	2/1.7 (64–128)	3/2.6 (64–128)	10/8.8 (64–512)	7/6.1 (64–256)	1 (*R. amblyommatis*); 1(*R. belli*)
	Dogs (10/68)	0/0	0/0	10/13.9 (64–512)	0/0	7 (*R. belli*)
Superagui Island	Humans (0/49)	0/0	0/0	0/0	0/0	–
	Dogs (10/56)	0/56	1/1.7 (256)	9/16.1 (128–2048)	0/0	1 (*R. parkeri*) 7 (*R. bellii*)
Guaraqueçaba (seashore mainland areas)	Humans (14/92)	4/4.4 (64–128)	5/5.4 (64–128)	4/4.4 (64–512)	11/11.9 (64–512)	1 (*R. bellii*)
	Dogs (22/89)	14/15.7 (64–1024)	16/18.0 (64–2048)	4/4.5 (512–2048)	4/4.5 (128–512)	7 (*R. parkeri*); 5 (*R. bellii*)
Pontal do Paraná (seashore mainland areas)	Humans (5/46)	0/0	1/2.2 (128)	3/6.5 (64–128)	2/4.3 (128–256)	1 (*R. amblyommatis*) 2 (*R. bellii*)
	Dogs (9/40)	2/5.0 (64–128)	0/0	9/22.5 (64–2048)	0/0	10 (*R. bellii*)
Total	Humans (40/328)	6/1.8 (64–128)	11/3.3 (64–128)	19/5.7 (64–512)	26/7.8 (64–512)	2 (*R. amblyommatis*) 4 (*R. bellii*)
	Dogs (62/282)	19/6.7 (64–1024)	21/7.4 (64–2048)	39/13.8 (64–2048)	8/2.8 (64–512)	12 (*R. parkeri*) 35 (*R. bellii*)

[a]A homologous reaction was determined when an endpoint titre to *Rickettsia* species was at least fourfold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species (or a very closely related species) involved in the highest endpoint titre was considered the possible antigen involved in a homologous reaction (PAIHR).

TABLE 2 Ticks collected from dogs of island and seashore mainland of southern Brazil

Study area	Tick species	Ticks tested for rickettsial infection Number of infected/Number of tested (% infection)
Peças Island	*Amblyomma ovale* (6 M + 4 F = 10)	2/7 (28.6%)
	Rhipicephalus sanguineus (57 M + 38 F = 95)	0/14 (0.0%)
	Rhipicephalus spp. (1 L)	–
Ilha do Mel Island	NA	–
Superagui Island	*Amblyomma ovale* (3 M + 2 F = 5)	0/1 (0.0%)
	Rhipicephalus sanguineus s.l (25 M + 23 F = 48)	1/35 (2.9%)
	Rhipicephalus spp. (5 N)	
Guaraqueçaba	*Amblyomma ovale* (5 M + 10 F + 1 N = 16)	5/10 (50.0%)
	Rhipicephalus sanguineus s.l (14 M + 11 F = 25)	1/15 (6.6%)
	Rhipicephalus spp. (5 L + 1 N = 6)	–
Pontal do Paraná	NA	–
Total	*Amblyomma ovale* (*N* = 31)	7/18 (38.9%)
	Rhipicephalus sanguineus s.l (*N* = 167)	2/64 (3.1%)
	Rhipicephalus spp. (*N* = 7)	–

Abbreviations: F, female; L, larvae; M, male; N, nymph.

All *ompA* products were DNA sequenced and by BLAST analysis, the *ompA* partial sequences of the four *A. ovale* ticks were identical to each other and 100% (414/414 bp) identical to corresponding sequence of *Rickettsia* sp. strain Atlantic rainforest from GenBank (MF536975.1) (Table 02). The amplicons of the *gltA* gene from *R. sanguineus* s.l and *A. ovale* ticks, which were negative for the *ompA* gene, did not generate good sequences. Rickettsial sequences generated in the present study were deposited in GenBank under the accession number ON783875.

Associated risk factors for human exposure to *Rickettsia* spp. were not statistically significant including household location ($p = .568$), owner of dog seropositive for *Rickettsia* spp. ($p = .800$), age ($p = .326$), education ($p = .744$), income ($p = .607$), access to forest areas ($p = .820$), frequency of accessing forest areas ($p = .761$), dog owner ($p = .782$), previous tick bite in household ($p = .493$), previous tick bite in peridomicile ($p = 1.0$) and previous tick bite in forest areas ($p = 1.0$). Furthermore, no variable presented significance <0.2 in the bivariate analysis. Therefore, it was not possible to perform the logistic regression (Table S1).

The statistical analysis for associated risk factors with *Rickettsia* spp. exposure in dogs was significant with access to natural areas ($p = .011$) and tick infestation ($p = .004$), but not with household location ($p = .537$), gender ($p = .858$), access to trails ($p = .167$), access to natural areas under supervision ($p = .984$), tick control regime ($p = .277$) and deworming ($p = .232$) (Table S2).

In the logistic regression analysis related to dogs at the initial model, three seropositivity predictors presented significance <0.2, including access to natural areas, infested by tick and access to trails. The initial model was submitted to backward stepwise approach that was determined after one step, removing one (access to trails) out of the three seropositivity predictors and retaining two (access to natural areas and infested by tick). Considering that tick history could be closely related to access to natural areas, the hypothesis assumed a collinearity between the two variables, in the logistic model. Nevertheless, multicollinearity was not confirmed since the estimated VIF was 1.02 and 1.43, respectively, for the historic of ticks and access to natural areas. Thus, the two variables were included in the final logistic regression model.

4 | DISCUSSION

The present study reports serological findings and molecular assays of *Rickettsia* spp. in humans, dogs and associated ticks on the islands and seashore mainland areas in a new likely transmission area of *R. parkeri* strain Atlantic rainforest in Paraná State, southern Brazil. Although seven non-fatal human cases were reported in a nearby seashore mainland area in 2020, the human seroprevalence of *Rickettsia* spp. antibodies herein was lower than seven of 15 (46.7%) sampled in the

Atlantic Forest area of Santa Catarina, a neighbouring state in southern Brazil (Barbieri et al., 2014). Despite the *A. ovale* infection rate with *Rickettsia* spp. presented herein (7/18; 38.9%) was higher than in Santa Catarina State (4/43; 9.3%), the higher human seroprevalence in this previous study may be related to overtime exposure to *Amblyomma* spp. ticks infected by *R. parkeri* strain Atlantic rainforest in this BSF endemic area, which has been recognized since 2003 (Barbieri et al., 2014). On other hand, human seroprevalence herein was higher than four of 41 (9.8%) dog owners of another seashore Atlantic Forest area at the Espírito Santo State, with no previous human cases of *R. parkeri* infection reported by the state health secretary and less than 1% infected ticks in Espírito Santo State (Faccini-Martínez et al., 2020). Thus, variances in seroprevalence among previous and present studies may be explained by differences in infection rate of *Rickettsia* spp. in ticks.

No statistical differences were found herein between human seropositivity and associated risk factors including household location, owner of dog seropositive for *Rickettsia* spp., age, education, income, access to forest areas, frequency of accessing forest areas, dog owner, previous tick bite in household, previous tick bite in peridomicile and previous tick bite in forest areas. A previous study has demonstrated differences under same longitude in ixodofauna distribution, which are rickettsial vectors of the SFG in Brazil, but only in different altitudes (Barbieri et al., 2015). Thus, results herein have indicated uniform human exposure to *Rickettsia* spp. on islands and seashore mainland of southern Brazil, which may be explained by similar latitude and altitude of these locations. Nonetheless, 40 of 328 (12.2%) positive samples may not be enough to statistically detect differences in exposure, and further studies should be conducted to fully establish whether associated risk factors play a role in these seashore areas.

Likewise, dogs of islands and seashore mainland have shown no association between *Rickettsia* spp. exposure and risk factors including household location, gender, access to trails, access to natural areas under owner supervision and tick control regime as associated risk factors. An increased dog seropositivity was associated with access to natural areas and tick infestation occurrence, statistically higher than observed in owners on islands and seashore mainland areas. Such findings corroborated with previous studies that have found association of seropositivity of SFG with dogs living nearby natural areas, unrestricted access to the natural areas and *Amblyomma* spp. tick parasitism (Barbieri et al., 2014; Costa et al., 2017; da Silva Rocha Fournier et al., 2020; Krawczak, Binder, et al., 2016). In addition, the largest fragment of Atlantic rainforest biome of Brazil has been located throughout islands and seashore mainland of Paraná State, which may contribute to *Rickettsia* spp. maintenance by enzootic cycles.

As *Rickettsia parkeri* strain Atlantic rainforest has presented deleterious effect on engorged females of *A. ovale* (Krawczak, Agostinho, et al., 2016), vertebrate host amplifiers have been necessary for persistent perpetuation of rickettsial cycle in *A. ovale* under natural conditions. As previously proposed, amplification and circulation of *R. parkeri* strain Atlantic rainforest under natural conditions have been maintained by small rodents as primary reservoir and *Amblyomma ovale* ticks as main vectors within the Atlantic rainforest biome (Krawczak & Labruna, 2018; Nieri-Bastos et al., 2016).

In the present study, at least 22.0% of owned dogs were seropositive to *Rickettsia* spp., with PAIHR for *R. belli* and *R. parkeri*, with lower seropositivity when compared to previous studies with dogs from Atlantic rainforest areas of *A. ovale* and *R. parkeri* circulation, which varied from 49% to 67.3% of dog seropositivity in Espírito Santo and Santa Catarina States (Barbieri et al., 2014; Faccini-Martínez et al., 2020). However, results herein are similar to the seroprevalence (19.7%) detected in dogs residing in areas with human cases of non-classic BSF, caused by *R. parkeri* strain Atlantic rainforest, in Rio Grande do Sul, southern Brazil (Krawczak, Binder, et al., 2016).

The *A. ovale* was the second most frequent tick in dogs herein. Out of total, three of eight (37.5%) parasitized dogs presented serological evidence for exposure to *R. parkeri* strain Atlantic rainforest. Considering that dogs are the most suitable domestic sentinel for BSF in areas of transmission by *A. ovale* (Barbieri et al., 2014; Krawczak, Binder, et al., 2016; Nieri-Bastos et al., 2016), results have indicated that islands and seashore mainland presented favourable conditions for the BSF occurrence in the ecoepidemiological scenario involving *A. ovale* ticks. Although 122 of 328 (37.2%) persons referred previous tick bite, no human spoliation was found. Nevertheless, *A. ovale* tick has been recognized as one of the most anthropophilic tick species in the Neotropical region and may be likely the responsible vector herein (Guglielmone et al., 2006).

Thus, concomitant human and dog serological results have indicated mutual exposure to *Rickettsia parkeri* strain Atlantic rainforest infection in islands and seashore mainland, southern Brazil. Despite no reportedly amplifiers of *R. parkeri* strain Atlantic rainforest, dogs infested by *A. ovale* presented a 10-fold higher risk of infection when compared to those not infested by *A. ovale* (da Silva Rocha Fournier et al., 2020).

Ticks were only found in dogs, and identified as *R. sanguineus* and *A. ovale*. Parasitism with *A. ovale* in dogs has been reported in other seashore areas of Paraná State, along with *R. sanguineus* and *Amblyomma aureolatum* (Silva, Garcia, Rodrigues, Andreotti, & Dittrich, 2017). Although two *R. sanguineus* from dogs sampled were found infected by *Rickettsia* spp., the participation of dogs in the BSF cycle and the risk of human infection must be considered (Moerbeck et al., 2016; Szabó, Pinter, et al., 2013). *Rickettsia parkeri* strain Atlantic rainforest infection was confirmed in four *A. ovale* specimens, corroborating to previous report of *A. ovale* infection in a dog from nearby preserved area of Atlantic rainforest biome in Paraná State seashore (Durães et al., 2021), and other Brazilian states within the Atlantic rainforest (Acosta et al., 2018; Barbieri et al., 2014; de Oliveira et al., 2019; Faccini-Martínez et al., 2020). Domestic dogs with access to areas of Brazilian Atlantic Forest have frequently demonstrated parasitism of *A. ovale*, which may increase the risk of infection by *Rickettsia* spp. (different from the classic FSB) in owners due to the anthropophilic habits of the immature forms of this tick species (Barbieri et al., 2014; Krawczak, Binder, et al., 2016; Szabó, Nieri-Bastos, et al., 2013).

5 | CONCLUSION

Considering the previous tick collection and health informs of seashore Atlantic Forest areas of Paraná State, the serological and molecular findings in the present study have confirmed previous tick and clinical case reports and enlarged the geographical occurrence of *A. ovale* infected by *R. parkeri* strain Atlantic rainforest in oceanic islands and seashore mainland cities of Paraná State, indicating a new likely transmission area of this new rickettsial infection in owners and dogs of southern Brazil. In addition, dogs may play a role in the herein region connecting enzootic cycles that occur in the wild ecotope with the peridomicile and domicile.

AUTHOR CONTRIBUTIONS

Louise Bach Kmetiuk, Felipe da Silva Krawczak and Alexander Welker Biondo conceptualized the idea of the study. Louise Bach Kmetiuk, Warley Vieira de Freitas Paula, Gracielle Teles Pádua, Luiza Gabriella Ferreira de Paula, Felipe da Silva Krawczak and Alexander Welker Biondo designed methodology. Louise Bach Kmetiuk, Warley Vieira de Freitas Paula, Gracielle Teles Pádua, Luiza Gabriella Ferreira de Paula, Vamilton Álvares Santarém, Felipe da Silva Krawczak and Alexander Welker Biondo performed validation. Warley Vieira de Freitas Paula, Gracielle Teles Pádua, Luiza Gabriella Ferreira de Paula, Rogério Giuffrida, Vamilton Álvares Santarém and Felipe da Silva Krawczak performed formal analysis. Louise Bach Kmetiuk, Ruana Renostro Delai, Aaronson Ramathan Freitas, João Henrique Farinhas and Alexander Welker Biondo curated the data. Louise Bach Kmetiuk, Felipe da Silva Krawczak and Alexander Welker Biondo wrote the original draft of the manuscript. Louise Bach Kmetiuk, Warley Vieira de Freitas Paula, Gracielle Teles Pádua, João Henrique Farinhas, Luiza Gabriella Ferreira de Paula, Rogério Giuffrida, Vamilton Álvares Santarém, Andrea Pires dos Santos, Fabiano Borges Figueiredo, Felipe da Silva Krawczak and Alexander Welker Biondo reviewed and edited the manuscript. Fabiano Borges Figueiredo, Felipe da Silva Krawczak and Alexander Welker Biondo performed supervision. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study area available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Ethical Appreciation at Ethics Committee in Human Health of the Brazilian Ministry of Health (protocol 46994521.0.0000.0102) and by the Ethics Committee of Animal Use (protocol number 036/2021) of the Federal University of Parana.

ORCID

Luiza Gabriella Ferreira de Paula https://orcid.org/0000-0002-6058-0495

Andrea Pires dos Santos https://orcid.org/0000-0002-1037-970X

Alexander Welker Biondo https://orcid.org/0000-0002-4182-5821

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SUPPORTING INFORMATION

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