

UNIVERSIDADE FEDERAL DO PARANÁ

AARONSON RAMATHAN FREITAS

“EFEITO ILHA”: SOROPREVALÊNCIA DE ANTICORPOS ANTI-
TOXOPLASMA GONDII EM MORADORES E SEUS CÃES EM
COMUNIDADES INSULARES DO LITORAL DO PARANÁ

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Aos povos originários de todo o Brasil que resistem a todos os desafios, com
muita resiliência e munidos da força da tradição.

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*“O otimismo é uma estratégia para criar um futuro melhor.
Porque a menos que você acredite que o futuro pode ser
melhor, é improvável que você assuma a
responsabilidade de criá-lo”*

Noam Chomsky

RESUMO

Introdução: A toxoplasmose é considerada uma das zoonoses mais negligenciadas em todo o mundo, afetando um terço da população mundial, e até o momento nenhum estudo se concentrou em comunidades tradicionais de pescadores e seus cães que vivem em ilhas costeiras. As comunidades tradicionais de pescadores artesanais no Brasil foram definidas como culturalmente diferenciadas e sob vulnerabilidade, juntamente com as populações indígenas e afro-brasileiras (quilombos).

Métodos: Anticorpos IgG anti - *Toxoplasma gondii* foram selecionados pelo teste de anticorpos fluorescentes indiretos (RIFI). Modelos de regressão logística univariável e multivariável foram realizados para avaliar os riscos para toxoplasmose.

Resultados: Em geral, a soropositividade anti- *T. gondii* foi observada em 59/328 (18,0%) dos proprietários e 66/283 (23,3%) dos cães pelo teste de imunofluorescência indireta (RIFI). Não foi observada diferença estatística entre proprietários ($p=0,360$) e cães ($p=0,655$) de ilhas e áreas continentais. De acordo com a análise bivariada, o consumo de água de nascentes locais ($p=0,016$; OR=2,11) foi fator de risco associado à soropositividade para *T. gondii*. O modelo de regressão logística mostrou significativamente que proprietários com hábito de consumo de água de nascente eram mais provavelmente soropositivos ($p=0,014$; OR=2,14). Na análise multivariada, a presença de anticorpos anti - *T. gondii* em cães foi associada a proprietários soropositivos ($p=0,008$; OR=2,81), consumo domiciliar de carne bovina ($p=0,042$; OR=1,7) e frango ($p=0,026$; OR=2,9). Não foram encontradas diferenças significativas de transmissão de *T. gondii* entre ilhas e áreas continentais para os proprietários ou seus cães.

Conclusões: Em conclusão, a soropositividade para toxoplasmose em comunidades de pescadores tem sido influenciada pela fonte de água e consumo de carne, e não por habitar ilhas, presença de cães, gatos ou ambos.

Palavras -chave: Soroprevalência; Toxoplasmose; Zoonose

ABSTRACT

Background: Toxoplasmosis is considered one of the most neglected zoonoses worldwide, affecting a third of the world's population, and to date no studies have focused on traditional communities of fishermen and their dogs living on coastal islands. Traditional artisanal fishing communities in Brazil were defined as culturally differentiated and under vulnerability, along with indigenous and Afro-Brazilian populations (quilombos).

Methods: Anti-*Toxoplasma gondii* IgG antibodies were screened by indirect fluorescent antibody test (IFAT). Univariable and multivariable logistic regression models were performed to assess the risks for toxoplasmosis.

Results: Overall, anti-*T. gondii* seropositivity was observed in 59/328 (18.0%) owners and 66/283 (23.3%) dogs by indirect immunofluorescent antibody test (IFAT). No statistical difference was observed between owners ($p=0.360$) and dogs ($p=0.655$) of islands and mainland areas. According to the bivariate analysis, water from local springs consumption ($p=0.016$; OR= 2.11) was associated risk factor for *T. gondii* seropositivity. The logistic regression model has significantly shown that owners with habit of spring water consumption were more likely seropositive ($p=0.014$; OR=2.14). At the multivariate analysis, presence of anti-*T. gondii* antibodies in dogs was associated to seropositive owners ($p=0.008$; OR=2.81), household consumption of beef meat ($p=0.042$; OR=1.7) and chicken ($p=0.026$; OR=2.9). No significant differences of *T. gondii* transmission were found between islands and mainland areas for either owners or their dogs.

Conclusions: In conclusion, seropositivity to toxoplasmosis in fisherman communities has been influenced by water source and meat consumption, and not by inhabiting islands, presence of dogs, cats or both.

Keywords: Seroprevalence; Toxoplasmosis; Zoonosis

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Lista de abreviaturas

COVID-19 – Doença infecciosa causada pelo coronavírus SARS-CoV-2

RIFI - Teste de Imunofluorescência Indireta

IFAT - Indirect Fluorescent Antibody Test

UFPR – Universidade Federal do Paraná

PUCPR – Pontifícia Universidade Católica do Paraná

OR – Odds Ratio

MAT - Modified Agglutination Test

ELISA - Enzyme-linked Immunosorbent Assay

IgG - Imunoglobulina G

IgM - Imunoglobulina M

KM – quilômetros/quilometres

RPM – Rotações por minuto

TX – Texas

EUA – Estados Unidos da América

PBS - Tampão fosfato-salino ou phosphate buffered saline

pH - Potencial hidrogeniônico

mm – Milímetros

Lista de símbolos

% - Porcentagem

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1 INTRODUÇÃO

As comunidades tradicionais no Brasil têm sido definidas como grupos culturalmente diferenciados e vulneráveis, com formas próprias de organização social, ocupando o território nativo e utilizando os recursos naturais como condição para suas expressões, gerados e transmitidos pela tradição (1).

Embora historicamente caracterizado, principalmente, pelo auto-reconhecimento e herança rudimentar ou não documentada, o enfrentamento da pandemia da COVID-19 (distanciamento social, proteção, prevenção e vacinação) recentemente garantiu o reconhecimento e a prioridade de tais comunidades por lei federal, incluindo indígenas, quilombolas (afrodescendentes brasileiros), assentamentos e comunidades de pescadores artesanais (2).

A toxoplasmose é uma zoonose de distribuição mundial que afeta um terço da população causada pelo *Toxoplasma gondii*, um protozoário que tem como hospedeiros definitivos gatos e outros felídeos, que pode liberar oocistos fecais que podem infectar uma variedade de hospedeiros intermediários, incluindo seres humanos (3). Como os oocistos de *T. gondii* eliminados nas fezes de animais podem ser ambientalmente viáveis, restando a infectividade por um longo tempo, particularmente sob condições climáticas ideais, como áreas tropicais (4), uma abordagem *One Health* deve ser feita para abordar holisticamente seres humanos, animais e ambientes saúde para avaliar a transmissão da toxoplasmose (5) em populações insulares tradicionais.

Estudos anteriores mostraram a disseminação do parasita em Fernando de Noronha, destino turístico insular brasileiro com sobreposição de ambientes humanos-animais (6). Nesses estudos, uma alta soroprevalência de *T. gondii* foi encontrada em 172/341 (50,4%) seres humanos em ilhéus, relacionada à alta sorologia da população felina e contaminação ambiental de oocistos (6,7). Nesse cenário, o efeito ilha deve ser hipotetizado para descrever uma potencial transmissão exacerbada de patógenos devido ao isolamento e exposição contínua em ilhas, principalmente no ambiente, com ocorrência diária e múltiplas ciclovias favorecidas pelo clima adequado (8). Os cães têm sido considerados sentinelas da toxoplasmose humana devido à sua domiciliação e compartilhamento de domicílio, expostos principalmente às mesmas fontes de água e comida caseira (9–11).

No Brasil, a soroprevalência em cães variou de 7,95% (12) a 40,0% (13). Embora a toxoplasmose tenha sido considerada uma das seis doenças parasitárias negligenciadas (doença de Chagas, ciclosporiase, cisticercose, toxocaríase, toxoplasmose e tricomoníase) que requerem ações de saúde pública mundial (14), nenhum estudo abrangente até o momento avaliou concomitantemente proprietários de ilhas e continentes e seus cães quanto à presença de anticorpos anti- *T. gondii*.

Assim, o objetivo do presente estudo foi detectar a presença de anticorpos anti-*T. gondii* em seres humanos e seus cães que vivem em comunidades tradicionais de pescadores de áreas insulares e litorâneas do sul do Brasil e comparar soroprevalência do patógeno em ambientes insulares e continentais.

REFERÊNCIAS

1. POVOS E COMUNIDADES TRADICIONAIS — MINISTÉRIO DA CIDADANIA Secretaria Especial do Desenvolvimento Social [Internet]. [citado em 8 de novembro de 2021]. Disponível em: <http://mds.gov.br/assuntos/seguranca-alimentar/direito-a-alimentacao/povos-e-comunidades-tradicionais>
2. Plano Nacional de Operacionalização da Vacinação contra Covid-19 — Português (Brasil) [Internet]. [citado em 5 de novembro de 2021]. Disponível em: <https://www.gov.br/saude/pt-br/coronavirus/publicacoes-tecnicas/guias-e-planos/plano-nacional-de-vacinacao-covid-19/view>
3. Dubey JP, Cerqueira-Cézar CK, Murata FHA, Kwok OCH, Yang YR, Su C. All about toxoplasmosis in cats: the last decade. *Veterinary Parasitology*. 2020 julho;283:109145.
4. Chacín-Bonilla L, Sánchez-Chávez Y, Estévez J, Larreal Y, Molero E. Prevalence of human toxoplasmosis in san carlos island, venezuela. *INCI* [Internet]. 2003 Ago [citado 2022 Nov 27]; 28(8): 457-462.
5. Pettan-Brewer C, Martins AF, de Abreu DPB, Brandão APD, Barbosa DS, Figueroa DP, et al. From the Approach to the Concept: One Health in Latin America-Experiences and Perspectives in Brazil, Chile, and Colombia. *Front. Public Health*, 14 September 2021.1;9:687110.
6. Magalhães FJR, Ribeiro-Andrade M, Souza FM, Lima Filho CDF, Biondo AW, Vidotto O, et al. Seroprevalence and spatial distribution of *Toxoplasma gondii* infection in cats, dogs, pigs and equines of the Fernando de Noronha Island, Brazil. *Parasitology International*. 2017 abril;66(2):43–6.
7. Carvalho M da C, Ribeiro-Andrade M, Melo RPB de, Guedes DM, Pinheiro Junior JW, Cavalcanti EFTSF, et al. Cross-sectional survey for *Toxoplasma gondii* infection in humans in Fernando de Noronha island, Brazil. *Rev Bras Parasitol Vet = Brazilian J Vet Parasitol Orgao Of do Col Bras Parasitol Vet*. 2021;30(3):e005121.
8. Delai RR, Freitas AR, Kmetiuk LB, Merigueti YFFB, Ferreira IB, Lescano SAZ, et al. One Health approach on human seroprevalence of anti-*Toxocara* antibodies, *Toxocara* spp. eggs in dogs and sand samples between seashore mainland and island areas of southern Brazil. *One*

- Health [Internet]. 2021;13:100353. Disponível em: <https://www.sciencedirect.com/science/article/pii/S2352771421001439>
9. Acha PN, Szyfres B. Zoonosis y enfermedades transmissibles comunes al hombre ya los animales: Volumen 1: bacteriosis and micosis. 2001;398–398.
 10. Silva RC da, Souza LC de, Langoni H, Tanaka EM, Lima VY de, Silva AV da. Risk factors and presence of antibodies to *Toxoplasma gondii* in dogs from the coast of São Paulo State, Brazil. *Pesqui Veterinária Bras* [Internet]. 2010 [citado em 5 de novembro de 2021];30(2):161–6. Disponível em: <http://www.scielo.br/j/pvb/a/w3GQsW5MKQPnvTj6RwDGmLN/?lang=en>
 11. Navarrete MG, Cordeiro MD, Batista Y, Alonso JC, Márquez M, Roque E, et al. Serological detection of *Toxoplasma gondii* in domestic dogs in the western region of Cuba. *Relatórios Vet Parasitol Reg Stud* [Internet]. 2017;9:9–12. Disponível em: <https://www.sciencedirect.com/science/article/pii/S2405939016301812>
 12. Cunha GR da, Pellizzaro M, Martins CM, Rocha SM, Yamakawa AC, Silva EC da, et al. Spatial serosurvey of anti-*Toxoplasma gondii* antibodies in individuals with animal hoarding disorder and their dogs in Southern Brazil. *PLoS Um*. 2020;15(5):e0233305.
 13. Meireles LR, Galisteo AJJ, Pompeu E, Andrade HFJ. *Toxoplasma gondii* disseminando-se em área urbana avaliada por soroprevalência em cães e gatos de vida livre. *Trop Med Int Health*. 2004 ago;9(8):876–81.
 14. CDC - Parasitas - Infecções Parasitárias Negligenciadas (NPIs) nos Estados Unidos [Internet]. [citado em 24 de março de 2022]. Disponível em: <https://www.cdc.gov/parasites/mpi/index.html>

2 ARTIGO CIENTÍFICO

Seropositivity of Anti-*Toxoplasma gondii* Antibodies in Owners and Their Dogs Living on Island and Mainland Seashore Areas of Southern Brazil

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ABSTRACT

Although toxoplasmosis has been considered among the most neglected zoonoses worldwide, no study has focused on the frequency and associated risk factors of owners and their dogs living on an island and mainland seashore areas. Accordingly, anti-*Toxoplasma gondii* IgG antibodies were screened by indirect fluorescent antibody test (IFAT) in owners and dogs from three oceanic islands and two nearby mainland harbor areas, with associated risk factors for toxoplasmosis assessed by univariable and multivariable logistic regression models. Overall, anti-*T. gondii* seropositivity was observed in 59/328 (18.0%)

owners and 66/283 (23.3%) dogs, with no statistical difference between owners ($p = 0.360$) and dogs ($p = 0.655$) from islands and mainland areas. Consumption of local water springs ($p = 0.016$; OR = 2.11) was an associated risk factor for *T. gondii* seropositivity, and owners with the habit of spring water intake were twice more likely seropositive ($p = 0.014$; OR = 2.14). Presence of anti-*T. gondii* antibodies in dogs was associated with seropositive owners ($p = 0.008$; OR = 2.81), household consumption of beef meat ($p = 0.042$; OR = 1.7) and chicken ($p = 0.026$; OR = 2.9). Despite being lower than the worldwide prevalence, toxoplasmosis seropositivity in owners and their dogs in southern Brazil was influenced by the positive owner, water source, and meat consumption, and not by inhabiting islands or seashore mainland areas, presence of dogs, cats, or both. In addition, drinking water quality should always be considered a critical risk factor for toxoplasmosis on islands.

Keywords: Brazil; One Health; pets and human health; serosurvey; toxoplasmosis; zoonosis

2.1 INTRODUCTION

Toxoplasmosis has been described as a worldwide distributed zoonosis caused by *Toxoplasma gondii*, a protozoan having cats and other Felidae as definitive hosts, which shed oocysts into feces to infect a variety of intermediate hosts, including dogs and human beings [1]. As shed *T. gondii* oocysts may be environmentally viable and retain longtime infectivity, particularly under optimal weather conditions, a One Health approach should be applied to concomitantly assess the human, animal, and environmental role on pathogen transmission [2].

Previous studies have already shown *T. gondii* overspreading in the Brazilian oceanic island of Fernando de Noronha, a major national preservation park and touristic destination 545 km far (340 miles) from the Brazilian mainland seashore, with a human seroprevalence of 172/341 (50.4%) inhabitants, along with high prevalence of 248/348 (71.3%) pet and 135/247 (54.7%) feral cat populations [3,4]. In this confined scenario of islands and seashore areas, a potential exacerbated transmission may occur due to favorable climate and daily exposure, as previously shown in the same areas studied herein for toxocariasis

[5]. Dogs have been considered sentinels for human toxoplasmosis due to domiciliation and household sharing, mostly exposed the same homemade food and water sources [6–8]. Although seroprevalence studies of dog toxoplasmosis in Brazil have ranged from 7.95% [9] to 40.0% [10], Fernando de Noronha Island has shown surprising seropositivity of 156/320 (48.8%) dogs [4]. In addition, human toxoplasmosis has been considered one of the six neglected parasitic diseases, along with Chagas, cyclosporiasis, cysticercosis, toxocariasis and trichomoniasis, demanding worldwide public health action [11]. Despite the consideration of toxoplasmosis as one of the most neglected zoonoses worldwide, no study to date has focused on the frequency and associated risk factors of owners and their dogs living on island and mainland seashore areas.

2.2 MATERIAL AND METHODS

2.2.1 Study design

The current study was designed as a cross-sectional seroepidemiological approach to owner and dog populations inhabiting oceanic islands and seashore mainland areas in the Paraná State, southern Brazil, from July 2019 to February 2020. The study herein was approved by the Ethics Committee of Animal Use at the Federal University of Parana (protocol 036/2021) and the Ethics Committee in Human Health at the Brazilian Ministry of Health (protocol 46994521.0.0000.0102).

2.2.2 Local of study

The serological survey was performed on three oceanic islands, Superagui Island, Ilha do Mel Island, and Peças Island, and two seashore mainland areas of the cities of Guaraqueçaba and Pontal do Paraná, located in the Paraná State, southern Brazil. The three Islands were environmentally protected units at the time, with Ilha do Mel Island State Park and Superagui Island National Park located within the largest unfragmented area of the Brazilian Atlantic Forest biome [12].

The largest site, Ilha do Mel Island (25°32'03.16" S and 48°18'015.67" W), comprises two major uninhabited conservation parks (Ecological Station and State Park), protected by environmental laws and representing 93.4% of the total

area of 2762.0 hectares [12]. Despite a local community of traditional culture with only 1100 permanent inhabitants, the island was ranked as the second biggest state touristic destination with 200,000 visitors annually. Currently distributed in five villages, local residents rely primarily on tourism for living [13].

The Superagui Island (25°27'025.31" S and 48°14'046.05" W) holds the Superagui National Park, a conservation unit adjacent to local traditional artisanal fisherman communities. The sampling point of Barra do Superagui with approximately 700 inhabitants and 350 dogs, was the major community at the time [14]. The smallest site, Peças Island (25°27'022" S and 48°20'007" W) were classified as fully environmentally protected area by the Brazilian government, particularly due to a dolphin nursery area [14]. Historically established during slavery, the Peças Island was inhabited by around 350 persons living on artisanal fishing and tourism at the time.

In addition, the touristic and commercial harbors of Pontal do Paraná (25°40'026" S and 48°30'039" W) and Guaraqueçaba (25°18'025" S and 48°19'044" W) cities, two of the nearest seashore areas located at the northern Parana State coastal region, were sampled for mainland comparison (Figure 1) [13,14].

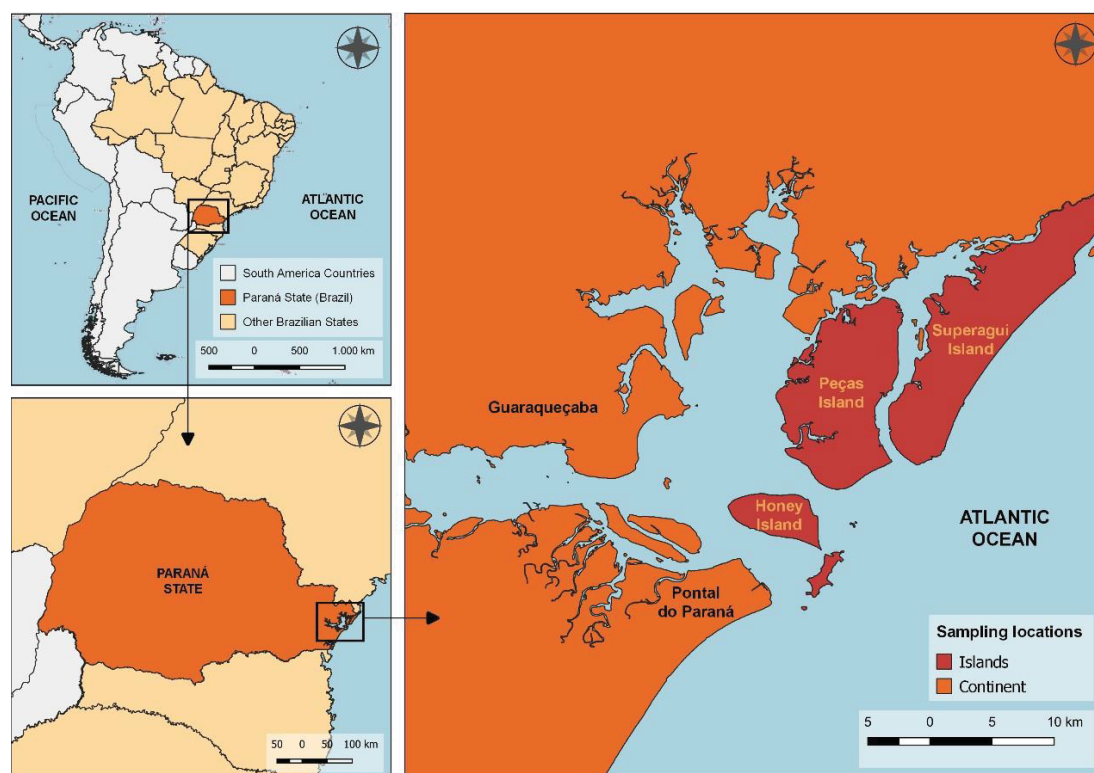


Figure 1. Sampling locations and frequency of anti-*T. gondii* antibodies in humans and their dogs from island and seashore mainland areas of southern Brazil.

2.2.3 Blood samples Collection

Populations inhabiting oceanic islands and seashore mainland areas were chosen by convenience, as owners were informed about the human and dog volunteer samplings by city officials when bringing their dogs for a series of free-of-charge campaigns of physical examination followed by high-quality high-volume spaying/neutering. Volunteer owners were then individually sampled by cephalic puncture after signed consent and completion of an epidemiological questionnaire, conducted by certified physicians and nurses at the local Basic Health Unit. Dogs were sampled by jugular puncture after signed owner consent and conducted by certified veterinarians. All samples were then placed in serum-separating tubes, centrifuged at 1500 RPM for five minutes, serum separated and stored at -20°C until processing.

2.2.4 Serological testing

Owner and dog serum samples were assayed for specific IgG antibodies against *T. gondii* by Indirect Fluorescent Antibody Test (IFAT) in five serial dilutions of phosphatebuffered saline pH 7.2 (PBS: 8.2 g NaCl: 1.9 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 0.3 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) solution, including 1:16, 1:64, 1:256, 1:1024 and 1:4096. Immunofluorescence slides were priorly sensitized with 0.1% formaldehyde to inactivated *T. gondii* tachyzoites (RH strain), and then applied for fluorescence using a commercial anti-IgG (human or dog) specific antibody conjugated with fluorescein isothiocyanate (Bethyl-Montgomery, TX, USA). Readings were performed by a certified researcher using an immunofluorescence microscope.

Seropositive samples were confirmed with antibody titers ≥ 16 for *T. gondii*, as the established cutoff point, and final serum titers were determined by the highest dilution with $\geq 50\%$ tachyzoites still showing fluorescence. Positive and negative human and dog controls were added to each slide to ensure quality control in all readings. The Indirect Fluorescent Antibody Test (IFAT) was performed in this study, as it is recommended as the gold standard, described

with greater specificity for the diagnosis of *T. gondii* [15,16] and comparable sensitivity to the Enzyme-linked Immunosorbent Assay (ELISA) and Modified Agglutination Test (MAT) [16–18]. Serum samples from the owner and dog were screened for specific IgG antibodies against *T. gondii* by the Indirect Fluorescent Antibody Test (IFAT). Serum samples from the owner and dog were screened for specific IgG antibodies against *T. gondii* by the Indirect Fluorescent Antibody Test (IFAT) in five serial dilutions of phosphate-buffered saline pH 7.2 (PBS: 8.2 g NaCl: 1.9 g Na₂HPO₄·7H₂O and 0.3 g NaH₂PO₄·H₂O) solution, including 1:16, 1:64, 1:256, 1:1024 and 1:4096, according to other studies that advocated this diagnostic method [19–22]. Immunofluorescence slides were previously sensitized with 0.1% formaldehyde to inactivated tachyzoites of *T. gondii* (RH strain), and then applied for fluorescence using a commercial anti-IgG specific antibody (human or canine) conjugated to fluorescein isothiocyanate (Bethyl Laboratories, Inc., Montgomery, TX, USA). Readings were taken by a certified researcher using an immunofluorescence microscope.

2.2.5 Epidemiological data collection

Human-associated risk factor analyses were based on an epidemiological questionnaire assessing potential exposure to *T. gondii*, which included owner age, gender, education level, household income and location, animal (dog or cat) ownership, drinking water source, food washing before eating, other raw or undercooked meat consumption. Dog-associated risk factors analyses were also based on epidemiological questionnaire data, including residence location, sex, cohoused with other dogs or cats, daily diet, drinking water source, raw meat intake, beach access and hunting habits.

2.2.6 Statistical analysis

A data set was constructed according to place of sampling (islands versus mainland areas) with the stratified outcome, where data with any inconsistency type was discharged after analysis from the data set. Seropositivity to *T. gondii* and potential associated risk factors were tested by variable categorization. Questionnaire responses such as “unknown” or “not informed” were considered

as lost information and removed from analyses when of insignificant impact (<10% of total). Variable was disposed in tables of double entry after categorization and tested for associations with anti-*T. gondii* seropositivity by Pearson's chi-squared test. When one or more table spots presented expected values lower than 5, Fisher's exact test was applied as an alternative.

Variables showing statistical significance equal to or lower than 0.2 were submitted to the multivariate logistic regression model, using the stepwise backward-type selection method as a source for the best estimative of Akaike information criteria. Likelihood reasons per interval were projected in both univariate and multivariate models at 95% confidence (odds ratio), with all analyses considering a 5% significance level and conducted using the R package [23,24].

In the logistic regression analysis, the final solution of backward stepwise approach was determined after one step, removing one (consumption of water consumption) out of the five seropositivity predictors including gender, water consumption, spring water consumption, and wash fruits/vegetables; only using water for washing fruits and vegetable for owners. In dogs, final solution was determined after two steps, with deletion of two (ingestion of cattle beef and ingestion of fish) out of five predictors (seropositive owner; home food diet; cattle, chicken, or fish meat).

2.3 RESULTS

Antibodies to *T. gondii* were detected in 59/328 (18.0%; 95% CI: 14.1–22.4%) owners (Figure 1). Despite a higher seropositivity on islands with 37/194 (19.1%; 95% CI: 14.2–25.2%) seropositive owners when compared to 22/134 (16.4%; 95% CI: 11.1–23.6%) positive owners on seashore mainland areas, no statistical difference was observed ($p = 0.562$; OR = 0.8).

According to the univariate analysis, the consumption of treated water ($p=0.047$; OR=0.5) was as a protective factor, while water from local springs ($p=0.016$; OR= 2.1) a risk factor for seropositivity to *T. gondii*. By the logistic regression only the consumption of spring water ($p=0.027$; OR=2.0) was significantly associated to *T. gondii* seropositivity (Table 1).

Antibodies to *T. gondii* were detected in 66/283 (23.3%; 95% CI: 18.8–28.6%) dogs (Figure 1). Despite a slightly higher seropositivity in dogs on seashore mainland with 38/154 (24.7%; 95% CI: 18.5–32.1%) when compared to 28/129 (21.7%; 95% CI: 15.5–29.6%) on island dogs, no statistical difference was observed ($p = 0.655$).

Logistic regression revealed the consumption of chicken meat ($p=0.028$; OR=2.9) and having a seropositive owner as risk factor for *T. gondii* seropositivity in dogs (Table 2). The evaluation of breed, age and dog hunting habits was not possible due to the lack of precision information given by owner dogs, representing more than 10% of lost information. Thus, the variables were removed from analyses.

1

Table 01. Associated risk factors of anti-*Toxoplasma gondii* antibodies detected by IFAT test in humans from island and seashore mainland areas of southern Brazil (N= 328).

Variable	Bivariate Analysis				Multivariate Analysis	
	anti- <i>T. gondii</i> antibodies		OR (95% IC)	P value	OR (95% IC)	P value
	seropositive No. (%)	Seronegative No. (%)				
	59/328 (18.0)		269/328 (82.0)			
Household location						
Seashore mainland	22 (37.3)	112 (41.6)	Ref			
Island	37 (62.7)	157 (58.4)	0.8 (0.47-1.49)			
Gender			0.098			
Female	31 (52.5)	175 (65.1)	Ref.		Ref	
Male	28 (47.5)	94 (34.9)	1.7 (0.94-2.98)		1.6 (0.87-2.85) 0.149	
Age (years old)			0.322			
>18	55 (93.2)	258 (95.9)	Ref.			
<18	4 (6.8)	11 (4.1)	0.6 (0.19-0.20)			
Education level			0.682			
> Elementary school	37 (62.7)	179 (66.5)	Ref.			
≤ Elementary school	22 (37.3)	90 (33.5)	1.2 (0.65-2.12)			
Income			0.627			
> 1 minimum wage	40 (70.2)	165 (65.7)	Ref			
≤ 1 minimum wage	17 (29.8)	86 (34.3)	0.8 (0.43-1.51)			
Dog owner			0.856			
No	7 (11.9)	27 (10.0)	Ref.			
Yes	52 (88.1)	242 (90.0)	0.8 (0.35-2.15)			

Cat owner	0.579		
No	39 (66.1)	164 (61.2)	Ref.
Yes	20 (33.9)	104 (38.8)	0.8 (0.44-1.46)
Water consumption			
Treated water	0.047*		
No	27 (45.8)	84 (31.2)	Ref.
Yes	32 (54.2)	185 (68.8)	0.5 (0.30-0.96)
Well water	1.0		
No	52 (88.1)	236 (87.7)	Ref.
Yes	7 (11.9)	33 (12.3)	1.0 (0.38-2.23)
Spring water	0.016		
No	33 (55.9)	196 (72.9)	Ref.
Yes	26 (44.1)	73 (27.1)	2.1 (1.17-3.78)
Wash fruits/vegetables			
Before consumption	0.118		
No	4 (6.8)	7 (2.6)	Ref.
Yes	55 (93.2)	260 (97.4)	0.4 (0.10-1.49)
Only water	0.177		
No	14 (23.7)	90 (33.8)	Ref.
Yes	45 (76.3)	176 (66.2)	1.6 (0.87-3.24)
Water and vinegar	0.546		
No	48 (81.4)	204 (76.7)	Ref.
Yes	11 (18.6)	62 (23.3)	0.8 (0.35-1.51)
Water with sodium hypochlorite	0.297		
No	56 (94.9)	238 (89.5)	Ref.
Yes	3 (5.08)	28 (10.5)	0.5 (0.11-1.42)
Consumption of raw or undercooked meat	0.3		

No	36 (61.0)	185 (69.0)	Ref.	
Yes	23 (39.0)	83 (31.0)	1.4 (0.78-2.55)	
Cattle				0.276
No	38 (64.4)	193 (72.6)	Ref.	
Yes	21 (35.6)	73 (27.4)	1.5 (0.79-2.65)	
Pig				1.0
No	56 (94.9)	249 (93.6)	Ref.	
Yes	3 (5.1)	17 (6.4)	0.8 (0.18-2.57)	
Chicken				0.746
No	57 (96.6)	252 (94.7)	Ref.	
Yes	2 (3.4)	14 (5.3)	0.7 (0.09-2.53)	
Fish				0.924
No	49 (83.1)	225 (84.6)	Ref.	
Yes	10 (16.9)	41 (15.4)	1.1 (0.50-2.34)	

2 *Variable has fit the criterion for logistic regression ($p < 0.2$) but it has not selected by the backward stepwise logistic regression model (R package).

Home cooked		0.054	
No	19 (28.8)	93 (43.1)	Ref.
Yes	47 (71.2)	123 (56.9)	1.9 (1.03-3.45)
Meat consumption		1.7 (0.93-3.20)	
Cattle		0.042*	
No	50 (75.8)	189 (87.1)	Ref.
Yes	16 (24.2)	28 (12.9)	2.2(1.06-4.28)
Pig		0.220	
No	62 (93.9)	212 (97.7)	Ref.
Yes	4 (6.06)	5 (2.30)	2.7(0.63-11.1)
Chicken		0.026	
No	57 (86.4)	206 (94.9)	Ref.
Yes	9 (13.6)	11 (5.07)	3.0 (1.12-7.56)
Fish		0.101*	
No	60 (90.9)	209 (96.3)	Ref.
Yes	6 (9.09)	8 (3.69)	2.6 (0.81-7.97)
Water consumption			
Tap water		0.243	
No	29 (43.9)	76 (35.0)	Ref.
Yes	37 (56.1)	141 (65.0)	0.7 (0.39-1.21)
Well water		0.923	
No	59 (89.4)	197 (90.8)	Ref.
Yes	7 (10.6)	20 (9.22)	1.2 (0.44-2.84)
Beach access		0.673	
No	23 (34.8)	84 (38.7)	Ref.
Yes	43 (65.2)	133 (61.3)	1.2 (0.67-2.12)

*Variables fitting the criterion for logistic regression ($p < 0.2$) but no selected by the backward stepwise logistic regression model (R package).

2.4 DISCUSSION

To the authors' knowledge, this was the first concomitant study on the occurrence of *T. gondii* antibodies in owners and their dogs living on islands and seashore mainland areas. The overall owner seropositivity in this study of the 18.0% was lower than most previous serosurveys, including the 49.8% in San Carlos Island, Venezuela [25] and all other Brazilian studies, with the 68.7% in the Pará State, northern Brazil [26], the 50.4% in the Fernando de Noronha Island, far northeastern Brazil [3], the 41.5% [27] and the 36.8% [9] in the same Parana State, southern Brazil, and average of the 32.7% in southern and central-western Brazil [28]. Seropositivity in the present study was higher than the 12.4% in Panama [29] and the 5.1% in provinces of China [30].

As previously shown in a nationwide USA survey, risk for IgG seropositivity increased with age and was higher in males, in persons living below the poverty level, and in persons with less than high school education [31]. Despite such results may reflect differences in cultural, socio-economic, sanitary, and religious characteristics, along with presence of domestic and wild animals, water source, animal and human health, seropositivity in this study was not statistically influenced by gender, age, educational level or family income. Besides age and educational level association to seropositivity, Fernando de Noronha Island has also presented significantly higher human and dog toxoplasmosis seropositivity than the present study, possibly due to a higher density of domestic cats as definitive protozoan hosts [3]. Although feral cat control programs have been conducted since 2007 in Fernando de Noronha, free-range and feral populations were estimated in a total of 1287 cats in 2017 [32]. In addition, climate differences between the northeastern Fernando de Noronha and the southeastern islands of the present study may have impacted on environmental persistence and spreading of *T. gondii*. While Fernando de Noronha presents tropical climate with dry (August–January) and rainy (February–July) seasons, with an average annual temperature of 26–27 °C and average rainfall of 1400 mm/year, the islands in this study present a super-humid

tropical-subtropical climate with no dry season, average temperature of 22 °C and average rainfall of 2500 mm/year [33]. Although a previous study has shown no evident infectivity loss of *T. gondii* oocysts maintained between 10 and 25 °C for 200 days in water [34], lower rainfall and mild dry season may provide better opportunities for environmental persistence and transmission. Thus, further studies should be conducted to better understand whether human and dog toxoplasmosis in seashore and island areas may be associated with local pet and feral cat populations, rainfall and/or rainy/dry season interchange, household distance from seashore, touristic visitation, professional exposure to sand (boatmen, waiters and other beach workers) and beach walking frequency.

As previously established, the main transmission routes of toxoplasmosis may include tissue cyst ingestion in raw or undercooked meat of infected animals, ingestion of fresh vegetables or water contaminated with *T. gondii* oocysts from cat feces [35,36]. In this study, the human water source was the only variable showing significant differences, with spring water presenting twice more likelihood of *T. gondii* IgG antibodies. In addition, piped water has been more likely contaminated with oocysts than river and roof water, compared to other drinking-water sources [29]. Water springs may also be contaminated by *T. gondii* feces from wild felids reservoirs [37]. As human anti-*T. gondii* antibodies were also associated with drinking rain or well water (OR = 2.4) in the Fernando de Noronha Island [3], drinking water quality should be always considered a critical risk factor for toxoplasmosis on islands.

Rainy seasons may also facilitate the dispersion of oocysts in the environment, increasing exposure to *T. gondii* infection, even those not owning cat at home, but drinking water from water springs with felid access as may occur in this study [3]. Waterborne outbreaks of toxoplasmosis have been frequently associated with inappropriate home-filtered or unfiltered water consumption in Brazil [38]. In a previous study, unfiltered municipally treated water was epidemiologically related with 155 cases of toxoplasmosis in Southern Brazil in 2001 [39]. In another study, consumption of home-filtered water by faucet-mount filtered was suggested as risk

factor for toxoplasmosis outbreak occurred in Northeastern Brazil in 2006, probably associated with one step of filtration occurs when the tap is unlocked [40]. In a recent study of potential source of 809 toxoplasmosis cases in Southern Brazil, bioassay with mice and piglets suggested *T. gondii* contamination in public treated water [41]. In urban areas, *T. gondii* oocysts may be resistant to usual water treatment with chlorinated products and decantation, probably associated with inner layers of the oocyst and sporocyst walls, as water supply used in these areas may usually come from distant unprotected areas and lead to human outbreaks by oocyst contamination of drinking water [42,43]. In addition, a recent systematic review has shown that only radiation and pressure were effective in destroying oocysts, while disinfectants in water has insufficiently or no effect on *T. gondii* oocysts [44].

No association was observed in the present study when comparing the consumption of raw meat and seropositivity, which may suggest that animal protein consumption was mostly based on intake of cooked or non-contaminated fish/seafood, which may have reduced the likelihood of ingesting contaminated chicken or beef meat. Thus, although consumption of local spring water was a risk factor in the present study, the relatively lower seropositivity of *T. gondii* in human beings when compared to other studies in Brazil may be consequence of dietary habits, based on cooked and/or non-contaminated protein source (mainly fish/seafood), as chicken meat consumption and having a seropositive owner were risk factors for dog seropositivity, corroborating with such hypothesis. Not surprisingly, our research group has recently shown that dietary habits of homeless persons and persons with animal hoarding behavior may also impact in lower seropositivity than the general population, mostly due to low intake of raw or uncooked meat and fresh vegetables [9,45].

Nonetheless, the study has shown the 39.0% of seropositive individuals declaring consumption of chicken and/or beef meat. No study to date was conducted with domestic animals, such as cattle and chicken, in island and seashore mainland of Parana State. A study with 386 serum samples of free-range chickens of northwest Parana has shown 102/386 (26.4%) seropositive for *T. gondii* by IFA,

64/386 (16.6%) by MAT and 47/386 (12.2%) in both tests [46]. In addition, samples of 38/119 (31.9%) seropositive free-range chickens used for isolation were considered positives in mouse bioassay, with high diversity of isolates ($n = 18$) [46]. A recent review has shown that cattle *T. gondii* seroprevalence ranged from 25.8 to 48.5% in Parana State [47].

Although 124/327 (37.9%) owners declared having cat at home, no risk association was observed on seropositivity to *T. gondii* antibodies. A previous study in Northern Brazil has shown that, despite no statistical significance observed for water source or water treatment, having a cat represented 1.95-fold more chance to be seropositive [26]. Dogs have also been proposed as toxoplasmosis sentinels for human infection, mostly due to sharing inhouse spaces and related exposure to food and water sources [6–8]. The overall seropositivity in dogs in this study (23.3%) was lower than the 40.8% in Bahia State, northeastern Brazil [48], 40.0% [10] and the 25.4% in Sao Paulo State, southeastern Brazil [8], the 36.5% in Bahia State, northeastern Brazil [49], the 34% in Rio de Janeiro State, southeastern Brazil [50], and the 32.7% in southern and central-western Brazil [28]; only higher than the 16.3% [27] and the 7.95% in the same Parana State, southern Brazil [9]. In addition, the observed seropositivity herein was lower than the 72.7% in Cuba [7] and higher than the 18.6% [51] and the 21.6% in China [52].

In the present study, dog seropositivity has not been associated with cats living in the same household. Still controversial, contact with cats may [53] or may not be considered a significant risk factor for dogs [9,28,53]. However, dogs with seropositive owners in this study have shown 2.81-fold more likelihood of seropositivity than those with seronegative owners ($p = 0.008$), which may be related to owner-dog sharing of food and water intake, emphasizing the One Health nature of toxoplasmosis. Although dogs eating homemade food were more likely seropositive in a previous study, varying from 1.70 to 7.00-fold in Sao Paulo [8], such risk was not be present in another report [9]. Despite dog meat consumption may vary as *T. gondii* infection source and raw rat meat has previously increased seropositivity by 5.19-fold [28], no raw meat was a risk factor in this study, exception

for chicken meat, probably uncooked, increasing serology by 2.90-fold. Intervention actions were indicated to local health services, including sanitary measures on appropriate food cooking and water source, which may control and prevent future infections [8].

Although owned dogs cohoused to cats were not at higher risk, cats have been well established to play crucial role on indirect epidemiologic chain of *T. gondii* cycle by shedding oocysts into their feces, contaminating soil and water sources [25]. Despite cat presence in islands may increase human and dog seropositivity, and dogs may serve as important sentinels for human exposure, cat contact or presence at home were not associated with infection in mainland urban settings [3,27].

As limitations, comparisons of *T. gondii* transmission between owners and their dogs living on islands and seashore mainland areas may be impaired due to household changing, since persons may have been born or previously lived in islands and moved to continent, or vice versa, or may work in daily boat transportation or commercial trading, with frequent mainland-island movement. In addition, the present study has been limited to investigate only *T. gondii* antibodies in owned dogs, not assessing unowned dogs and pet or feral cats, which may play a role in toxoplasmosis transmission due to free outdoors access, particularly nearby spring water source. In addition, the variables age, dog hunting behavior and dog breed were analysed only in the univariate analyses (Table S1). Data concerning age and dog hunting behavior showed expressive lost information (18.4 and 11.3%, respectively). Increase of risk for seropositivity was observed in dogs aging more than one year, but only one dog younger than a year was positive. Consequently, the range of CI 95% for the Odds Ratio was extremely large (1 to 8 years-old: OR = 8.79; CI 95% = 1.81–212, older than 8 years: OR = 16.4; CI 95% = 2.68–433) leading to a potential imprecision of results. Despite dog breed showed only 9/283 (3.2%) dogs with missing information, imprecision of answers given by owners made this variable not fitted for the final model of logistic regression.

Finally, educative programs should be established to reduce *Toxoplasma gondii* risk of exposure among travelers, sightseers and residents in endemic areas, particularly by avoiding spring water consumption [54].

2.5 CONCLUSIONS

The present study is the first concurrent report of anti-*T. gondii* in owners and correspondent dogs of islands and seashore mainland areas. Although different risk factors may interfere on both human and animal infection occurrence, individually or together, the present study has shown different associated risk factors for owners and their dogs. Human being *T. gondii* exposure has been associated with spring water consumption, and dog exposure was associated with seropositive owner, household consumption of beef meat and chicken. Dogs of seropositive owners were more likely seropositive than those of seronegative owners, probably related to owner-dog sharing of food and water, emphasizing the One Health nature of toxoplasmosis. In addition, drinking water quality should be always considered a critical risk factor for toxoplasmosis on islands.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/tropicalmed7100252/s1>, Table S1: Univariate analysis for accessing bred, age and hunting behavior as risk factor for anti-*Toxoplasma gondii* antibodies detected by IFAT test in dogs from island and seashore mainland areas of southern Brazil (N = 283). The variables were analyzed separately due to the number of missing (age and hunting behavior: lost of information higher than 10%) or imprecision of data regarding bred provided by dog's owners.

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A.P.D.B., R.C.d.S., R.G. and V.A.S.; data curation, A.R.F., R.R.D., L.B.K., R.M., A.P.D.B., I.R.d.B.-F. and A.W.B.; writing—original draft preparation, A.R.F., L.B.K., R.G., V.A.S. and A.W.B.; writing—review and editing, A.R.F., L.B.K., E.C.d.S., R.G., F.B.F., A.P.D.S., V.A.S. and A.W.B.; supervision, C.T.P., A.P.D.S., V.A.S. and A.W.B. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Povos e Comunidades Tradicionais—Ministério da Cidadania Secretaria Especial do Desenvolvimento Social, (n.d.). Available online: <http://mds.gov.br/assuntos/seguranca->

alimentar/direito-a-alimentacao/povos-e-comunidades-tradicionais (accessed on 8 November 2021).

2. Plano Nacional de Operacionalização da Vacinação contra COVID-19—Português (Brasil), (n.d.). Available online: <https://www.gov.br/saude/pt-br/coronavirus/publicacoes-tecnicas/guias-e-planos/plano-nacional-de-vacinacao-covid-19/view> (accessed on 5 November 2021).

3. Carvalho, M.D.C.; Ribeiro-Andrade, M.; de Melo, R.P.B.; Guedes, D.M.; Junior, J.W.P.; Cavalcanti, E.F.T.S.F.; Magalhães, F.J.R.; Mota, R.A. Cross-sectional survey for *Toxoplasma gondii* infection in humans in Fernando de Noronha island, Brazil. *Rev. Bras. Parasitol. Vet.* 2021, 30, e005121. [CrossRef]

4. Magalhães, F.J.; Ribeiro-Andrade, M.; Souza, F.M.; Filho, C.D.L.; Biondo, A.W.; Vidotto, O.; Navarro, I.T.; Mota, R.A. Seroprevalence and spatial distribution of *Toxoplasma gondii* infection in cats, dogs, pigs and equines of the Fernando de Noronha Island, Brazil. *Parasitol. Int.* 2017, 66, 43–46. [CrossRef]

5. Delai, R.R.; Freitas, A.R.; Kmetiuk, L.B.; Merigueti, Y.F.F.B.; Ferreira, I.B.; Lescano, S.A.Z.; Gonzáles, W.H.R.; Brandão, A.P.D.; de Barros-Filho, I.R.; Pettan-Brewer, C.; et al. One Health approach on human seroprevalence of anti-*Toxocara* antibodies, *Toxocara* spp. eggs in dogs and sand samples between seashore mainland and island areas of southern Brazil. *One Health* 2021, 13, 100353. [CrossRef]

6. Acha, P.N.; Szyfres, B. Zoonosis y Enfermedades Transmisibles Comunes al Hombre y a los Animales; Volumen I: Bacteriosis y micosis; Pan American Health Organization: Washington, DC, USA, 2001; p. 398.

7. Navarrete, M.G.; Cordeiro, M.D.; Batista, Y.; Alonso, J.C.; Márquez, M.; Roque, E.; Fonseca, A. Serological detection of *Toxoplasma gondii* in domestic dogs in the western region of Cuba. *Veter. Parasitol. Reg. Stud. Rep.* 2017, 9, 9–12. [CrossRef]

8. Silva, R.; De Souza, L.C.; Langoni, H.; Tanaka, E.M.; De Lima, V.Y.; Silva, A. Risk factors and presence of antibodies to *Toxoplasma gondii* in dogs from the coast of São Paulo State, Brazil. *Pesqui. Veterinária. Bras.* 2010, 30, 161–166. [CrossRef]

9. Da Cunha, G.R.; Pellizzaro, M.; Martins, C.M.; Rocha, S.M.; Yamakawa, A.C.; Da Silva, E.C.; Dos Santos, A.P.; Morikawa, V.M.; Langoni, H.; Biondo, A.W. Spatial serosurvey of anti-*Toxoplasma gondii* antibodies in individuals with animal hoarding disorder and their dogs in Southern Brazil. *PLoS ONE* 2020, 15, e0233305. [CrossRef]

10. Meireles, L.R.; Galisteo, A.J.; Pompeu, E.; Andrade, H.F. *Toxoplasma gondii* spreading in an urban area evaluated by seroprevalence in free-living cats and dogs. *Trop. Med. Int. Health* 2004, 9, 876–881. [CrossRef]
11. CDC-Parasites-Neglected Parasitic Infections (NPIs) in the United States, (n.d.). Available online: <https://www.cdc.gov/parasites/npi/index.html> (accessed on 24 March 2022).
12. Parque Estadual da Ilha do Mel (PEIM) | Instituto Água e Terra, (n.d.). Available online: <http://www.iat.pr.gov.br/Pagina/Parque-Estadual-da-Ilha-do-Mel-PEIM> (accessed on 24 November 2021).
13. IBGE | Cidades@ | Paraná | Pontal do Paraná | Panorama, (n.d.). Available online: <https://cidades.ibge.gov.br/brasil/pr/pontal-do-parana/panorama> (accessed on 24 November 2021).
14. IBGE | Cidades@ | Paraná | Guaraqueçaba | Panorama, (n.d.). Available online: <https://cidades.ibge.gov.br/brasil/pr/guaraquecaba/panorama> (accessed on 24 November 2021).
15. Cola, G.A.; Garcia, J.L.; Da Costa, L.; Ruffolo, B.; Navarro, I.T.; Freire, R.L. Freire, Comparison of the indirect fluorescent antibody test and modified agglutination test for detection of anti-*Toxoplasma gondii* antibodies in rats. *Semin. Ciências Agrárias* 2010, 31, 717–722. [CrossRef]
16. Comparação do ensaio imunoenzimático indireto (ELISA-teste) com a reação da imunofluorescência indireta na detecção de anticorpos IgG anti-*Toxoplasma gondii* em ovinos naturalmente infectados. *Ci. Vet. Tróp.* 2014, 17, 16–20. Available online: <https://pesquisa.bvsalud.org/portal/resource/pt/vti-688292> (accessed on 11 June 2022).
17. Uchôa, C.M.A.; Duarte, R.; Laurentino-Silva, V.; Alexandre, G.M.C.; Ferreira, H.G.; Amendoeira, M.R.R. Padronização de ensaio imunoenzimático para pesquisa de anticorpos das classes IgM e IgG anti-*Toxoplasma gondii* e comparação com a técnica de imunofluorescência indireta. *Rev. Soc. Bras. Med. Trop.* 1999, 32, 661–669. [CrossRef]
18. Franco, W.A.C.; Bergamaschi, D.P.; Richtzenhain, L.J.; Nogueira, Y.; Camargo, L.M.A.; de Souza, S.L.P.; Gennari, S.M. Evaluation of the performance of the modified direct agglutination test (MAT) for detection of *Toxoplasma gondii* antibodies in dogs. *Braz. J. Veter. Res. Anim. Sci.* 2003, 40, 452–456. [CrossRef]

19. Dubey, J.P.; Hattel, A.L.; Lindsay, D.; Topper, M.J. Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission. *J. Am. Veter. Med Assoc.* 1988, 193, 1259–1263.
20. Dubey, J.P. *Toxoplasmosis of Animals and Humans*; CRC Press: Boca Raton, FL, USA, 2016.
21. Mantovan, K.B.; Luiz, C.G.S.; Menozzi, B.D.; Nilsson, M.G.; Latosinski, G.S.; Langoni, H. Inquérito soroepidemiológico para toxoplasmose, leptospirose e leishmaniose visceral canina. *Veterinária Zootec.* 2021, 28, 1–10. [CrossRef]
22. Calfele, J.L.R.; Langoni, H.; Mantovan, K.B. Detecção de imunoglobulinas anti-*Toxoplasma gondii* e anti-*Neospora caninum* em bovinos no rio grande do sul, brasil. *Veterinária Zootec.* 2021, 28, 1–7. [CrossRef]
23. R: The R Project for Statistical Computing, (n.d.). Available online: <https://www.r-project.org/> (accessed on 4 November 2021).
24. Subirana, I.; Sanz, H.; Vila, J. Building Bivariate Tables: The compare Groups Package for R. *J. Stat. Softw.* 2014, 57, 1–16. [CrossRef]
25. Prevalence of Human Toxoplasmosis in san Carlos Island, Venezuela, (n.d.). Available online: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-18442003000800005 (accessed on 4 November 2021).
26. Ramos, R.C.F.; Palmer, J.P.S.; Dib, L.V.; Lobão, L.F.; Pinheiro, J.L.; Dos Santos, C.R.; Uchôa, C.M.A.; Bastos, O.M.P.; Da Silva, H.P.; Fonseca, A.B.M.; et al. Soropositividade e fatores de risco associados à infecção por *Toxoplasma gondii* em pacientes atendidos no Laboratório Municipal de Oriximiná, estado do Pará, Brasil. *Rev. Pan Amaz. Saúde* 2021, 12, 1–11. [CrossRef]
27. Benitez, A.D.N.; Martins, F.D.C.; Mareze, M.; Santos, N.; Ferreira, F.P.; Martins, C.M.; Garcia, J.L.; Mitsuka-Breganó, R.; Freire, R.L.; Biondo, A.W.; et al. Spatial and simultaneous representative seroprevalence of anti-*Toxoplasma gondii* antibodies in owners and their domiciled dogs in a major city of southern Brazil. *PLoS ONE* 2017, 12, e0180906. [CrossRef]
28. Machado, F.P.; Kmetiuk, L.B.; Teider-Junior, P.I.; Pellizzaro, M.; Yamakawa, A.C.; Martins, C.M.; Bach, R.V.W.; Morikawa, V.M.; De Barros-Filho, I.R.; Langoni, H.; et al. Seroprevalence of anti-*Toxoplasma gondii* antibodies in wild boars (*Sus scrofa*), hunting dogs, and hunters of Brazil. *PLoS ONE* 2019, 14, e0223474. [CrossRef]

29. Etheredge, G.D.; Michael, G.; Muehlenbein, M.P.; Frenkel, J.K. The roles of cats and dogs in the transmission of *Toxoplasma* infection in Kuna and Embera children in eastern Panama. *Rev. Panam. Salud Pública* 2004, 16, 176–186. [CrossRef]
30. Xin, S.; Su, R.; Jiang, N.; Zhang, L.; Yang, Y. Low Prevalence of Antibodies Against *Toxoplasma gondii* in Chinese Populations. *Front. Cell. Infect. Microbiol.* 2020, 10, 302. [CrossRef]
31. Jones, J.L.; Kruszon-Moran, D.; Elder, S.; Rivera, H.N.; Press, C.; Montoya, J.G.; McQuillan, G.M. *Toxoplasma gondii* Infection in the United States, 2011–2014. *Am. J. Trop. Med. Hyg.* 2018, 98, 551–557. [CrossRef]
32. Dias, R.A.; Abrahão, C.R.; Micheletti, T.; Mangini, P.R.; Gasparotto, V.P.D.O.; Pena, H.F.D.J.; Ferreira, F.; Russell, J.C.; Silva, J.C.R. Prospects for domestic and feral cat management on an inhabited tropical island. *Biol. Invasions* 2017, 19, 2339–2353. [CrossRef]
33. Fernando de Noronha (PE) | Cidades e Estados | IBGE. Available online: <https://www.ibge.gov.br/cidades-e-estados/pe/fernando-de-noronha.html> (accessed on 7 June 2022).
34. Dubey, J.P. *Toxoplasma gondii* oocyst survival under defined temperatures. *J. Parasitol.* 1998, 84, 862. [CrossRef]
35. Almeria, S.; Dubey, J. Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. *Res. Veter. Sci.* 2021, 135, 371–385. [CrossRef]
36. Smith, N.C.; Goulart, C.; Hayward, J.A.; Kupz, A.; Miller, C.M.; van Dooren, G.G. Control of human toxoplasmosis. *Int. J. Parasitol.* 2021, 51, 95–121. [CrossRef]
37. Pinto-Ferreira, F.; Caldart, E.T.; Freire, R.L.; Mitsuka-Breganó, R.; De Freitas, F.M.; Miura, A.C.; Mareze, M.; Martins, F.D.C.; Urbano, M.; Seifert, A.L.; et al. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. *Rev. Bras. Parasitol. Veterinária* 2018, 27, 327–337. [CrossRef]
38. Bahia-Oliveira, L.; Gomez-Marin, J.; Shapiro, K. *Toxoplasma gondii*. In *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project)*; Michigan State University: East Lansing, MI, USA, 2019. [CrossRef]
39. De Moura, L.; Bahia-Oliveira, L.M.G.; Wada, M.Y.; Jones, J.L.; Tuboi, S.H.; Carmo, E.H.; Ramalho, W.M.; Camargo, N.J.; Trevisan, R.; Graça, R.M.T.; et al. Waterborne

Toxoplasmosis, Brazil, from Field to Gene. *Emerg. Infect. Dis.* 2006, 12, 326–329. [CrossRef]

40. e Silva, G.N.D.R.; Branco, M.D.R.F.C.; Rodrigues, Z.M.R.; dos Santos, A.M.; Pereira, P.R.M.; da Silva, M.D.S.; Nunes, A.T.D.S.; Júnior, A.R.G.; Medeiros, M.N.L.; Azevedo, C.D.M.P.E.S.D.; et al. Toxoplasmosis outbreak in Brazil, 2006-Revisited. *Parasite Epidemiol. Control* 2019, 7, e00117. [CrossRef]

41. Minuzzi, C.E.; Fernandes, F.D.; Portella, L.P.; Bräunig, P.; Sturza, D.A.F.; Giacomini, L.; Salvagni, E.; Ribeiro, J.D.S.; Silva, C.R.; Difante, C.M.; et al. Contaminated water confirmed as source of infection by bioassay in an outbreak of toxoplasmosis in South Brazil. *Transbound. Emerg. Dis.* 2020, 68, 767–772. [CrossRef]

42. Eng, S.B.; Werker, D.H.; King, A.S.; Marion, S.A.; Bell, A.; Issac-Renton, J.L.; Irwin, G.S.; Bowie, W.R. Computer-Generated Dot Maps as an Epidemiologic Tool: Investigating an Outbreak of Toxoplasmosis. *Emerg. Infect. Dis.* 1999, 5, 815–819. [CrossRef]

43. Dumètre, A.; Dubey, J.P.; Ferguson, D.J. Effect of household bleach on the structure of the sporocyst wall of *Toxoplasma gondii*. *Parasite* 2021, 28, 68. [CrossRef]

44. Pinto-Ferreira, F.; Paschoal, A.T.P.; Pasquali, A.K.S.; Bernardes, J.C.; Caldart, E.T.; Freire, R.L.; Mitsuka-Breganó, R.; Navarro, I.T. Techniques for inactivating *Toxoplasma gondii* oocysts: A systematic review. *Orgao Off. Do Col. Bras. Parasitol. Vet.* 2021, mboxemph30, e026420. [CrossRef]

45. Felipetto, L.G.; Teider-Junior, P.I.; Da Silva, F.F.V.; Yamakawa, A.C.; Kmetiuk, L.B.; Couto, A.C.D.; Martins, C.M.; Vaz, E.S.; Ullmann, L.S.; Langoni, H.; et al. Serosurvey of Anti-*Toxoplasma gondii* Antibodies in Homeless Persons of São Paulo City, Southeastern Brazil. *Front. Public Health* 2020, 8, 580637. [CrossRef]

46. Pena, H.F.J.; Alves, B.F.; Soares, H.S.; Oliveira, S.; Ferreira, M.N.; Bricarello, P.A.; Machado, T.M.P.; Castro, B.B.P.; Gennari, S.M. Free-range chickens from Santa Catarina state, southern Brazil, as asymptomatic intermediate hosts for *Toxoplasma gondii* clonal type I and typical Brazilian genotypes. *Veter. Parasitol. Reg. Stud. Rep.* 2018, 13, 55–59. [CrossRef]

47. Gomes, D.F.C.; Krawczak, F.D.S.; De Oliveira, C.H.S.; Júnior, F.; Fernandes, E.; Lopes, W.D.Z.; Sevá, A.D.P.; Gennari, S.M. *Toxoplasma gondii* in cattle in Brazil: A review. *Rev. Bras. Parasitol. Vet.* 2020, 29, e015719. [CrossRef]

48. Deiró, A.G.D.J.; Montargil, S.M.A.; Carvalho, F.S.; Munhoz, A.D.; Albuquerque, G.R. Antibody occurrence of Anti-*Toxoplasma gondii*, *Leishmania sp.* and *Ehrlichia canis* in dogs in Bahia State. *Semin. Ciências Agrárias* 2018, 39, 199–210. [CrossRef]
49. Occurrence of anti-*Toxoplasma gondii* antibodies and the risk factors associated with canine infection at ilhéus-itabuna region in the state of bahia. *Braz. J. Vet. Med.* 2010, 32, 115–121. Available online: <https://rbmv.org/BJVM/article/view/840> (accessed on 5 November 2021).
50. Arruda, I.F.; Millar, P.R.; Barbosa, A.D.S.; Abboud, L.C.D.S.; dos Reis, I.C.; Moreira, A.S.d.C.; Guimarães, M.P.d.P.; Amendoeira, M.R.R. *Toxoplasma gondii* in domiciled dogs and cats in urban areas of Brazil: Risk factors and spatial distribution. *Parasite* 2021, 28, 56. [CrossRef]
51. Sheng, Z.; Jin, Y.; Yao, Y.; El-Ashram, S.; Shen, J.; Wang, X.-L.; Ji, Y. Seroprevalence of *Toxoplasma gondii* Infection in Pet Dogs in Anhui Province, China. *Iran. J. Parasitol.* 2020, 15, 446–451. [CrossRef]
52. Duan, G.; Tian, Y.-M.; Li, B.-F.; Yang, J.-F.; Liu, Z.-L.; Yuan, F.-Z.; Zhu, X.-Q.; Zou, F.-C. Seroprevalence of *Toxoplasma gondii* infection in pet dogs in Kunming, Southwest China. *Parasites Vectors* 2012, 5, 118. [CrossRef]
53. Frenkel, J.K.; Parker, B.B. An Apparent Role of Dogs in the Transmission of *Toxoplasma gondii*: The Probable Importance of Xenosmophilia. *Ann. N. Y. Acad. Sci.* 1996, 791, 402–407. [CrossRef]
54. Sepúlveda-Arias, J.C.; Gómez-Marin, J.E.; Bobi'c, B.; Naranjo-Galvis, C.A.; Djurković-Djaković, O. Toxoplasmosis as a travel risk. *Travel Med. Infect. Dis.* 2014, 12, 592–601. [CrossRef]

3 CONCLUSÃO

Este é o primeiro estudo comparando a soroprevalência de *T. gondii* em seres humanos e seus cães, em comunidades insulares e costa continental. A soroprevalência entre tutores e seus cães foi associada ao fator de risco “água não tratada”, algo comum nas comunidades insulares, por ter um deficit de políticas públicas voltadas ao saneamento básico.

Dessa maneira, maiores estudos sobre os ambientes e rotinas dos moradores devem ser avaliados em uma próxima etapa deste estudo.

REFERÊNCIAS

ACHA, P.N.; SZYFRES, B. Zoonosis y Enfermedades Transmisibles Comunes al Hombre y a los Animales; Volumen I: Bacteriosis y micosis; Pan American Health Organization: Washington, DC, USA, 2001; p. 398.

ALMERIA, S.; DUBEY, J. Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. *Res. Veter. Sci.* 2021, 135, 371–385.

ARRUDA, I.F.; MILLAR, P.R.; BARBOSA, A.D.S.; ABBOUD, L.C.D.S.; DOS REIS, I.C.; MOREIRA, A.S.D.C.; GUIMARÃES, M.P.D.P.; AMENDOEIRA, M.R.R. *Toxoplasma gondii* in domiciled dogs and cats in urban areas of Brazil: Risk factors and spatial distribution. *Parasite* 2021, 28, 56.

BAHIA-OLIVEIRA, L.; GOMEZ-MARIN, J.; SHAPIRO, K. *Toxoplasma gondii*. In *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project)*; Michigan State University: East. Lansing, MI, USA, 2019.

BENITEZ, A.D.N.; MARTINS, F.D.C.; MAREZE, M.; SANTOS, N.; FERREIRA, F.P.; MARTINS, C.M.; GARCIA, J.L.; MITSUKA-BREGANÓ, R.; FREIRE, R.L.; BIONDO, A.W.; et al. Spatial and simultaneous representative seroprevalence of anti-*Toxoplasma gondii* antibodies in owners and their domiciled dogs in a major city of southern Brazil. *PLoS ONE* 2017, 12, e0180906.

CALLEFE, J.L.R.; LANGONI, H.; MANTOVAN, K.B. Detecção de imunoglobulinas anti- *Toxoplasma gondii* e anti- *Neospora caninum* em bovinos no rio grande do sul, brasil. *Veterinária Zootec.* 2021, 28, 1–7.

CARLOS, R. S. A., ALBUQUERQUE, G. R., BEZERRA, R. A., SICUPIRA, P. M. L., MUNHOZ, A. D., & LOPES, C. W. G. Occurrence of anti-*Toxoplasma gondii* antibodies and the risk factors associated with canine infection at ilhéus-itabuna region in the state of bahia. *Braz. J. Vet. Med.* 2010, 32, 115–121. Available online:

<https://rbmv.org/BJVM/article/view/840> (accessed on 5 November 2021).

CARVALHO, M.D.C.; RIBEIRO-ANDRADE, M.; DE MELO, R.P.B.; GUEDES, D.M.; JUNIOR, J.W.P.; CAVALCANTI, E.F.T.S.F.; MAGALHÃES, F.J.R.; MOTA, R.A. Cross-sectional survey for *Toxoplasma gondii* infection in humans in Fernando de Noronha island, Brazil. *Rev. Bras. Parasitol. Vet.* 2021, 30, e005121.

CDC-Parasites-Neglected Parasitic Infections (NPIs) in the United States, (n.d.). Available online: <https://www.cdc.gov/parasites/npi/index.html> (accessed on 24 March 2022).

CHACÍN-BONILLA, L.; SÁNCHEZ-CHÁVEZ, Y.; ESTÉVEZ, J.; LARREAL, Y.; MOLERO, E. Prevalence of human toxoplasmosis in san carlos island, venezuela. INCI [Internet]. 2003 Ago [citado 2022 Nov 27] ; 28(8): 457-462.

CHACÍN-BONILLA, L.; SÁNCHEZ-CHÁVEZ, Y.; ESTÉVEZ, J.; LARREAL, Y.; MOLERO, E. Prevalence of Human Toxoplasmosis in san Carlos Island, Venezuela, (n.d.). Available online: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-18442003000800005 (accessed on 4 November 2021).

COLA, G.A.; GARCIA, J.L.; DA COSTA, L.; RUFFOLO, B.; NAVARRO, I.T.; FREIRE, R.L. FREIRE, Comparison of the indirect fluorescent antibody test and modified agglutination test for detection of anti-*Toxoplasma gondii* antibodies in rats. *Semin. Ciências Agrárias* 2010, 31, 717–722.

DA CUNHA, G.R.; PELLIZZARO, M.; MARTINS, C.M.; ROCHA, S.M.; YAMAKAWA, A.C.; DA SILVA, E.C.; DOS SANTOS, A.P.; MORIKAWA, V.M.; LANGONI, H.; BIONDO, A.W. Spatial serosurvey of anti-*Toxoplasma gondii* antibodies in individuals with animal hoarding disorder and their dogs in Southern Brazil. *PLoS ONE* 2020, 15, e0233305.

DE MOURA, L.; BAHIA-OLIVEIRA, L.M.G.; WADA, M.Y.; JONES, J.L.; TUBOI, S.H.;

CARMO, E.H.; RAMALHO, W.M.; CAMARGO, N.J.; TREVISAN, R.; GRAÇA, R.M.T.; et al. Waterborne Toxoplasmosis, Brazil, from Field to Gene. *Emerg. Infect. Dis.* 2006, 12, 326–329.

DEIRÓ, A.G.D.J.; MONTARGIL, S.M.A.; CARVALHO, F.S.; MUNHOZ, A.D.; ALBUQUERQUE, G.R. Antibody occurrence of Anti-*Toxoplasma gondii*, *Leishmania* sp. and *Ehrlichia canis* in dogs in Bahia State. *Semin. Ciências Agrárias* 2018, 39, 199–210.

DELAI, R.R.; FREITAS, A.R.; KMETIUK, L.B.; MERIGUETI, Y.F.F. B.; FERREIRA, I. B.; LESCANO, S.A.Z.; et al. One Health approach on human seroprevalence of anti-*Toxocara* antibodies, *Toxocara* spp. eggs in dogs and sand samples between seashore mainland and island areas of southern Brazil. *One Health* [Internet]. 2021;13:100353. Disponível em: <https://www.sciencedirect.com/science/article/pii/S2352771421001439>

DIAS, R.A.; ABRAHÃO, C.R.; MICHELETTI, T.; MANGINI, P.R.; GASPAROTTO, V.P.D.O.; PENA, H.F.D.J.; FERREIRA, F.; RUSSELL, J.C.; SILVA, J.C.R. Prospects for domestic and feral cat management on an inhabited tropical island. *Biol. Invasions* 2017, 19, 2339–2353.

DUAN, G.; TIAN, Y. M.; LI, B. F.; YANG, J. F.; LIU, Z. L.; YUAN, F. Z.; ZHU, X. Q.; ZOU, F.C. Seroprevalence of *Toxoplasma gondii* infection in pet dogs in Kunming, Southwest China. *Parasites Vectors* 2012, 5, 118.

DUBEY, J. P.; CERQUEIRA-CÉZAR, C. K.; MURATA, F. H. A.; KWOK, O. C. H.; YANG, Y. R.; SU, C. All about toxoplasmosis in cats: the last decade. *Veterinary Parasitology*. 2020 julho;283:109145.

DUBEY, J.P. *Toxoplasma gondii* oocyst survival under defined temperatures. *J. Parasitol.* 1998, 84, 862.

DUBEY, J.P. *Toxoplasmosis of Animals and Humans*; CRC Press: Boca Raton, FL,

USA, 2016.

DUBEY, J.P.; HATTEL, A.L.; LINDSAY, D.; TOPPER, M.J. Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission. *J. Am. Veter. Med Assoc.* 1988, 193, 1259–1263.

DUMÈTRE, A.; DUBEY, J.P.; FERGUSON, D.J. Effect of household bleach on the structure of the sporocyst wall of *Toxoplasma gondii*. *Parasite* 2021, 28, 68.

ENG, S.B.; WERKER, D.H.; KING, A.S.; MARION, S.A.; BELL, A.; ISSAC-RENTON, J.L.; IRWIN, G.S.; BOWIE, W.R. Computer-Generated Dot Maps as an Epidemiologic Tool: Investigating an Outbreak of Toxoplasmosis. *Emerg. Infect. Dis.* 1999, 5, 815–819.

ETHEREDGE, G.D.; MICHAEL, G.; MUEHLENBEIN, M.P.; FRENKEL, J.K. The roles of cats and dogs in the transmission of *Toxoplasma* infection in Kuna and Embera children in eastern Panama. *Rev. Panam. Salud Pública* 2004, 16, 176–186.

FELIPETTO, L.G.; TEIDER-JUNIOR, P.I.; DA SILVA, F.F.V.; YAMAKAWA, A.C.; KMETIUK, L.B.; COUTO, A.C.D.; MARTINS, C.M.; VAZ, E.S.; ULLMANN, L.S.; LANGONI, H.; et al. Serosurvey of Anti-*Toxoplasma gondii* Antibodies in Homeless Persons of São Paulo City, Southeastern Brazil. *Front. Public Health* 2020, 8, 580637.

FRANCO, W.A.C.; BERGAMASCHI, D.P.; RICHTZENHAIN, L.J.; NOGUEIRA, Y.; CAMARGO, L.M.A.; DE SOUZA, S.L.P.; GENNARI, S.M. Evaluation of the performance of the modified direct agglutination test (MAT) for detection of *Toxoplasma gondii* antibodies in dogs. *Braz. J. Veter. Res. Anim. Sci.* 2003, 40, 452–456.

FRENKEL, J.K.; PARKER, B.B. An Apparent Role of Dogs in the Transmission of *Toxoplasma gondii*: The Probable Importance of Xenosmophilia. *Ann. N. Y. Acad.*

Sci. 1996, 791, 402–407.

GOMES, D.F.C.; KRAWCZAK, F.D.S.; DE OLIVEIRA, C.H.S.; JÚNIOR, F.; FERNANDES, E.; LOPES, W.D.Z.; SEVÁ, A.D.P.; GENNARI, S.M. *Toxoplasma gondii* in cattle in Brazil: A review. Rev. Bras. Parasitol. Vet. 2020, 29, e015719.

IBGE | Cidades@ | Paraná | Guaraqueçaba | Panorama, (n.d.). Available online: <https://cidades.ibge.gov.br/brasil/pr/guaraquecaba/panorama> (accessed on 24 November 2021).

IBGE | Cidades@ | Paraná | Pontal do Paraná | Panorama, (n.d.). Available online: <https://cidades.ibge.gov.br/brasil/pr/pontal-do-parana/panorama> (accessed on 24 November 2021).

IBGE. Fernando de Noronha (PE) | Cidades e Estados | Available online: <https://www.ibge.gov.br/cidades-e-estados/pe/fernando-de-noronha.html> (accessed on 7 June 2022).

JONES, J.L.; KRUSZON-MORAN, D.; ELDER, S.; RIVERA, H.N.; PRESS, C.; MONTOYA, J.G.; MCQUILLAN, G.M. *Toxoplasma gondii* Infection in the United States, 2011–2014. Am. J. Trop. Med. Hyg. 2018, 98, 551–557.

MACHADO, F.P.; KMETIUK, L.B.; TEIDER-JUNIOR, P.I.; PELLIZZARO, M.; YAMAKAWA, A.C.; MARTINS, C.M.; BACH, R.V.W.; MORIKAWA, V.M.; DE BARROS-FILHO, I.R.; LANGONI, H.; et al. Seroprevalence of anti-*Toxoplasma gondii* antibodies in wild boars (*Sus scrofa*), hunting dogs, and hunters of Brazil. PLoS ONE 2019, 14, e0223474.

MAGALHÃES, F.J.R.; RIBEIRO-ANDRADE, M.; SOUZA, F.M.; LIMA FILHO, C.D.F.; BIONDO, A.W.; VIDOTTO, O.; et al. Seroprevalence and spatial distribution of *Toxoplasma gondii* infection in cats, dogs, pigs and equines of the Fernando de Noronha Island, Brazil. Parasitology International. 2017 abril;66(2):43–6.

MANTOVAN, K.B.; LUIZ, C.G.S.; MENOZZI, B.D.; NILSSON, M.G.; LATOSINSKI, G.S.; LANGONI, H. Inquérito soroepidemiológico para toxoplasmose, leptospirose e leishmaniose visceral canina. *Veterinária Zootec.* 2021, 28, 1–10.

MEIRELES, L.R.; GALISTEO, A.J.; POMPEU, E.; ANDRADE, H.F. *Toxoplasma gondii* spreading in an urban area evaluated by seroprevalence in free-living cats and dogs. *Trop. Med. Int. Health* 2004, 9, 876–881.

MINUZZI, C.E.; FERNANDES, F.D.; PORTELLA, L.P.; BRÄUNIG, P.; STURZA, D.A.F.; GIACOMINI, L.; SALVAGNI, E.; RIBEIRO, J.D.S.; SILVA, C.R.; DIFANTE, C.M.; et al. Contaminated water confirmed as source of infection by bioassay in an outbreak of toxoplasmosis in South Brazil. *Transbound. Emerg. Dis.* 2020, 68, 767–772.

NAVARRETE, M.G.; CORDEIRO, M.D.; BATISTA, Y.; ALONSO, J.C.; MÁRQUEZ, M.; ROQUE, E.; FONSECA, A. Serological detection of *Toxoplasma gondii* in domestic dogs in the western region of Cuba. *Veter. Parasitol. Reg. Stud. Rep.* 2017, 9, 9–12.

Parque Estadual da Ilha do Mel (PEIM) | Instituto Água e Terra, (n.d.). Available online: <http://www.iat.pr.gov.br/Pagina/Parque-Estadual-da-Ilha-do-Mel-PEIM> (accessed on 24 November 2021).

PENA, H.F.J.; ALVES, B.F.; SOARES, H.S.; OLIVEIRA, S.; FERREIRA, M.N.; BRICARELLO, P.A.; MACHADO, T.M.P.; CASTRO, B.B.P.; GENNARI, S.M. Free-range chickens from Santa Catarina state, southern Brazil, as asymptomatic intermediate hosts for *Toxoplasma gondii* clonal type I and typical Brazilian genotypes. *Veter. Parasitol. Reg. Stud. Rep.* 2018, 13, 55–59.

PETTAN-BREWER, C.; MARTINS, A.F.; DE ABREU, D.P.B.; BRANDÃO, A.P.D.; BARBOSA, D.S.; FIGUEROA, D.P.; et al. From the Approach to the Concept: One Health in Latin America-Experiences and Perspectives in Brazil, Chile, and

Colombia. *Front. Public Health*, 14 September 2021.1;9:687110.

PINTO-FERREIRA, F.; CALDART, E.T.; FREIRE, R.L.; MITSUKA-BREGANÓ, R.; DE FREITAS, F.M.; MIURA, A.C.; MAREZE, M.; MARTINS, F.D.C.; URBANO, M.; SEIFERT, A.L.; et al. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. *Rev. Bras. Parasitol. Veterinária* 2018, 27, 327–337.

PINTO-FERREIRA, F.; PASCHOAL, A.T.P.; PASQUALI, A.K.S.; BERNARDES, J.C.; CALDART, E.T.; FREIRE, R.L.; MITSUKA-BREGANÓ, R.; NAVARRO, I.T. Techniques for inactivating *Toxoplasma gondii* oocysts: A systematic review. *Orgao Off. Do Col. Bras. Parasitol. Vet.* 2021, mboxemph30, e026420.

Plano Nacional de Operacionalização da Vacinação contra Covid-19 — Português (Brasil) [Internet]. [citado em 5 de novembro de 2021]. Disponível em: <https://www.gov.br/saude/pt-br/coronavirus/publicacoes-tecnicas/guias-e-planos/plano-nacional-de-vacinacao-covid-19/view>

Povos e Comunidades Tradicionais—Ministério da Cidadania Secretaria Especial do Desenvolvimento Social, (n.d.). Available online: <http://mds.gov.br/assuntos/seguranca-alimentar/direito-a-alimentacao/povos-e-comunidades-tradicionais> (accessed on 8 November 2021).

R: The R Project for Statistical Computing, (n.d.). Available online: <https://www.r-project.org/> (accessed on 4 November 2021).

RAMOS, R.C.F.; PALMER, J.P.S.; DIB, L.V.; LOBÃO, L.F.; PINHEIRO, J.L.; DOS SANTOS, C.R.; UCHÔA, C.M.A.; BASTOS, O.M.P.; DA SILVA, H.P.; FONSECA, A.B.M.; et al. Soropositividade e fatores de risco associados à infecção por *Toxoplasma gondii* em pacientes atendidos no Laboratório Municipal de Oriximiná, estado do Pará, Brasil. *Rev. Pan Amaz. Saúde* 2021, 12, 1–11.

RIZZO, H.; CARVALHO, J.S.; HORA, J.H.C.; RODRIGUES, F.M.; FRAGA, G. J. M.;

VILLA LOBOS, E.M.C. Comparação do ensaio imunoenzimático indireto (ELISA-teste) com a reação da imunofluorescência indireta na detecção de anticorpos IgG anti-Toxoplasma gondii em ovinos naturalmente infectados. Ci. Vet. Tróp. 2014, 17, 16–20. Available online: <https://pesquisa.bvsalud.org/portal/resource/pt/vti-688292> (accessed on 11 June 2022).

SEPÚLVEDA-ARIAS, J.C.; GÓMEZ-MARIN, J.E.; BOBIĆ, B.; NARANJO-GALVIS, C.A.; DJURKOVIĆ-DJAKOVIĆ, O. Toxoplasmosis as a travel risk. Travel Med. Infect. Dis. 2014, 12, 592–601.

SHENG, Z.; JIN, Y.; YAO, Y.; EL-ASHRAM, S.; SHEN, J.; WANG, X.-L.; JI, Y. Seroprevalence of Toxoplasma gondii Infection in Pet Dogs in Anhui Province, China. Iran. J. Parasitol. 2020, 15, 446–451.

SILVA, G.N.D.R.; BRANCO, M.D.R.F.C.; RODRIGUES, Z.M.R.; DOS SANTOS, A.M.; PEREIRA, P.R.M.; DA SILVA, M.D.S.; NUNES, A.T.D.S.; JÚNIOR, A.R.G.; MEDEIROS, M.N.L.; AZEVEDO, C.D.M.P.E.S.D.; et al. Toxoplasmosis outbreak in Brazil, 2006-Revisited. Parasite Epidemiol. Control 2019, 7, e00117.

SILVA, R.C DA.; SOUZA, L.C DE.; LANGONI, H.; TANAKA, E.M.; LIMA, V.Y DE.; SILVA, A.V da. Risk factors and presence of antibodies to *Toxoplasma gondii* in dogs from the coast of São Paulo State, Brazil. Pesqui Veterinária Bras [Internet]. 2010 [citado em 5 de novembro de 2021];30(2):161–6. Disponível em: <http://www.scielo.br/j/pvb/a/w3GQsW5MKQPnvTj6RwDGmLN/?lang=en>

SMITH, N.C.; GOULART, C.; HAYWARD, J.A.; KUPZ, A.; MILLER, C.M.; VAN DOOREN, G.G. Control of human toxoplasmosis. Int. J. Parasitol. 2021, 51, 95–121. [CrossRef]

SUBIRANA, I.; SANZ, H.; VILA, J. Building Bivariate Tables: The compare Groups Package for R. J. Stat. Softw. 2014, 57, 1–16.

UCHÔA, C.M.A.; DUARTE, R.; LAURENTINO-SILVA, V.; ALEXANDRE, G.M.C.;

FERREIRA, H.G.; AMENDOEIRA, M.R.R. Padronização de ensaio imunoenzimático para pesquisa de anticorpos das classes IgM e IgG anti-Toxoplasma gondii e comparação com a técnica de imunofluorescência indireta. Rev. Soc. Bras. Med. Trop. 1999, 32, 661–669.

XIN, S.; SU, R.; JIANG, N.; ZHANG, L.; YANG, Y. Low Prevalence of Antibodies Against Toxoplasma gondii in Chinese Populations. Front. Cell. Infect. Microbiol. 2020, 10, 302.

APÊNDICES

APÊNDICE 1

Delai RR, Freitas AR, Kmetiuk LB, Meriguetti YFFB, Ferreira IB, Lescano SAZ, et al. One Health approach on human seroprevalence of anti-*Toxocara* antibodies, *Toxocara* spp. eggs in dogs and sand samples between seashore mainland and island areas of southern Brazil. One Heal [Internet]. 2021;13:100353. Available from: <https://www.sciencedirect.com/science/article/pii/S2352771421001439>

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One Health approach on human seroprevalence of anti-*Toxocara* antibodies, *Toxocara* spp. eggs in dogs and sand samples between seashore mainland and island areas of southern Brazil

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ABSTRACT

Toxocarosis, caused by *Toxocara* spp. nematodes, is among the top 5 neglected parasitic diseases worldwide; however, no comprehensive study to date has serologically compared infections in people and their dogs and environmentally contaminated soil or sand of mainland and island locations. Accordingly, this study aimed to assess the seroprevalence of anti-*Toxocara* antibodies in traditional human seashore populations, the presence of eggs in dogs' feces and hair, and the presence of eggs in environmental samples from islands compared to the adjacent mainland of southern Brazil. Overall, 212/328 (64.6%) people were positive for *Toxocara* spp. antibodies, including 125/190 (65.8%) island and 87/138 (63.0%) mainland residents. For dog samples, 12/115 (10.43%) were positive for the presence of *Toxocara* spp. eggs, all from dogs living in islands, and 22/104 (21.15%) dog hair samples contained eggs of *Toxocara* spp. Environmental contamination with *Toxocara* spp. eggs was observed in 50/130 (38.46%) samples from all sampled sites. No significant association was found between risk factors (age, sex, educational level, monthly income, owning dogs or cats, ingestion of treated water, and consumption of raw or uncooked meat) and *Toxocara* spp. seropositivity. The present study is the first concurrent report on people, their dogs, and environmental contamination of *Toxocara* spp. The high prevalence we observed in the seashore populations of both in island and mainland areas may be caused by exposure to contaminated sand and climatic factors favoring frequent exposure to *Toxocara* spp. In conclusion, seashore lifestyle and living conditions of both island and mainland areas may have predisposed higher contact with infected pets and contaminated soil, favoring the high prevalence of toxocarosis.

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1. Introduction

Toxocariasis is a neglected zoonotic disease caused by nematode parasites of the genus *Toxocara* that have a worldwide distribution [1]. Toxocariasis is one of top five neglected parasitic diseases targeted for public health action [1,2]. A systematic review and meta-analysis estimated the global anti-*Toxocara* spp. seroprevalence to be 19%, meaning that approximately one-fifth of the human population has been exposed to toxocariasis agents [3]. Human toxocariasis is commonly an asymptomatic infection; however, larvae migration may cause a range of diseases affecting organs in visceral toxocariasis, eyes in ocular toxocariasis, or the central nervous system in neurotoxocariasis [4].

Considered accidental hosts, humans may acquire infection by ingestion of embryonated or larvated eggs of *Toxocara canis* and *T. cati*, present in soil and contaminated food [2]. Foodborne toxocariasis may occur by ingestion of encapsulated *Toxocara* larvae in raw or uncooked tissues of paratenic hosts, contaminated water, or contact with dog hair [5,6].

The main source of human infection is the ingestion of *Toxocara* spp. eggs in soil, particularly by children due to their higher exposure to contaminated soil in public parks, schoolyards, and playground sandboxes [7]. As definitive hosts of *Toxocara* spp., dogs and cats have reportedly played essential roles in transmitting *T. canis* and *T. cati*, respectively, by shedding eggs in feces into the environment [6]. A systematic review and meta-analysis verified a global prevalence of 21% of *Toxocara* spp. eggs in public places, with the highest prevalence associated with high geographic longitude, low latitude, and high humidity [8]. Soil contamination has also been observed on beaches. In southeastern Brazil, a study showed the presence of *Toxocara* spp. eggs in 59.4% of the soil samples evaluated [9]. Another study in southeast coast of Brazil evaluated the environmental contamination of sandy soil of an urban beach and a virgin beach, one that remains in natural state, without exploration or exploitation by humans. It was reported that 38/120 (31.7%) of urban beach samples were positive for eggs of *Toxocara* spp. while all the samples from the virgin beach were negative. This difference was attributed to the presence of dogs and garbage [10] on urban beaches.

A systematic metanalysis review on the global seroprevalence of human *Toxocara* spp. infection has identified several associated risk factors found in seashore areas, including social vulnerability and low income, unhealthy dog populations with access to public areas, close human contact with dogs, cats, or soil, lower latitude, consumption of raw meat, consumption of untreated drinking water, young age, higher humidity and precipitation, and higher temperature [3]. Therefore, human populations living along mainland seashores and islands may be highly exposed, particularly in developing countries, probably due to low income, poor housing and sanitation, poor hygiene practices, presence of free-roaming dogs, and lack of medical and veterinary health care.

One Health has been applied as a tool to solve problems such as zoonoses by research groups in Brazil, taking advantage of the National Health System, which has provided simultaneous human-animal-soil samplings on different vulnerable populations and their animals, including animal hoarders, homeless, incarcerated, indigenous and traditional island populations. With this approach, domestic animals have been concurrently surveyed along with their owners and environment, holistically providing new pathogen roles in different settings. An island-effect hypothesis has been proposed to describe the overlap of associated risk factors for human *Toxocara* spp. infection in traditional island populations. In the scenario herein, island-effect is hypothesized to describe potentially exacerbated pathogen transmission due to isolation and continuous exposure in islands, particularly for environmental, foodborne, or vector-borne transmission with a daily occurrence and multiple cycle pathways favored by the climate. The term, island-effect, has been used in other three situations: (1) *Foster's rule*, also island-effect, where populations of animals in isolated islands may

change in size; (2) *Urban heat island*, to designate a spatial-temporal distribution of temperature in urban areas; and (3) *Nut island effect*, as management principle for an isolated team which lowers its efficiency. Previous studies have shown parasite dissemination in Fernando de Noronha, a top Brazilian island tourist destination with overlapping human-animal environments. In such studies, a high *T. gondii* seroprevalence was found in 172/341 (50.4%) human islanders, related to high serology of the cat population and environmental contamination of oocysts [11,12].

Finally, no study to date has serologically compared continental (mainland) and island seroprevalence for *Toxocara* spp. along with a survey to detect *Toxocara* spp. eggs in dog feces and sand samples, and additionally assess island-effect occurrence. Accordingly, the present study aimed to assess toxocariasis exposure and the presence of eggs in dogs and the environment in an island compared to the adjacent mainland seashore of southern Brazil.

2. Material and methods

2.1. Ethics

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 Paraná.

2.2. Study design

This study represents a cross-sectional seroepidemiological approach describing human and dog populations living on oceanic islands and seashore mainland cities in the state of Paraná, southern Brazil. The study was conducted from July 2019 through February 2020.

2.3. Study areas

The study was conducted on three oceanic islands, Ilha do Mel island, Superagui Island, and Peças Island; and two mainland seashore cities, Pontal do Paraná and Guaraqueçaba, all within the Paraná State (Table 1). The islands are environmentally preserved conservation units. The Ilha do Mel island State Park and the Superagui National Park are covered by the largest continuous remnant of the Atlantic Forest in Brazil.

Table 1
Locations and coordinates of island and mainland of Paraná State, southern Brazil, where people, dogs, and sand were sampled.

Location	Coordinates	Population	Sampling	%
Islands				
Ilha do Mel island	25°34'12"S and 48°18'57"W	1094	113	10.3
Superagui	25°27'19"S and 48°14'50"W	700	49	7.0
Peças	25°27'22"S and 48°20'07"W	350	28	8.0
Mainland				
Pontal do Paraná	25°40'26"S and 48°30'39"W	5000 ^a	46	0.92
Guaraqueçaba	25°18'25"S and 48°19'44"W	2182 ^b	92	4.21
Total			328	

^a Only considered neighborhood area of the mainland port of Pontal do Sul. Pontal do Paraná City had an estimated 27,915 inhabitants at the time.

^b Considered only the mainland port at the city urban area. Guaraqueçaba city had an overall estimated 7594 inhabitants at the time.

2.4. Population

Socioeconomic characteristics varied with location. On the islands of Superagui and Peças, the population was comprised of traditional fisherman communities, with strong environment connections that rely on natural resources for living, mostly fishing and craftsmanship. Although ecological tourism in these two islands has grown in the past five years, both islands lack adequate health, education, and infrastructure systems, with most supplies (eg. clothes and construction materials) coming from the mainland by boat.

Both Superagui and Peças islands are part of Guaraqueçaba city which is located within an environmentally protected area. It is one of the seven seashore cities of Paraná State, and is semi-isolated relying mainly on sea transportation or use of a 90 km (56 miles) unpaved road that takes 3 h to travel each way by passenger car. This makes for difficult living conditions but contributes to environmental preservation [13]. With 95% of its area under permanent environmental protection and a mainland port in Ponta do Paraná city, tourism is the main form of income on Ilha do Mel island. The island has two major seashore non-communicating villages (New Brasília and Encantadas) and the population increases 5-fold at high season [14].

Ponta do Paraná city is the closest and mainland source for daily supplies for Ilha do Mel island, Superagui islands, and Peças islands. In addition, some of the health and education personal come daily from mainland cities to the islands.

2.5. Sample collection

2.5.1. Blood sampling

Human participants were sampled following signed consent and completion of an epidemiological questionnaire. Human blood samples were collected by cephalic venipuncture, dog and cat blood samples were collected by jugular venipuncture. Blood samples placed in sterile vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) and serum separator gel. Packed cell volume (PCV) and total plasma proteins (TPP) were measured by centrifugation and refractometry, respectively. All samples were also collected in tubes without anti-coagulant and kept at room temperature (25 °C) until visible clot retraction, centrifuged at 800g for five minutes, and serum separated and kept at -20 °C until processing. The samples stored in tubes containing serum separator gel were kept at room temperature (25 °C) until visible clots formed and were then centrifuged at 800g for five minutes to separate the serum, kept at -20 °C until processing.

2.5.2. Dog feces and hair samplings

Using physical restraint, dog fecal samples were collected from the rectum, placed into individual plastic bags, and kept under refrigeration (4 °C) until processing. Hair sections from the dog's lower back and perineal regions were carefully cut (to avoid skin injury) by sterile scalpel blades. Samples were placed in plastic bags and kept under refrigeration (4 °C) until processing, as previously established [15].

2.5.3. Soil sampling

Soil samples were randomly collected on each island consisting of ten samples: five beach and five trail. During sampling, characteristics of sites were recorded, including the presence of children and adults in direct contact with the soil, the presence of dogs and cats, and the presence of feces at the site. Approximately 100 g of soil sample at a depth of 5 to 10 cm was collected, as previously established [16], placed into a plastic bag, and stored under refrigeration (4 °C) until processing [7].

2.6. Serological tests

2.6.1. *Toxocara canis* excretory-secretory (TES) antigen preparation

Adult forms of *T. canis* were obtained from naturally infected puppies

that spontaneously released the parasites. Adult female nematodes were exposed to 1% sodium hypochlorite for 5 min to remove debris, followed by washing with 0.9% saline for three minutes. After washing, the anterior third of the worm was sectioned to collect eggs [17].

The eggs were incubated in 2% formalin for approximately 30 days at 28 °C, and hatched larvae were incubated at 37 °C in serum-free Eagle medium, according to a previously described protocol [18]. The culture supernatant was removed weekly, treated with 5.0 µL/mL of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF; 200 mM), concentrated in a commercially available kit (Amicon Ultra Centrifugal Filter Unit, Millipore, Danvers, MA, USA), dialyzed with distilled water, centrifuged 18,500 g for 60 min at 4 °C, and filtered with 0.22 µm filter membranes (Millipore). The protein concentration was determined using the method of Lowry et al. [19].

2.6.2. Pre-adsorption of sera with *Ascaris suum* adult worm extract

Serum samples were pre-adsorbed with *A. suum* adult worm extract (AWE) following an established protocol [18], to remove antibodies elicited by exposure to *Ascaris* spp. that could cross-react with *Toxocara* antigens, and, consequently, to enhance the specificity of the ELISA assay [20]. Adult nematodes, recovered from the intestine of slaughtered pigs, were macerated in distilled water, and one part 1.5 M NaOH was added to nine parts of water to make a final concentration of 0.15 M. After incubation at room temperature for 2 h, the pH of the material was neutralized with 6 M HCl and centrifuged (at 18,500 g for 20 min at 4 °C). After removing lipids with ether, the supernatant was filtered through 0.22 µm filter membranes. All serum samples were pre-incubated for 30 min at 37 °C with an AWE solution (25.0 µg/µL) in 0.01 M phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 (PBS-T) (Sigma, St. Louis, MO, USA).

2.6.3. Indirect enzyme-linked immunosorbent assay (ELISA)

Polystyrene 96-well microtiter plates (Corning, Costar, New York, USA) were coated for 1 h at 37 °C and then for 18 h at 4 °C, with 1.9 µg/µL per well of TES antigens in 0.06 M carbonate-bicarbonate buffer, at pH 9.6 and subsequently blocked for 1 h at 37 °C with 3% commercial skimmed milk PBS-Tween 5%. After adsorption with *A. suum* somatic antigen, anti-Human IgG (Fc-specific) peroxidase antibody produced in goat (Sigma A6029) was added at a 1:5000 dilution (45 min at 37 °C, performing three 5-min washing).

The reaction was revealed using the substrate o-phenylenediamine (0.4 mg/mL, Sigma). The reaction was interrupted by adding 2 N sulfuric acid. Positive and negative controls were included in each plate. Absorbance was read at 492 nm, defining the cut-off as the mean absorbance of 96 negative control sera plus three standard deviations. The present test has shown 78.3% sensitivity and 92.3% of specificity, as previously reported [21,22]. Antibody levels were expressed as reactivity indices (RI), which were calculated as the ratio between the absorbance values of each sample and the cut-off value, set at 0.400.

2.7. Fecal and hair analyses

Fecal samples were examined using two standard techniques: centrifuge sedimentation and flotation in hyper-saturated sodium chloride [23].

Hair samples were examined for *Toxocara* spp. eggs according to a previously described protocol [15] with modifications [24]. Each sample was transferred into 50 mL falcon tubes. Hair was rinsed (distilled water plus 0.2 mL of Tween 80) and kept overnight. Next, additional distilled water (20 mL) was added, and the tubes were rigorously homogenized for 3 min with a vortex. A second washing was performed (two drops of Tween 80 in 40 mL of distilled water). Then, the material was filtered through 300 µm, 212 µm, and 38 µm metallic sieves. After filtration, the hair was discarded, and the washing material obtained (38 µm sieve) was centrifuged 800 xg for five min. A total of 500 µL of resulting sediment was transferred to a slide and microscopically

analyzed at 10× and 40× magnifications.

2.8. Soil analysis

Soil analysis followed the protocol described by Otero et al. [7], with some modifications. From each soil point collected, 20 g were weighed, rinsed with anionic detergent (100 mL of Tween 80 5%), homogenized (1 min), and allowed to stand for 12 h. The contents were then sieved (300 µm, 212 µm, 90 µm, and 38 µm), and samples were washed in running water (10 min). The soil retained in the sieve of 38 µm was collected and transferred to a graduated tube and centrifuged (800 xg for 5 min). Following centrifugation, the sediment (500 µL) was evaluated. The remaining material was subjected to a centrifuge-flotation technique using a zinc sulfate solution ($d = 1.35 \text{ g/cm}^3$).

Dog hair and soil samples were evaluated under optical microscopy (10× and 40× objective) for counting and morphological evaluation of the eggs. *Toxocara* spp. eggs were classified according to the stage of development: viable (intact egg with content), non-viable (egg not intact or with damaged wall), embryonating (egg with two or more cell divisions), and embryonated (egg containing larvae of the parasite).

2.9. Epidemiological data collection

Epidemiological analysis of human characteristics was based on a questionnaire that assessed potential exposure to *Toxocara* and included the geographical location of residence, age, sex, education level, household income, animal ownership, drinking water source/type, washing fruits and vegetables before consumption, sanitizing hand before meals, consumption of raw or undercooked meat. For the epidemiological analysis of the characteristics of dogs and cats, the questionnaire gathered data on sex, breed, animal origin, geographical location of residence, diet, raw meat intake, drinking water source/type, access to trails and beaches and forests, hunting habits, ectoparasite presence and control, deworming, vomiting, weight loss, and diarrhea.

2.9.1. Statistical analyses

The data were organized in spreadsheets, and the analytical process started with a descriptive exploration of the databases. The Chi-square test was used to verify the association between *Toxocara* spp. results in humans and the collection sites. Fisher exact test was used to verify the association between the *Toxocara* spp. results of humans in relation to their dogs. The Mann-Whitney test was used to compare *Toxocara* spp. serology of humans and their PCV and TTP results.

Bivariate analysis was performed for all independent variables, calculating the odds ratio (OR) and confidence interval (CI) and the *P* value (*P*). Multivariate analysis was performed by fitting the variables in a multilevel logistic regression model, in which spatial dependence was tested. The best-fitting model was considered the one that included significantly associated variables ($P < 0.05$) and minimized the Akaike Information Criterion (AIC) value.

As computational support, R software [25] was used. All tests performed considered a two-way significance level (α) of 5% and a 95% CI.

3. Results

Out of 328 human samples, 190 (57.9%) were collected on islands and 138 (42.1%) on the seashore mainland. Overall, 212/328 (64.6%) were positive for anti-*Toxocara* spp. (CI = 59.46–69.80), including 125/190 (65.79%) on islands (CI = 58.04–72.54) and 87/138 (63.04%) on the mainland (CI = 54.99–71.09).

Seropositivity was 194/301 (64.5%) in adults and 16/22 (72.7%) in children. All individuals were clinically healthy at the time of sampling, with no self-reported clinical complain. Results were reported to the City Secretary of Health, and future visits recommended for seropositive persons (including specialty exams as ophthalmology, cardiology, and

neurology), to provide medical assistance and appropriate treatment, if necessary. No statistical difference was observed between the anti-*Toxocara* antibody result and PCV ($P = 0.786$) or TTP ($P = 0.352$) in humans. The descriptive statistics for these variables are shown in Supplementary Table 1.

Regarding PCV and TTP, the descriptive analysis of parameters in humans, dogs, and cats, according to the result for *Toxocara* spp. are shown in Supplementary Table 2.

No associated risk factor assessed by the questionnaire was statistically significant for anti-*Toxocara* antibodies in humans, either in the bivariate or multivariate analysis (Table 2).

Out of dog fecal samples, 70/115 (60.9%) were collected on islands and 45/115 (39.1%) on the mainland. The 12/115 (10.43%) positive for *T. canis* (CI = 4.84–16.02) were all from dogs living on islands (17.1%, CI = [8, 31–25.97]). Out of cat fecal samples, 1/15 (6.7%) was collected on island and 14/15 (93.3%) were from the mainland; all were negative for *Toxocara* spp.

Hair samples were collected from 75/104 (72.1%) dogs from islands and 29/104 (27.9%) from mainland areas. Overall, 22/104 (21.2%) hair samples contained eggs of *Toxocara* spp. Viable eggs were observed in 19/22 (86.4%) samples, either in cell division or not yet embryonated, and 3/22 (13.6%) were non-viable or degenerated. Descriptive analysis of dog and cat samples is presented in Supplementary Table 3.

No statistical association was observed between the sampling site and human toxocarosis among five sites ($P = 0.172$) or between islands and mainlands ($P = 0.641$). Such comparison was not performed for pets since all infected dogs lived on islands, and no infected cats were found. Sample distribution according to sampling site and results for human anti-*Toxocara* antibodies and *Toxocara* spp. eggs in pets are shown in Supplementary Table 4.

Overall, 295/328 (89.9%) individuals were dog owners, of whom 103/295 (34.9%) had their dog sampled. While 7/103 (6.8%) positive owners had positive dogs, 25/103 (24.3%) negative owners had negative dogs. Different results between owners and their dogs were found in 71/103 cases (68.9%). No statistical difference was found between *Toxocara* spp. Seropositivity in owners and presence of eggs in dogs ($P = 0.325$). The complete association analyzes between owner and dog results is presented in Supplementary Table 5. No association was found between human seropositivity to *Toxocara* spp. and positive dogs for fecal *Toxocara* spp. (OR = 0.5303; 95% CI = 0.1540–1.827; $P = 0.3246$).

Environmental contamination (recovery of *Toxocara* spp. eggs) was observed in 50/130 (38.5%) samples from all sites. In Ilha do Mel island, sampling was performed in the two largest island communities, Encantadas and Brasília. A total of 3/10 (30%) beach and 8/10 (80%) trail sets had recovery of *Toxocara* spp. eggs in Encantadas, while 5/10 (50%) beach and 10/10 (100%) trail sets were positive in Brasília. On the other two islands, 1/10 (10%) beach and 6/10 (60%) trail sets were positive on Superagui Island, and 6/10 (60%) beach sets were positive on Peças Island, with no trail samples. On mainland, a total of 4/10 (40%) sets were positive on central square, including 1/10 (10%) soccer field, with 0/10 (0%) positive beach set in Guaraqueçaba. In Pontal do Sul, three sites were sampled, the wharf to Ilha do Mel island 4/10 (40%), the access trail to the main beach 2/10 (20%), and the beach, where 0/10 (0%) sets were positive. (Table 3).

In all sites, children and adults in direct contact with the soil were observed because the sites were beaches for bathers and the trails give access to the communities (houses, grocery stores, churches, schools, and health centers). The presence of dogs and cats and the presence of feces at the sites were also observed.

4. Discussion

This study shows an overall 212/328 (64.6%) seroprevalence for anti-*Toxocara* antibodies in populations of both islands and mainland cities in southern Brazil. Compared to other seroprevalence studies in Brazil, the prevalence herein was higher, except for one study that

Table 2
Bivariate analysis of associated risk factors for anti-Toxocara antibodies in populations of mainland and island of southern Brazil.

Variables	Population			ELISA positive			OR	95% CI		P value
	No.	Total	%	No.	Total	%		Lower limit	Upper limit	
Site										
Island	190	328	57.93	125	212	58.96	1.13	0.71	1.78	0.608
Mainland	138	328	42.07	87	212	41.04				
Age										
Children/teens	22	323	6.81	16	210	7.62	1.47	0.56	3.87	0.435
Adults/elderly	301	323	93.19	194	210	92.38				
Sex										
Female	209	328	63.72	131	212	61.79	0.78	0.49	1.26	0.327
Male	119	328	36.28	81	212	38.21				
Education										
≤ Elementary school	110	328	33.54	75	212	35.38	1.27	0.78	2.06	0.340
> Elementary school	218	328	66.46	137	212	64.62				
Income										
≤ 1 minimum wage	108	315	34.29	75	205	36.59	1.35	0.82	2.21	0.241
> 1 minimum wage	207	315	65.71	130	205	63.41				
Dog owner										
Yes	295	328	89.94	191	212	90.09	1.05	0.48	2.19	0.899
No	33	328	10.06	21	212	9.91				
Cat owner										
Yes	125	326	38.34	87	211	41.23	1.42	0.89	2.30	0.147
No	201	326	61.66	124	211	58.77				
Treated water										
No	111	328	33.84	79	212	37.26	1.56	0.96	2.58	0.078
Yes	217	328	66.16	133	212	62.74				
Wash fruits/vegetables										
No	11	327	3.36	9	211	4.27	2.54	0.64	16.84	0.238
Yes	316	327	96.64	202	211	95.73				
Wash hands										
No	11	328	3.35	8	212	3.77	1.48	0.42	6.85	0.570
Yes	317	328	96.65	204	212	96.23				
Consumption of raw or uncooked meat										
Yes	102	327	31.19	67	211	31.75	1.08	0.66	1.77	0.768
No	225	327	68.81	144	211	68.25				

Table 3
Environmental contamination with *Toxocara* spp. eggs in island and mainland areas, in different sets and locations, southern Brazil.

	Location					Total
	Beach	Trail	Central Square	Soccer Field	Wharf	
Island						
Illa do Mel island - Encantadas	3/10	8/10	NS	NS	NS	11/20 (55.0%)
Illa do Mel island - Brasília	5/10	10/10	NS	NS	NS	15/20 (75.0%)
Superagui Island	1/10	6/10	NS	NS	NS	7/20 (35.0%)
Papas Island	6/10	NS	NS	NS	NS	6/10 (60.0%)
Mainland						
Guarapuaçu	0/10	NS	4/10	1/10	NS	5/30 (16.7%)
Postal do Sul	0/10	2/10	NS	NS	4/10	6/30 (20.0%)
Total	15/60 (25.0%)	26/40 (65.0%)	4/10 (40.0%)	1/10 (10.0%)	4/10 (40.0%)	50/130 (38.5%)

*NS: not sampled.

reported 247/344 (71.8%) seropositive adults living in a rural area in the Rio Grande do Sul State (RS), also in southern Brazil [26]. Reports with lower prevalence reported include 194/376 (51.6%) in urban recreation areas of north-central mesoregion, Paraná State, Brazil [27], 18/280 (6.43%) of pregnant women presented at the Obstetric Center, RS, Brazil [28], and other Brazilian regions [29–31]. To the author's knowledge, the prevalence of seropositivity in children/teens observed herein is the highest reported to date in Brazil, according to a recent review [32].

In this study, no significant association was found between risk factors (age, sex, educational level, monthly income, owning dogs or cats, ingestion of treated water, and consumption of raw or uncooked meat) and *Toxocara* seropositivity in humans. In a survey reporting 382/413 (92.49%) seropositive pregnant women in Nigeria, the lack of significant association between risk factors and *Toxocara* spp. seropositivity was related to the difficulty of obtaining statistical significance due to high seropositivity [33]. Thus, the high prevalence reported

herein may have also impaired assessment of associated risk factors for toxocariasis.

The high prevalence observed in the shore populations, both in island and mainland areas, may be caused by contaminated sand and climatic factors favoring a frequent exposure to *Toxocara* spp. Seropositivity for anti-*Toxocara* antibodies in humans has been considered common in tropical and subtropical countries, including Brazil [34]. The tropical climate and living conditions have reportedly predisposed survival of pathogens such as *Toxocara* spp. in the environment [3,35].

According to a meta-analysis and compared to this study, anti-*Toxocara* seroprevalence was higher in children (OR = 1.9) than in adults, [3] likely secondary to frequent ingestion of infective *Toxocara* spp. eggs from soil/sand [1,34]. A serosurvey has shown a high prevalence of 144/166 (86.8%) in children aged 7–12 years in the Marshall Islands [35]. Some studies have shown that professionals, such as waste pickers and workers in contact with animals, are at high risk of toxocariasis due to exposure to contaminated soil [36,37]. The population of this study

was composed of traditional communities living on fishing and touristic activities and having frequent soil contact. Most soil samples assessed in this study were contaminated with *Toxocara* spp. eggs, some of them appearing viable. Furthermore, the presence of free-roaming dogs accessing public places, including trails and beaches, was a common observation during the samplings. Thus, contact with soil containing *Toxocara* spp. eggs may have exposed most of the island and mainland populations independent of the age of the inhabitants.

Human contact with dogs or cats has also been considered a risk factor for toxocarosis, most likely in non-dewormed pets [1,34]. In the present study, most people interviewed declared owning at least a dog, mostly unleashed and free-roaming. The fecal and hair analysis showed the presence of dogs infected by *T. canis*. Regular and systematic pet deworming should be recommended to mitigate toxocarosis transmission and reduce environmental contamination by *Toxocara* spp. eggs, including household backyards, trails, and beaches.

Consuming undercooked/raw meat from paratenic hosts has been considered another risk factor (OR: 1.59; 95%CI: 1.03–2.47) associated with toxocarosis [3], most frequently in Asian countries due to cultural behavior of ingesting raw meat [38]. Despite being readily available, fish consumption may present a low risk of foodborne toxocarosis, according to a recent experimental study [39]. In addition, most study participants declared beef as the primary source of animal protein, and the vast majority did not have a habit of eating raw/undercooked meat. Thus, the lack of association of ingesting raw meat and toxocarosis may reflect the low impact of transmission via flesh in the studied population independent of animal sources.

Consumption of improper or contaminated drinking water has also been considered a risk factor for toxocarosis [3,34]. Despite the social vulnerability observed in the studied population herein, participants mostly declared drinking treated water, washing hands before meals, and washing vegetables before consumption. Although not statistically significant, such habits may have a protective effect for infection by *Toxocara* spp.

Anthropic actions related to close associations domestic animals may favor disease cycles, particularly zoonoses, that could impact public health, native fauna, and the natural environment. Geographically isolated areas, with overlapping human and animal populations, may increase potential impact, in an island-effect of increased disease transmission, but this was not observed in this study for the soil-borne pathogen, *Toxocara* spp.

As limitations, the present study may have not detected an island-effect of exacerbated *Toxocara* spp. transmission due to cross-household individuals, since persons may have been born or previously lived in islands and moved to mainland, or vice-versa, or may have worked in daily boat transportation and commercial trading, with frequent mainland-island circulation. In addition, proximity of the mainland and islands may have also decreased the degree of isolation, with mainland distance of 4 km (2.5 miles) from the Ilha do Mel island, 10 km (6.2 miles) from the Peças Island, and 10 km (6.2 miles) from the Superagui island. Finally, frequency of walking and playing on trails, trailside households, soccer activity on sand fields and beaches, as well as daily swimming, surfing, and other sand-contact activities have not been fully assessed.

5. Conclusions

The present study is the first concurrent report of anti-*Toxocara* spp. serology in people and the presence of *Toxocara* spp. eggs in their dogs and environmental samples in islands and mainland areas. The high seroprevalence observed in this study suggests that seashore lifestyle and living conditions in both island and mainland areas may have predisposed higher contact with infected pets and contaminated soil, favoring toxocarosis. The island-effect of increased disease transmission was not observed for the soil-borne pathogen *Toxocara* spp.

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Declaration of Competing Interest

We declare that we do not have any conflict of interest associated with manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2021.100353>.

References

- [1] G. Ma, C.V. Holland, T. Wang, A. Hofmann, C.-K. Fan, R.M. Mairou, P.J. Hotez, R. B. Gasser, Human toxocarosis, *Lancet Infect. Dis.* 18 (2018) e14–e24, [https://doi.org/10.1016/S1473-3099\(17\)30331-5](https://doi.org/10.1016/S1473-3099(17)30331-5).
- [2] P.A.M. Overgaauw, F. van Knapen, Veterinary and public health aspects of *Toxocara* spp., *Vet. Parasitol.* 193 (2013) 398–403, <https://doi.org/10.1016/j.vetpar.2012.12.035>.
- [3] A. Rostami, S.M. Elahi, C.V. Holland, A. Taghipour, M. Khalili-Farshchi, Y. Fekri, V.F. Oursini, P.J. Hotez, R.B. Gasser, Seroprevalence estimates for toxocarosis in people worldwide: a systematic review and meta-analysis, *PLoS Negl. Trop. Dis.* 13 (2019), e0007809.
- [4] S. McGuinness, K. Leder, Global burden of Toxocarosis: a common neglected infection of poverty, *Curr. Trop. Med. Rep.* 1 (2014), <https://doi.org/10.1007/s40475-013-0012-5>.
- [5] H.L.C. de Azevedo, G.L. Rastorfer, M.S. Peço, T. Gallina, M.M. Villela, M.O. de Nobre, C.J. Scaini, M.E.A. Bessa, Presence of *Toxocara canis* eggs on the hair of dogs: a risk factor for Visceral Larva Migrans, *Vet. Parasitol.* 174 (2010) 115–118, <https://doi.org/10.1016/j.vetpar.2010.07.016>.
- [6] C.V. Holland, Knowledge gaps in the epidemiology of Toxocara: the enigma remains, *Parasitology* 144 (2017) 81–94, <https://doi.org/10.1017/S0033182015001407>.
- [7] D. Otter, A.M. Alves, R. Nijme, J. Rodifenna, P. Overgaauw, L. Madeira de Carvalho, Environmental contamination with *Toxocara* spp. eggs in public parks and playgrounds of Greater Lisbon, Portugal, *J. Infect. Public Health* 11 (2017), <https://doi.org/10.1016/j.jiph.2017.05.002>.
- [8] Y. Fekri, R.B. Gasser, A. Rostami, C.S. Fan, S.M. Ghassami, M. Javarian, M. Bayani, B. Amooei, B. Moradi, Toxocara eggs in public places worldwide - a systematic review and meta-analysis, *Environ. Pollut.* 242 (2018) 1467–1475, <https://doi.org/10.1016/j.envpol.2018.07.067>.
- [9] S. Rocha, R. Pinto, A. Floriano, L. Teixeira, B. Basili, A. Martins, S. Costa, M. Caserio, Environmental analyses of the parasitic profile found in the sandy soil from the Santos municipality beaches, SP, Brazil, *Rev. Inst. Med. Trop. São Paulo* 53 (2011) 277–281, <https://doi.org/10.1590/S0036-46652011000500007>.
- [10] E.L. Rauceo, C. Gómez-Hernández, L. Queiroz, B. Moura, N. Nogueira, G. Ferreira, K. Rezende-Oliveira, Parasite detection in sand from bays on the North Coast of São Paulo State, Brazil, *Rev. Parasitol. Trop. J. Trop. Pathol.* 49 (2020), <https://doi.org/10.5216/rpt.v49i3.63783>.
- [11] F.J.R. Magalhães, M. Ribeiro-Azade, F.M. Souza, C.D.F. Lima Filho, A.W. Biondo, O. Vidotto, L.T. Navarro, R.A. Mota, Seroprevalence and spatial distribution of *Toxoplasma gondii* infection in cats, dogs, pigs and equines of the Fernando de Noronha Island, Brazil, *Parasitol. Int.* 66 (2017) 43–46, <https://doi.org/10.1016/j.parint.2016.11.014>.
- [12] M.C. da Cunha, M. Ribeiro-Azade, R.P.R. de Melo, D.M. Guedes, J.W. Pinheiro Junior, E.F.T.S.F. Cavalcanti, F.J.R. Magalhães, R.A. Mota, Cross-sectional survey for *Toxoplasma gondii* infection in humans in Fernando de Noronha island, Brazil, *Rev. Bras. Parasitol. Vet.* 30 (2021), e005121, <https://doi.org/10.1590/S1984-29612021062>.

- [13] P.H.C. da Garcia, Acupuntura ecoturística em área de proteção ambiental: o caso de Guaraqueçaba (PR), 2010, <https://doi.org/10.1186/D.116.2010.TD6-11082010-100436>.
- [14] Plano de Manejo - Parque Estadual Ilha do Mel | Instituto Água e Terra. <http://www.iat.gov.br/Paginas/Plano-de-Manejo-Parque-Estatal-Ilha-do-Mel>, 2012 (n.d.), accessed July 25, 2021.
- [15] G. Roddie, C. Holland, P. Stafford, A. Wolfe, Contamination of fox hair with eggs of *Toxocara canis*, *J. Helminthol.* 82 (2008) 293–296, <https://doi.org/10.1017/S0022140X08996954>.
- [16] M. Choochinda, F. Mikaeli, S.M. Sadjadj, S. Ehsani, S. Inmananesh, Molecular characterization of *Toxocara* spp. eggs isolated from public parks and playgrounds in Shiraz, Iran, *J. Helminthol.* 93 (2019) 306–312, <https://doi.org/10.1017/S0022140X18000354>.
- [17] C.-X. Fan, R.-S. Liu, C.-C. Huang, W.-C. Chung, C.-W. Liao, W.-Y. Du, E.-E. Su, Seroepidemiology of *Toxocara canis* infection among mountain aboriginal adults in Taiwan, *Ann. J. Trop. Med. Hyg.* 71 (2004) 216–221.
- [18] G.R. Elefant, S.H. Shimizu, M.C.A. Sanchez, C.M.A. Jacob, A.W. Ferreira, A serological follow-up of toxocarosis patients after chemotherapy based on the detection of IgG, IgA, and IgE antibodies by enzyme-linked immunosorbent assay, *J. Clin. Lab. Anal.* 20 (2006) 164–172, <https://doi.org/10.1002/jcla.20126>.
- [19] O.H. Lowry, M.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [20] A. Romasanta, J.L. Romero, M. Arias, R. Sánchez-Andrade, C. López, J.L. Soler, P. Díaz, P. Díez-Baños, P. Morondo, A. Paz-Silva, Diagnosis of parasitic zoonoses by immunoenzymatic assays—analysis of cross-reactivity among the secretory/secretory antigens of *Fasciola hepatica*, *Toxocara canis*, and *Acanthamoeba* spp., *Immunol. Invest.* 32 (2003) 131–142, <https://doi.org/10.1081/imm-120022974>.
- [21] L. Glickman, P. Schantz, R. Dombronski, R. Cypess, Evaluation of serodiagnostic tests for visceral leishmaniasis, *Am. J. Trop. Med. Hyg.* 27 (1978) 492–498, <https://doi.org/10.4269/ajtmh.1978.27.492>.
- [22] J. Fillard, J.-F. Magnaval, Laboratory diagnosis of human toxocarosis, *Vet. Parasitol.* 193 (2013) 327–336, <https://doi.org/10.1016/j.vetpar.2012.12.028>.
- [23] D.D. Bowman, *Georgis' Parasitology for Veterinarians*, 2014, p. 496.
- [24] Y.F.F.B. Merigueti, 37702473886, <http://attes.cnpq.br/8569158472614202>, Frequência e fatores associados à contaminação de pó de cinza por ovos de *Toxocara* spp. na região de Presidente Prudente, São Paulo. <http://bdtd.unoeste.br:8080/jspui/bitstream/jspui/1015>, 2017 (accessed July 25, 2021).
- [25] R Core Team, European Environment Agency, a.d. <https://www.eea.europa.eu/da/ta-and-maps/indicators/oxygen-consuming-substances-in-rivers/e-development-co-re-team-2006>, 2020 (accessed July 25, 2021).
- [26] A. Amato, M. Mareiro Villalón, A. Sena-Lopes, N. Farias, L. Faria, L. Avila, M. Berns, S. Borenk, Seroepidemiology of *Toxoplasma gondii* and *Toxocara canis* in a human rural population of Southern Rio Grande do Sul, *Rev. Inst. Med. Trop. São Paulo* 60 (2018), e28, <https://doi.org/10.1590/s1678-9946201860028>.
- [27] C. Colli, G. Rubinsky-Elefant, M. Paladín, D. Falavigna, E. Guilherme, S. Mattia, S. Marques de Araújo, E. Ferreira, I. Previdelli, A. Falavigna-Guilherme, Serological, clinical and epidemiological evaluation of toxocarosis in urban areas of south Brazil, *Rev. Inst. Med. Trop. São Paulo* 52 (2010) 69–74, <https://doi.org/10.1590/S0036-46852010000200002>.
- [28] P.C. Santos, L.M. Lehmann, C. Lorenzi, C. Hirsch, P.L. Tolosa, G.T. Mattos, P. S. Cardoso, G.R. Klafke, M.F.A. Berns, C.V. Gonçalves, C.J. Scaini, The seropositivity of *Toxocara* spp. antibodies in pregnant women attended at the university hospital in Southern Brazil and the factors associated with infection, *PLoS One* 10 (2015), e0131058.
- [29] E.C. Negri, V.A. Santarém, G. Rubinsky-Elefant, R. Giuffrida, Anti-*Toxocara* spp. antibodies in an adult healthy population: serosurvey and risk factors in Southeast Brazil, *Asian Pac. J. Trop. Biomed.* 3 (2013) 211–216, [https://doi.org/10.1016/S2221-1691\(13\)60052-0](https://doi.org/10.1016/S2221-1691(13)60052-0).
- [30] L.E. Prates-Carneiro, G. Rubinsky-Elefant, A.W. Ferreira, P.R. Araújo, C. Troloni, S.C. Zago, M. Katsura, L. Sato, A. Ito, A. Vaz, Seroepidemiology of toxoplasmosis, toxocarosis and cysticercosis in a rural settlement, São Paulo State, Brazil, *Pathog. Glob. Health* 107 (2013) 88–95, <https://doi.org/10.1179/204477213P.00000000079>.
- [31] L.C. Pereira, G.R. Elefant, Y.M. Nóbrega, T. Vitell, N. Nitz, L. Giandolfi, R. Pratesi, M. Hecht, *Toxocara* spp. seroprevalence in pregnant women in Brasília, Brazil, *Rev. Soc. Bras. Med. Trop.* 49 (2016) 641–643, <https://doi.org/10.1590/S0037-8682-0106-2016>.
- [32] P.P. Chieffi, S.A. Zevallós Lencina, G. Rodrigues E Fonseca, S.V. Dos Santos, Human Toxocarosis 2010 to 2020 contributions from Brazilian researchers, *Res. Rep. Trop. Med.* 12 (2021) 81–91, <https://doi.org/10.2147/RRTRM.S274733>.
- [33] K. Ikotun, O. Sowemimo, C.-M. Chou, K. Ajewole, T.-W. Chung, S. Asaria, O. Akintade, V. Gyung, T. Nwafor, E. Henry, C.-K. Fan, High seroprevalence of *Toxocara* antibodies in pregnant women attending an antenatal clinic at a university hospital in Ife-Ife, Nigeria, *Trans. R. Soc. Trop. Med. Hyg.* 114 (2020), <https://doi.org/10.1093/trstmh/trz116>.
- [34] G. Ma, A. Rostami, T. Wang, A. Hofmann, P.J. Hotez, R.B. Gasser, Global and regional seroprevalence estimates for human toxocarosis: a call for action, *Adv. Parasitol.* 109 (2020) 275–290, <https://doi.org/10.1016/j.apspr.2020.01.011>.
- [35] C.-J. Fu, T.-W. Chung, H.-S. Lin, C.-H. Wu, Y.-C. Liu, M.K. Langinur, M.-Y. Lu, W. W.-W. Hsiao, C.-K. Fan, Seroepidemiology of *Toxocara canis* infection among primary schoolchildren in the capital area of the Republic of the Marshall Islands, *BMC Infect. Dis.* 14 (2014) 261, <https://doi.org/10.1186/1471-2334-14-261>.
- [36] J.A. Haugma, L. Tarig, D.J. Horderik, A.H. Havelaar, Infectious disease risks associated with occupational exposure: a systematic review of the literature, *Occup. Environ. Med.* 69 (2012) 140–146, <https://doi.org/10.1136/oshwe-2011-100068>.
- [37] C. Alvarado-Espino, Toxocarosis in waste pickers: a case control seroprevalence study, *PLoS One* 8 (2013), e54897, <https://doi.org/10.1371/journal.pone.0054897>.
- [38] C.-M. Chou, C.-K. Fan, Chapter Twenty-two - seroprevalence of *Toxocara* spp. infection in Southeast Asia and Taiwan, in: D.D.S.T.-A. in P. Bowman (Ed.), *Toxocara and Toxocarosis*, Academic Press, 2020, pp. 449–463, <https://doi.org/10.1016/b978-0-12-819020-0.0020>.
- [39] E.A. de Oliveira, Y.F.F.B. Merigueti, L.B. Ferreira, I.S. Garcia, A.S. Pereira, R.S. de Santos, L.R. Kneidli, A.P. Dos Santos, A.W. Biondo, R. Giuffrida, V.A. Santarém, The role of Nile Tilapia (*Oreochromis niloticus*) in the life cycle of *Toxocara* spp., *Front. Vet. Sci.* 8 (2021), 685911, <https://doi.org/10.3389/fvets.2021.685911>.

APÊNDICE 2

Kmetiuk LB, Paula WVF, Pádua GT, Delai RR, Freitas AR, Farinhas JH, de Paula LGF, Giuffrida R, Pimpão CT, Álvares Santarém V, Dos Santos AP, Figueiredo FB, Krawczak FDS, Biondo AW. Epidemiology of *Rickettsia* spp. in Atlantic rainforest areas of island and seashore mainland, southern Brazil. *Transbound Emerg Dis*. 2022 Oct 5. doi: 10.1111/tbed.14723. Epub ahead of print. PMID: 36196667. Available from: <https://pubmed.ncbi.nlm.nih.gov/36196667/>

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

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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 an anthropophilic tick throughout South America (Guglielmoni et al., 2006), and human cases of *R. parkeri* strain in 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 (Bitencourt, 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, Ponta 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, & Bedara, 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 (Regnier, 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* *vinii* 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, Barques, & 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 <i>Rickettsia</i> species (% seroreactivity for human beings or dogs (range of endpoint titre))				No. samples with determined homologous reaction (PAIHR in parentheses) ^a
		<i>R. rickettsii</i>	<i>R. parkeri</i>	<i>R. bellii</i>	<i>R. amblyommatis</i>	
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. bellii)
	Dogs (10/68)	0/0	0/0	10/13.9 (64–512)	0/0	7 (R. bellii)
Superagui Island	Humans (0/49)	0/0	0/0	0/0	0/0	–
	Dogs (10/56)	0/5.6	1/1.7 (256)	9/16.1 (128–2048)	0/0	1 (R. parkeri); 7 (R. bellii)
Guaraqueçaba (seashore mainland area)	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)
Ponta do Paraná (seashore mainland area)	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 four fold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species for 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	<i>Amblyomma ovale</i> (6 M + 4 F = 10)	2/7 (28.6%)
	<i>Rhipicephalus sanguineus</i> (57 M + 38 F = 95)	0/14 (0.0%)
	<i>Rhipicephalus</i> spp. (1 L)	–
Ilha do Mel Island	NA	–
Superagui Island	<i>Amblyomma ovale</i> (3 M + 2 F = 5)	0/1 (0.0%)
	<i>Rhipicephalus sanguineus</i> s.l. (25 M + 23 F = 48)	1/35 (2.9%)
	<i>Rhipicephalus</i> spp. (5 N)	–
Guaraqueçaba	<i>Amblyomma ovale</i> (5 M + 10 F + 1 N = 16)	5/10 (50.0%)
	<i>Rhipicephalus sanguineus</i> s.l. (14 M + 11 F = 25)	1/15 (6.6%)
	<i>Rhipicephalus</i> spp. (5 L + 1 N = 6)	–
Pontal do Paraná	NA	–
Total	<i>Amblyomma ovale</i> (N = 31)	7/18 (38.9%)
	<i>Rhipicephalus sanguineus</i> s.l. (N = 167)	2/64 (3.1%)
	<i>Rhipicephalus</i> 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 S2). 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. *Rickettsia* 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 peridomestic ($p = 1.0$) 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-Martinez 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 biodiversity 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-Martinez 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 spillover was found. Nevertheless, *A. ovale* tick has been recognized as one of the most anthrophilic tick species in the Neotropical region and may be likely the responsible vector herein (Guglielmo 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-Martinez 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 FSF) in owners due to the anthrophilic 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 ecotone with the peridomestic 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 are 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 Paraná.

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REFERENCES

- Acosta, I., Luz, H., Faccini-Martínez, Á., Muñoz-Leal, S., Cerutti, Jr., C., & Labruna, M. (2018). First molecular detection of *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma ovale* ticks from Espírito Santo state, Brazil. *Revista Brasileira de Parasitologia Veterinária – Brazilian Journal of Veterinary Parasitology: Orgao Oficial Do Colegio Brasileiro de Parasitologia Veterinaria*, 27(3), 420–422. <https://doi.org/10.1590/S1984-296120180017>
- Barbieri, A. R. M., Filho, J. M., Nieri-Bastos, F. A., Souza, J. C., Szabó, M. P. J., & Labruna, M. B. (2014). Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. *Ticks and Tick-Borne Diseases*, 5(6), 848–853. <https://doi.org/10.1016/j.ttbdis.2014.07.010>
- Barbieri, J. M., Da Rocha, C. M., Bruhn, F. R., Cardoso, D. L., Pinter, A., & Labruna, M. B. (2015). Altitudinal Assessment of *Amblyomma aurantium* and *Amblyomma ovale* (Acarina: Ixodidae), Vectors of Spotted Fever Group Rickettsiosis in the State of São Paulo, Brazil. *Journal of Medical Entomology*, 52(5), 1170–1174. <https://doi.org/10.1093/jme/tjv073>
- Barros Battesti, D., Arzu, M., & Bechara, G. (2006). *Carapatos De Importancia Médico-veterinaria Da Região Neotropical: Um Guia Ilustrado Para Identificação De Espécies*. <https://repositorio.butantan.gov.br/handle/butantan/3153>
- Bitencourt, K., Amorim, M., de Oliveira, S. V., Voloch, C. M., & Gazzeta, G. S. (2019). Genetic diversity, population structure and rickettsias in *Amblyomma ovale* in areas of epidemiological interest for spotted fever in Brazil. *Medical and Veterinary Entomology*, 33(2), 256–268. <https://doi.org/10.1111/mve.12363>
- Brazil. 2022. *Brazilian Spotted Fever - Notified cases in Diseases Information System of Paraná, Brazil*. <http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sih/cnv/nuuf.def>
- Clifford, C. M., Anastas, G., & Elbi, A. (1961). *The larval ixodid ticks of the eastern United States (Acarina-Ixodidae)*. Miscellaneous publications of the Entomological Society of America, 2, 213–237.
- Costa, F. B., da Costa, A. P., Moraes-Filho, J., Martins, T. F., Soares, H. S., Ramirez, D. G., Dias, R. A., & Labruna, M. B. (2017). *Rickettsia amblyommatis* infecting ticks and exposure of domestic dogs to *Rickettsia* spp. in an Amazon-Cerrado transition region of northeastern Brazil. *PLoS ONE*, 12(6), e0179163. <https://doi.org/10.1371/journal.pone.0179163>
- da Silva, B. R., Garcia, M. V., da Silva Rodrigues, V., Andreotti, R., & Ditttrich, R. L. (2017). Ixodidae fauna of domestic dogs in Paraná, southern Brazil. *Brazilian Journal of Veterinary Parasitology*, 26(3), 375–377. <https://doi.org/10.1590/S1984-29612017021>
- da Silva Rocha Fournier, G. F., Pinter, A., Muñoz-Leal, S., Labruna, M. B., Lopes, M. G., Martins, T. F., Colacicó, L., Móra, C. R. S., Moraes-Filho, J., & Dias, R. A. (2020). Implications of domestic dogs in the epidemiology

- of *Rickettsia parkeri* strain Atlantic rainforest and *Rangelia vitalii* in South-eastern Brazil. *Brazilian Journal of Veterinary Parasitology*, 29(1), e022419. <https://doi.org/10.1590/S1984-29612020003>
- Dantas-Torres, F., Fernandes Martins, T., Muñoz-Leal, S., Onofrio, V. C., & Barros-Battesti, D. M. (2019). Ticks (Ixodidae: Argasidae, Ixodidae) of Brazil: Updated species checklist and taxonomic keys. *Ticks and Tick-Borne Diseases*, 10(6), 101252. <https://doi.org/10.1016/j.ttbdis.2019.06.012>
- de Oliveira, P. B., Harvey, T. V., Fehlbeg, H. F., Rocha, J. M., Martins, T. F., de Acosta, I. C. L., Labruna, M. B., Faccini, J. L. H., & Albuquerque, G. R. (2019). Serologic and molecular survey of *Rickettsia* spp. in dogs, horses and ticks from the Atlantic rainforest of the state of Bahia, Brazil. *Experimental & Applied Acarology*, 78(3), 431–442. <https://doi.org/10.1007/s10493-019-00397-x>
- Durães, L. S., Bittencourt, K., Ramalho, F. R., Nogueira, M. C., de Carvalho Nunes, E., & Gazêta, G. S. (2021). Biodiversity of potential vectors of rickettsiae and epidemiological mosaic of spotted fever in the State of Paraná, Brazil. *Frontiers in Public Health*, 9, 577789. <https://doi.org/10.3389/fpubh.2021.577789>
- Faccini-Martínez, Á. A., da Silva Krawczak, F., de Oliveira, S. V., Labruna, M. B., & Angerami, R. N. (2021). Rickettsioses in Brazil: Distinct diseases and new paradigms for epidemiological surveillance. *Revista Da Sociedade Brasileira de Medicina Tropical*, 54, e07322020. <https://doi.org/10.1590/0037-8682-0732-2020>
- Faccini-Martínez, Á. A., Muñoz-Leal, S., Krawczak, F. S., Acosta, I. C. L., Martins, T. F., Serpa, M. C. A., Barbieri, A. R. M., Tovar, J. R., Cerutti Junior, C., & Labruna, M. B. (2020). Epidemiological aspects of *Rickettsia parkeri* in the Atlantic forest biome of Espírito Santo state, Brazil. *Ticks and Tick-Borne Diseases*, 11(2), 101319. <https://doi.org/10.1016/j.ttbdis.2019.101319>
- Faccini-Martínez, Á. A., Vilges De Oliveira, S., Junior, C. C., & Labruna, M. B. (2018). Febre Maculosa por *Rickettsia parkeri* no Brasil: Condutas de vigilância epidemiológica, diagnóstico e tratamento. *Journal of Health & Biological Sciences*, 24(2), 299–312. <https://doi.org/10.12662/2317-3076/jhbs.v6i3.1940.p299-312.2018>
- Gerardi, M., Ramírez-Hernández, A., Binder, L. C., Krawczak, F. S., Gregori, F., & Labruna, M. B. (2019). Comparative susceptibility of different populations of *Amblyomma sculptum* to *Rickettsia rickettsii*. *Frontiers in Physiology*, 10, 653. <https://doi.org/10.3389/fphys.2019.00653>
- Guedes, E., Leite, R., Prata, M., Pacheco, R., Walker, D., & Labruna, M. (2006). Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. *Memórias Do Instituto Oswaldo Cruz*, 100, 841–845. <https://doi.org/10.1590/S0074-02762005000800004>
- Guglielmo, A. A., Beati, L., Barros-Battesti, D. M., Labruna, M. B., Nava, S., Venzal, J. M., Mangold, A. J., Szabó, M. P. J., Martins, J. R., González-Acuña, D., & Estrada-Peña, A. (2006). Ticks (Ixodidae) on humans in South America. *Experimental & Applied Acarology*, 40(2), 83–100. <https://doi.org/10.1007/s10493-006-9027-0>
- Horta, M. C., Labruna, M. B., Sangioni, L. A., Vianna, M. C. B., Gennari, S. M., Galvão, M. A. M., Mangold, A. J., Szabó, M. P. J., Martins, J. R., González-Acuña, D., & Walker, D. H. (2004). Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a Brazilian spotted fever-endemic area in the state of São Paulo, Brazil: Serologic evidence for infection by *Rickettsia rickettsii* and another spotted fever group *Rickettsia*. *The American Journal of Tropical Medicine and Hygiene*, 71(1), 93–97.
- Krawczak, F. S., Agostinho, W. C., Polo, G., Moraes-Filho, J., & Labruna, M. B. (2016). Comparative evaluation of *Amblyomma ovale* ticks infected and noninfected by *Rickettsia* sp. strain Atlantic rainforest, the agent of an emerging rickettsiosis in Brazil. *Ticks and Tick-Borne Diseases*, 7(3), 502–507. <https://doi.org/10.1016/j.ttbdis.2016.02.007>
- Krawczak, F. S., Binder, L. C., Oliveira, C. S., Costa, F. B., Moraes-Filho, J., Martins, T. F., Sponchiado, J., Melo, G. L., Gregori, F., Polo, G., Oliveira, S. V., & Labruna, M. B. (2016). Ecology of a tick-borne spotted fever in southern Brazil. *Experimental & Applied Acarology*, 70(2), 219–229. <https://doi.org/10.1007/s10493-016-0070-1>
- Krawczak, F. S., & Labruna, M. B. (2018). The rickettsial *Euryzomys russatus*, a competent amplifying host of *Rickettsia parkeri* strain Atlantic rainforest for the tick *Amblyomma ovale*. *Ticks and Tick-Borne Diseases*, 9(5), 1133–1136. <https://doi.org/10.1016/j.ttbdis.2018.04.013>
- Labruna, M. B., Horta, M. C., Aguiar, D. M., Cavalcante, G. T., Pinter, A., Gennari, S. M., & Camargo, L. M. A. (2007). Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro municipality, western Amazon, Brazil. *Vector Borne and Zoonotic Diseases*, 7(2), 249–255. <https://doi.org/10.1089/vbz.2006.0621>
- Labruna, M. B., Jorge, R. S. P., Sana, D. A., Jicoma, A. T. A., Kashivakura, C. K., Furtado, M. M., Ferro, C., Perez, S. A., Silveira, L., Santos, T. S., Jr., Marques, S. R., Morato, R. G., Nava, A., Adania, C. H., Teixeira, R. H., Gomes, A. A., Conforti, V. A., Azevedo, F. C., Prada, C. S., ... Barros-Battesti, D. M. (2005). Ticks (Acari: Ixodidae) on wild carnivores in Brazil. *Experimental & Applied Acarology*, 36(1–2), 149–163. <https://doi.org/10.1007/s10493-005-2563-1>
- Labruna, M. B., Whitworth, T., Horta, M. C., Bouyer, D. H., McBride, J. W., Pinter, A., Popov, V., Gennari, S. M., & Walker, D. H. (2004). *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. *Journal of Clinical Microbiology*, 42(1), 90–98. <https://doi.org/10.1128/JCM.42.1.90-98.2004>
- Mangold, A. J., Barges, M. D., & Mas-Coma, S. (1998). Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitology Research*, 84(6), 478–484. <https://doi.org/10.1007/s004360050433>
- Martins, T. F., Diniz-Reis, T. R., Libardi, G. S., Percequillo, A. R., Verdade, L. M., Matushima, E. R., & Labruna, M. B. (2015). Ticks (Acari: Ixodidae) identified from prey-predator interactions via faecal analysis of Brazilian wild carnivores. *Experimental & Applied Acarology*, 66(1), 119–125. <https://doi.org/10.1007/s10493-015-9886-3>
- 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 and Tick-Borne Diseases*, 1(2), 75–99. <https://doi.org/10.1016/j.ttbdis.2010.03.002>
- Moerbeck, L., Vizzoni, V. F., Machado-Ferreira, E., Cavalcante, R. C., Oliveira, S. V., Soares, C. A. G., Amorim, M., & Gazêta, G. S. (2016). *Rickettsia* (Rickettsiales: Rickettsiaceae) vector biodiversity in high altitude Atlantic Forest fragments within a semiarid climate: A new endemic area of spotted-fever in Brazil. *Journal of Medical Entomology*, 53(6), 1458–1466. <https://doi.org/10.1093/jme/tjw121>
- Nieri-Bastos, F. A., Horta, M. C., Barros-Battesti, D. M., Moraes-Filho, J., Ramirez, D. G., Martins, T. F., & Labruna, M. B. (2016). Isolation of the pathogen *Rickettsia* sp. strain Atlantic rainforest from its presumed tick vector, *Amblyomma ovale* (Acari: Ixodidae), from two areas of Brazil. *Journal of Medical Entomology*, 53(4), 977–981. <https://doi.org/10.1093/jme/tjw062>
- Parola, P., Paddock, C. D., Socolovschi, C., Labruna, M. B., Mediannikov, O., Kermif, T., Abdad, M. Y., Stenos, J., Bitam, I., Fournier, P. E., & Raoult, D. (2013). Update on tick-borne rickettsioses around the world: A geographic approach. *Clinical Microbiology Reviews*, 26(4), 657–702. <https://doi.org/10.1128/CMR.00032-13>
- Parola, P., & Raoult, D. (2001). Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Clinical Infectious Diseases*, 32(6), 897–928. <https://doi.org/10.1086/319347>
- Regnery, R. L., Spruill, C. L., & Plikaytis, B. D. (1991). Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *Journal of Bacteriology*, 173(5), 1576–1589. <https://doi.org/10.1128/jb.173.5.1576-1589.1991>
- Sangioni, L. A., Horta, M. C., Vianna, M. C. B., Gennari, S. M., Soares, R. M., Galvão, M. A. M., Schumaker, T. T., Ferreira, F., Vidotto, O., & Labruna, M. B. (2005). Rickettsial infection in animals and Brazilian spotted fever

- endemicity. *Emerging Infectious Diseases*, 11(2), 265–270. <https://doi.org/10.3201/eid1102040656>
- Szabó, M. P. J., Nieri-Bastos, F. A., Spolidorio, M. G., Martins, T. F., Barbieri, A. M., & Labruna, M. B. (2013). In vitro isolation from *Amblyomma ovale* (Acar: Ixodidae) and ecological aspects of the Atlantic rainforest *Rickettsia*, the causative agent of a novel spotted fever rickettsiosis in Brazil. *Parasitology*, 140(6), 719–728. <https://doi.org/10.1017/S0031182012002065>
- Szabó, M. P. J., Pinter, A., & Labruna, M. B. (2013). Ecology, biology and distribution of spotted-fever tick vectors in Brazil. *Frontiers in Cellular and Infection Microbiology*, 3, 1–9. <https://doi.org/10.3389/fcimb.2013.00027>

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