One Health 13 (2021) 100353

Contents lists available at ScienceDirect

One Health

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

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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ARTICLE INFO

Keywords:
One Health
Toxocariasis
Toxocara canis
Toxocara cati
Zoonoses

ABSTRACT

Toxocariasis, 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 toxocariasis.

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https://doi.org/10.1016/j.onehlt.2021.100353
Received 25 July 2021; Received in revised form 18 November 2021; Accepted 24 November 2021
Available online 25 November 2021

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 Parana.

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 habitants 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 Parana 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 Pontal 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 Brasilia 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 800*g* 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 800*g* for five minutes to separated 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 phenylmethylsulphonyl 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 HCI 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/uL 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 x*g* 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 x*g* 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 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, sanitizinghand 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 TPP ($P = 0.352$) 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 an 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 toxocariasis 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				
Cats 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					
	Beach	Trail	Central Square	Soccer Field	Wharf	Total
Island						
Ilha do Mel island – Encantadas	3/10	8/10	NS	NS	NS	11/20 (55.0%)
Ilha 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%)
Peças Island	6/10	NS	NS	NS	NS	6/10 (60.0%)
Mainland						
Guaraqueçaba	0/10	NS	4/10	1/10	NS	5/30 (16.7%)
Pontal 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 metanalysis 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 toxocariasis, 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 toxocariasis 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 toxocariasis [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 toxocariasis, 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 toxocariasis 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 toxocariasis [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 toxocariasis. The island-effect of increased disease transmission was not observed for the soil-borne pathogen *Toxocara* spp.

Funding

The research was funded through the Araucária Foundation for Scientific and Technological Development of Paraná State (SUS2020111000014).

Declaration of Competing Interest

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

Acknowledgments

The authors thank the State Secretary of Environment for assistance on blood sampling of human beings and dogs. Ruana Renostro Delai and Aaronson Ramathan Freitas have been supported by a Ministry of Health fellowship for their residency program on Shelter Medicine. Yslla Merigueti and Isabela B. Ferreira have been supported by the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) for her Post-graduation. The authors kindly thank the Suzanne Pratt for editing and improving the article.

Appendix A. Supplementary data

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

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