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Soil-Transmitted Parasites and Non-Pathogenic Nematodes in Different Regions of Porto Alegre City, Brazil: A Comparison between Winter and Summer

Marina Ziliotto, Joel Henrique Ellwanger * and José Artur Bogo Chies *

Laboratory of Immunobiology and Immunogenetics, Postgraduate Program in Genetics and Molecular Biology (PPGBM), Department of Genetics, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre 91501-970, Brazil; marinaztto@gmail.com

* Correspondence: joel.ellwanger@gmail.com (J.H.E.); jabchies@terra.com.br (J.A.B.C.)

Abstract: We assessed the prevalence of soil-associated parasites and non-pathogenic nematodes in eight public areas of Porto Alegre (Rio Grande do Sul state, southern Brazil), the most populous city in Rio Grande do Sul. Soil samplings were carried out during the winter of 2022 and summer of 2023: A total of 80 samples were collected in winter and 80 in summer (ten samples from each sampling site per season), totaling 160 soil samples. The frequency of microscopic non-pathogenic nematode larvae was significantly higher (p = 0.048) in winter (93.75%) than in summer (82.50%). Considering the pooled data from winter and summer (n = 160) for human pathogenic parasites, the following frequencies were observed (using microscopy analysis): hookworm (filariform) larvae (1.25%), hookworm (rhabditiform) larvae (11.25%), *Strongyloides* spp. (filariform) larvae (0.63%), *Strongyloides* spp. (rhabditiform) larvae (2.5%), hookworm eggs (10.63%), *Ascaris* spp. eggs (10.00%), and *Trichuris* spp. eggs (1.25%). Hookworm (rhabditiform) larvae were the most frequent parasitic structures (15.00%) in winter, and *A. lumbricoides* eggs were the most frequent parasitic structures (8.75%) in summer. No statistically significant difference was observed in the frequency of pathogenic parasites between the seasons ($p > 0.05$). *Toxoplasma gondii* DNA was assessed, but all soil samples tested negative in molecular analysis. Our results indicate that soil from many regions of Porto Alegre shows a high prevalence of soil-transmitted helminths, indicating the need for improvements in social conditions and environmental sanitation in the city. Our study also suggests that climate change may affect soil biodiversity, potentially harming non-pathogenic nematodes and favoring human pathogenic parasites.

Keywords: Brazil; climate; soil-transmitted helminths; environmental sanitation; one health; parasites

Citation: Ziliotto, M.; Ellwanger, J.H.; Chies, J.A.B. Soil-Transmitted Parasites and Non-Pathogenic Nematodes in Different Regions of Porto Alegre City, Brazil: A Comparison between Winter and Summer. *Parasitologia* **2024**, *4*, 1–14. https://doi.org/10.3390/parasitologia4010001

Academic Editor: Maria Victoria Periago

Received: 10 November 2023
Revised: 12 December 2023
Accepted: 18 December 2023
Published: 21 December 2023

1. Introduction

Parasites are organisms found in virtually all ecosystems, and parasitic infections can influence various aspects of the host's life, including health conditions and behavior [1,2]. Also, the effects of some parasitic infections on human health can trigger major economic losses. Neglected tropical diseases (NTD), including those caused by parasites, are more prevalent in the world's poorest and marginalized populations than in developed countries. In this context, NTD persists amongst these populations due to a range of socioeconomic and environmental issues. Vulnerable and poor populations have limited social and economic power to call attention to their health problems, facing a greater disease burden than other more privileged populations [3]. Moreover, anthropogenic environmental changes (e.g., deforestation, biodiversity loss, pollution, unplanned urbanization, and climate change) facilitate human exposure to several emerging and neglected pathogens, including parasites, which mainly affect populations with socioeconomic vulnerability [3,4].

Soil-transmitted helminths (STH) are a group of parasites with an obligate life cycle in the soil. Considering their distribution and public health importance, the main STH

are hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*), and whipworms (*Trichuris trichiura*). *Strongyloide stercoralis* is also an important STH species in countries such as Brazil. STH transmission occurs mainly through contact with feces or soil contaminated with parasite eggs, and the prevalence of STH infections is related to socioeconomic and environmental aspects, such as family income, schooling level, and environmental sanitation [5–7].

STH is part of the NTD group and is endemic to at least 120 countries [8]. STH infections cause high morbidity and are associated with impaired intellectual development and growth in children, iron-deficiency anemia, intestinal problems, and malnutrition [7,9]. In 2019, these parasitic infections were estimated to be responsible for the loss of 1.9 million disability-adjusted life years (DALYs) globally [10].

In addition to STH, other parasites with zoonotic potential are present in the soil, such as *Toxoplasma gondii* [11], a cosmopolitan obligate intracellular protozoan that parasitizes practically any warm-blooded animal. Humans infected with *T. gondii* can develop toxoplasmosis, which is a zoonosis with a wide geographical distribution. The prevalence of toxoplasmosis is influenced by human cultural patterns (e.g., food and hygiene habits), socioeconomic conditions, and access to environmental sanitation [12].

Inadequate water, sanitation, and hygiene (WASH) are critical drivers of the parasitic disease burden, especially among children from low-income and middle-income countries. The disease burden linked to inadequate WASH scores can be significantly reduced (potentially eliminated) by improving WASH indicators [13], thereby reducing human exposure to unhealthy environments [14]. Preventive chemotherapy and improvements in WASH scores are the main recognized measures for decreasing STH infections [15]. Although the morbidity associated with STH infections is controlled by preventive chemotherapy, the interruption of STH transmission cycles will only be effective when populations have access to adequate sanitation systems [16]. Sanitation, safe water, and hygiene are health-promoting components with different characteristics [17], but often approached each other because they bring complementary benefits. Sanitation is currently understood as a way of promoting health, quality of life, well-being, and sustainable development [18].

Undeniably, the expansion of the sanitation system in Brazil has had a positive impact on public health. Until 1930, infectious and parasitic diseases were responsible for 45% of the deaths in Brazil. This percentage was reduced to less than 5% in 2010, largely due to the expansion of the sanitation structures in the country [18]. However, this progress is both slow and insufficient. For example, Brazil occupies the 83rd position in the global sanitation ranking. In 2020, only 48.7% of the Brazilian population had access to safely managed sanitation, an increase of approximately only 13% compared to the data from 2000 [19]. Access to safe sanitation for less than 50% of the population is a serious and problematic situation for health, social, and environmental reasons.

Porto Alegre city, the capital of Rio Grande do Sul state (southern Brazil), showed important advances concerning environmental sanitation until 2015, when investments on sewage treatment stagnated. The lack of continuous investments caused the city to lose its position in the Sanitation Ranking of *Instituto Trata Brasil*, which evaluates the evolution of water, sewage, investment, and water loss indicators in the largest Brazilian cities. Porto Alegre was ranked 24th position in the 2017 ranking to the 43rd position in the 2022 ranking [20,21], which represents a significant setback.

Considering (i) the importance of access to adequate sanitation for the prevention of infections by STH and other parasites, and (ii) the lack of updated information on the environmental prevalence of these parasites in Porto Alegre, in this study we evaluated the presence of soil-transmitted parasites in eight regions of Porto Alegre. For comparison purposes, this evaluation was performed in both winter and at summer, using microscopy and molecular techniques. We also collected socio-environmental information (considering human, animal, and environmental factors) from each study site to help explain our findings from the One Health perspective.

2. Results

2.1. Characteristics of the Sampling Sites

Table 1 details geospatial information, temperature, weather conditions, and soil characteristics at the time of samplings for each study site in both seasons. In winter, the temperature varied between 13 °C and 21 °C (17 °C average), with cloudy weather being recorded in most sampling days. During summer, the average temperature was 26 °C, and most days presented sunny weather. The soil samples showed mostly organic composition.

Table 1. Basic information of each sampling site (winter and summer seasons).

Sampling Sites	Popular Name [1]	Latitude	Longitude	Temperature (Average), Winter	Temperature (Average), Summer	Weather Condition, Winter	Weather Condition, Summer	Soil Characteristic
Site 1	Alfândega Square	−30.028917	−51.230997	13 °C	26 °C	Cloudy	Cloudy	Organic; Sandy
Site 2	Mascarenhas de Moraes Park	−29.981681	−51.186776	20 °C	28 °C	Cloudy	Sunny	Organic; Sandy; Clayey
Site 3	Miguel Anibal Square	−30.012382	−51.132493	13 °C	25 °C	Cloudy	Cloudy	Organic; Sandy; Clayey
Site 4	Chico Mendes Park	−30.026889	−51.112667	21 °C	22 °C	Cloudy	Cloudy	Organic; Sandy; Clayey
Site 5	Doctor Jurandy Barcellos da Silva Square	−30.076251	−51.219721	20 °C	28 °C	Cloudy	Sunny	Organic; Sandy; Clayey
Site 6	Ipanema Beach [2]	−30.133273	−51.237503	18 °C	28 °C	Cloudy	Sunny	Organic; Sandy
Site 7	Saint'Hilaire Park	−30.097943	−51.113585	15 °C	27 °C	Cloudy	Sunny	Organic; Clayey
Site 8	Espigão Square	−30.241057	−51.086015	20 °C	25 °C	Sunny	Sunny	Organic; Sandy; Clayey

[1] Translated from Brazilian Portuguese popular names. [2] Freshwater beach.

Table 2 shows information concerning to socio-environmental characteristics of each sampling site (compiling data from both seasons). Considering vegetation, except the Saint'Hilaire Park (site 7), which has a well-preserved native vegetation, and Ipanema Beach (site 6), which is on the edge of Lake Guaíba, all other places have a predominance of ornamental vegetation, mixing native and exotic species. Three locations had inadequate environmental sanitation (sites 2, 6 and 7). The Mascarenhas de Moraes Park (site 2) had evident untreated sewage disposal in a stream present in the urban perimeter. In all sites, improper disposal of domestic waste was observed. We highlight Espigão Square (site 8), where incorrect disposal of hospital waste (many used syringes) was observed, in addition to an alarming amount of garbage disposed throughout the region. In almost all sites (n = 7; 87.5%), incorrectly discarded garbage formed potential artificial breeding ground for the development of mosquito larvae. Also, the presence of dogs was observed, directly or indirectly, in all sites. Considering synanthropic animals, the main indicators were pigeon feces, observed in six sites (75%). We also observed human feces at three sites (37.8%) (Table 2).

Table 2. Socio-environment-related characteristics of each sampling site (considering data collected during winter and summer seasons together).

Sampling Sites	Local Vegetation	Human Dwellings/Buildings	Noise Pollution	Environmental Sanitation	Domestic Sewage Disposal	Artificial Mosquito Larvae Breeding Grounds	Domestic Solid Waste	Industrial/Biological Sewage Disposal	Expected Human Circulation	Domestic and Farm Animals [1]	Synanthropic Animals [1]	Human Feces
Site 1	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Intense	Present, dogs	Present, pigeons and rodents	Present
Site 2	Ornamental	Regular	Absent/light sounds	Insufficient	Present	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Present
Site 3	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Infrequent	Present, dogs	Present, pigeons	Absent
Site 4	Ornamental	Regular	Absent/light sounds	Proper	Absent	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Absent
Site 5	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Frequent	Present, dogs and cats	Present, pigeons	Present
Site 6	Naturally absent	Regular	Present	Insufficient	Present	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Absent
Site 7	Natural grass-lands/shrubs and preserved arboreal forest	Irregular	Absent/lights sounds	Insufficient	Present	Absent	Present	Absent	Infrequent	Present, dogs and horses	Absent	Absent
Site 8	Ornamental and degraded arboreal forest	Regular	Present	Proper	Absent	Present	Present	Absent	Infrequent	Present, dogs	Absent	Absent

[1] Direct observation or signs indicating the presence of these animals.

2.2. Frequency of Parasite/Nematode-Positive Sites

Table 3 details results obtained with microscopy analyses. Each + mark in the table indicates the presence of at least one positive sample for non-pathogenic nematodes and pathogenic parasite (larvae or eggs) at the sampling site. Nematode larvae were observed in all sampling sites, both in summer and winter seasons. Considering polled results from winter and summer (number of + sites in both seasons), we observed hookworm eggs in seven (87.5%) study sites, *Ascaris* spp. eggs in six (75%) sites, and *Trichuris* spp. eggs in one (12.5%) site. We found hookworm rhabditiform larvae in 100% of the study sites, and hookworm filariform larvae (infective form) in two (25%) sites. We also observed *Strongyloides* spp. rhabditiform larvae in three (37.5%) sites, and filariform larvae in one (12.5%) site (combined winter + summer data).

Considering only the positive results obtained during winter, we observed hookworm filariform larvae at one (12.5%) sampling site, and hookworm rhabditiform larvae at seven (87.5%) sampling sites. Also, during the winter, *Strongyloides* spp. filariform larvae were observed at site 4, and *Strongyloides* spp. rhabditiform larvae at sites 1 and 2. Regarding parasite eggs, hookworm eggs were observed at almost all sites ($n = 7$; 87.5%), *A. lumbricoides* eggs at five sites (62.5%), and *T. trichiura*-like eggs were observed at only one site (12.5%) during winter (Table 3).

Also considering the number of positive sites, but now looking at summer data, hookworm filariform larvae was observed at one sampling site (12.5%), and hookworm rhabditiform larvae was observed at five sampling sites (62.5%). *Strongyloides* spp. filariform larvae were not observed during the summer, whereas *Strongyloides* spp. rhabditiform larvae were observed at two sites (25%). Hookworm eggs were observed at five sites (62.5%) and *A. lumbricoides* eggs were observed at six sites (75%). *Trichuris* spp. eggs were not observed during the summer (Table 3).

Table 3. Microscopic non-pathogenic nematodes and pathogenic parasite larvae and eggs observed at each sampling site during winter and summer seasons.

Observed Parasite Larvae and Eggs	Sampling Sites															
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8	
	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)	Winter (*n* = 10)	Summer (*n* = 10)
Non-pathogenic nematode larvae [1]	+(10)	+(9)	+(9)	+(8)	+(10)	+(9)	+(10)	+(10)	+(9)	+(9)	+(9)	+(3)	+(9)	+(8)	+(9)	+(10)
Hookworm (filariform) larvae								+(1)					+(1)			
Hookworm (rhabditiform) larvae		+(1)	+(1)		+(1)	+(1)	+(2)	+(2)	+(2)		+(2)	+(1)	+(1)		+(3)	+(1)
Strongyloides spp. (filariform) larvae							+(1)									
Strongyloides spp. (rhabditiform) larvae	+(1)	+(1)	+(1)							+(1)						
Hookworm eggs [2]	+(1)		+(1)		+(1)	+(2)	+(2)	+(1)	+(1)	+(1)	+(2)	+(1)	+(3)	+(1)		
Ascaris spp. eggs	+(1)	+(1)		+(1)	+(1)	+(1)	+(2)	+(2)	+(2)	+(1)	+(3)	+(1)				
Trichuris spp. eggs	+(2)															

Each + indicates the presence of at least one positive sample for non-pathogenic nematodes and pathogenic parasite (larvae or eggs) at the sampling site. The number of positive samples at each site is shown in parentheses. [1] Non-pathogenic to humans. Components of natural soil biodiversity. [2] Potentially *Necator* spp. and *Ancylostoma* spp. eggs.

2.3. Frequency of Microscopic Non-Pathogenic Nematodes and Pathogenic Parasites between Seasons

Table 4 shows the frequency of positive samples based on microscopic analysis, considering pooled data from winter and summer, as well as data from each season separately. The frequencies of non-pathogenic nematode larvae were significantly different between seasons ($p = 0.048$), with a higher frequency in winter (93.75%) than in summer (82.50%).

Table 4. Comparison of the proportions of positive samples between winter and summer for each parasite.

Observed Parasite Larvae and Eggs	Winter + Summer ($n = 160$)	Winter ($n = 80$)	Summer ($n = 80$)	Fisher's p-Value (Winter versus Summer)
Microscopic nematode larvae [1]	141 (88.13%)	75 (93.75%)	66 (82.50%)	**0.048**
Hookworm (filariform) larvae	2 (1.25%)	1 (1.25%)	1 (1.25%)	1.000
Hookworm (rhabditiform) larvae	18 (11.25%)	12 (15.00%)	6 (7.50%)	0.210
Strongyloides spp. (filariform) larvae	1 (0.63%)	1 (1.25%)	0 (0%)	1.000
Strongyloides spp. (rhabditiform) larvae	4 (2.5%)	2 (2.50%)	2 (2.50%)	1.000
Hookworm eggs [2]	17 (10.63%)	11 (13.75%)	6 (7.50%)	0.305
Ascaris spp. eggs	16 (10.00%)	9 (11.25%)	7 (8.75%)	0.793
Trichuris spp. eggs	2 (1.25%)	2 (2.50%)	0 (0%)	0.497

[1] Non-pathogenic for humans. Natural components of soil biodiversity. [2] Potentially *Necator* spp. and *Ancylostoma* spp. eggs. Statistically significant p-value is shown in bold.

Considering pathogenic parasites, pooled data from winter and summer showed that the most frequent parasitic structures were hookworm (rhabditiform) larvae (11.25%), hookworm eggs (10.63%), and *Ascaris* spp. eggs (10.00%) (Table 4). Hookworm (rhabditiform) larvae were the most frequent parasitic structures (15.00%) during winter, and *Ascaris* spp. eggs were the most frequent parasitic structures (8.75%) during summer. No statistical difference was observed in the frequency of pathogenic parasites between the seasons ($p > 0.05$ in all tests; Table 4).

2.4. Molecular Analysis

According to the results obtained from the PCR with specific primers, none of the 64 soil samples analyzed showed bands indicative of the presence of *T. gondii* DNA. Notably, positive and negative controls satisfactorily ensured the quality of PCR reactions.

3. Discussion

The United Nations has established water supply and sanitation as one of the Sustainable Development Goals [22], considering that environmental sanitation promotes human and environmental health, and controls multiple infectious and parasitic diseases [23,24]. The dynamics of WASH-related diseases are also affected by different features, such as population density, housing issues, concentration of pathogenic agents to which an individual is exposed, genetic and immune human susceptibility, and other factors [7,25,26]. The transmission of parasites in urban areas also depends on biological and socioeconomic factors. For instance, the prevalence of pathogenic parasites found in soil samples is strongly influenced by sewage treatment systems in cities [24]. Raw sewage can contain a large load of parasite eggs, contaminating soil and water where the sewage is improperly discharged. In addition, inequalities and inadequate housing can facilitate the contamination of the population in two ways: (i) irregular housing may be close to streams or water contaminated by untreated raw sewage, and (ii) lack of access to treated sewage is more common in slums and irregular housing, facilitating the contact of vulnerable populations with contaminated water and soil [27,28].

In this study, we evaluated the public areas in eight main regions of Porto Alegre city. Almost all sampling sites had adequate sanitation infrastructure, although evidence

of irregular garbage disposal was observed in all studied areas. As a matter of fact, the presence of an adequate sanitation infrastructure cannot, per se, totally prevents the occurrence of other situations that may compromise the environment and affect public health. One example is the presence of human and animal feces in the environment, even in areas with adequate sanitation. The presence of irregular garbage disposal and feces is not surprising, considering that the studied areas encompassed large public green areas, in the city, with a high circulation of people and animals. Nevertheless, we also observed the disposal of untreated sewage in the Mascarenhas de Moraes Park, evidencing the insufficient environmental sanitation in Porto Alegre.

The presence of non-pathogenic nematode larvae (components of the soil's natural biodiversity) [29,30] in 100% of the analyzed sites can be regarded as methodological control. In this sense, one can argue that the methodology was well executed since Rugai's method adapted for soil aims to recover live larvae [31]. Nevertheless, we observed a lower frequency of samples positive for non-pathogenic nematode larvae during summer than during winter. The abundance and structure of nematode population are sensitive to changes in the environment and season-related conditions [32,33]. Specifically, soil characteristics (moisture and temperature) are major factors that influence nematode populations, along with food availability, interactions with other organisms, and physical-chemical conditions of the environment [32]. Therefore, the differences between analyses carried out in the Rio Grande do Sul state may also be related to the climate and soil diversity of the state, a very biodiverse region covered by two biomes (Pampa and Atlantic Forest), and an extensive marine coast [7].

These results could also be related to the period of intense drought that Rio Grande do Sul experienced in the months prior to our summer samplings due to the occurrence of the oceanic and atmospheric phenomenon known as "La Niña", allied to the climatic consequences associated to Amazon deforestation [34,35]. These conditions may have contributed to a reduction in the population of microscopic soil nematodes. From a broader perspective this result clearly highlights that climate change will indeed have an important impact on soil biodiversity and nematode communities, as suggested elsewhere [36–39].

Considering the main STH, our results concerning microscopy analyzes (frequencies detailed in Table 4) are in line with other studies carried out in the Rio Grande do Sul state (reviewed by Ziliotto et al. [7]). Based on data compiled from studies performed in the state, hookworms were found in a range of 11.0% to 93.2% of environmental samples, *A. lumbricoides* in a range of 3.1% to 10.2%, and *T. trichiura* in a range of 0.8% to 32.5% of environmental samples [7]. Also, our observations of filariform and rhabditiform *Strongyloides* spp. larvae are not surprising since strongyloidiasis is endemic in Brazil [5].

The fact that we did not observe differences in STH prevalence between seasons (Table 4), although non-pathogenic nematode occurrence was affected (as discussed above), suggests that pathogenic soil-parasites are more resilient to climatic variations than are non-pathogenic soil fauna. This result is in line with studies suggesting that climate change contributes to biodiversity loss, favoring pathogenic organisms that are more resilient to environmental changes [4]. Also, an increase in temperatures and precipitations may favor the STH development in the soil [40].

To the best of our knowledge, the last study carried out in Porto Alegre with the objective of analyzing the presence of parasitic structures in soil samples was published in 2013 [41]. In this study, 100% of the analyzed sites had contaminated soil, with 40.8% of them showing parasitic structures. The frequency of *A. lumbricoides* eggs was 10.2%, and the frequency of *T. trichiura* eggs was 4.4% [41], both frequencies similar to those found in our study (Table 4). This suggests that parasite contamination in Porto Alegre's soil has remained stable over the last 10 years.

Another parasite of medical interest found in the soil by Vargas et al. [41] was *Toxocara* spp., with a frequency of 4.2%. In contrast, no *Toxocara* eggs were observed in our samples. Concerning *T. gondii*, no DNA positivity was observed for *T. gondii* in the samples. Considering the high incidence of *Toxocara* spp. and *T. gondii* observed in other studies [42–44],

we expected to find *Toxocara* spp. and *T. gondii* in our samples. We believe that the small amount of soil (0.2 ± 0.05 g) used in the DNA extraction kit could have limited the detection capacity of *T. gondii* DNA, potentially explaining our results. The technique used by Vargas et al. [41] includes a flotation process, which is commonly used to recover *Toxocara* eggs in the soil, due to the characteristics of the egg [45]. However, Rugai's method does not include this process. Differences between the microscopy-based parasitological methods used by us and those used by Vargas et al. [41] potentially explain the discrepancy in the results concerning *Toxocara* eggs.

In other Latin American countries, various techniques have been used to assess the presence of STH in soil samples [46–49]. For example, using washing, sedimentation, and flotation techniques, Falcone et al. [49] found the presence of parasites in 31% of soil samples from Argentina, including *A. lumbricoides* (1.1%) and hookworms (5.6%) [49]. Also, in the subtropical northern border of Argentina, Riveiro et al. [48] evaluated environmental contamination by parasites using a modified version of the Shurtleff and Averre method. The authors found that 37.5% of the soil samples were contaminated with parasites, with hookworms being the most prevalent parasite (28.8%) parasite. *Toxacara* spp. (6%), *Trichuris* spp. (3.8%) and *Ascaris* spp. (1.1%) were also found [48].

Important advances in the direction of sanitation promotion have been made in Brazil in recent decades, such as the National Basic Sanitation Law (2007) and the National Solid Waste Policy (2010) [18]. However, much work still needs to be done, especially considering Porto Alegre city. In contrast to diseases that do not have vaccines or treatments available, environmental interventions to reduce disease burden linked to inadequate WASH already exist [14] and can be immediately implemented in the presence of political will, intersectoral and public-private partnerships, and resource allocation [14]. Health sector and scientists must advocate the critical, and still underused, health-cost saving potential of sanitation [17]. Finally, we stress that human, animal, and environmental factors must be addressed together in health promotion policies focused on controlling parasitic infections [26].

4. Materials and Methods

4.1. Legal Aspects

Soil samplings were authorized by the Secretaria do Meio Ambiente e Infraestrutura of Porto Alegre city and by the Sistema de Autorização e Informação em Biodiversidade—SISBIO (Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio, Ministério do Meio Ambiente, Brazil): SISBIO No. 82718-1. The samplings performed at the Parque Natural Municipal Saint'Hilarie was also authorized by the institutional leaders. The animal taxa sampled in this study were registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen (Ministério do Meio Ambiente, Brazil): registration code A6C2812.

4.2. Study Area and Soil Sampling

Porto Alegre, located in the extreme south of Brazil, has an estimated population of 1,492,530 people, and includes areas covered by the Pampa and Atlantic Forest biomes [50]. With the objective of performing soil samplings at sites from various landscapes in Porto Alegre's territory, we based our sampling on the division of the eight planning regions of Porto Alegre city [51], as shown in Figure 1. For each of the eight regions, a park or public square with intense circulation of humans and animals was chosen for the soil samples. Specifically, the study sites were: Alfândega Square ("Praça da Alfândega"; site 1), Mascarenhas de Moraes Park ("Parque Mascarenhas de Moraes"; site 2), Miguel Anibal Genta Square ("Praça Miguel Anibal Genta"; site 3), Chico Mendes Park ("Parque Chico Mendes"; site 4), Doctor Jurandir Barcellos da Silva Square ("Praça Doutor Jurandir Barcellos da Silva"; site 5), Ipanema Beach ("Praia de Ipanema", a freshwater beach; site 6), Saint'Hilarie Park ("Parque Natural Municipal Saint'Hilarie"; site 7), and Espigão Square ("Praça do Espigão"; site 8). Table 1 details basic information on each sampling site.

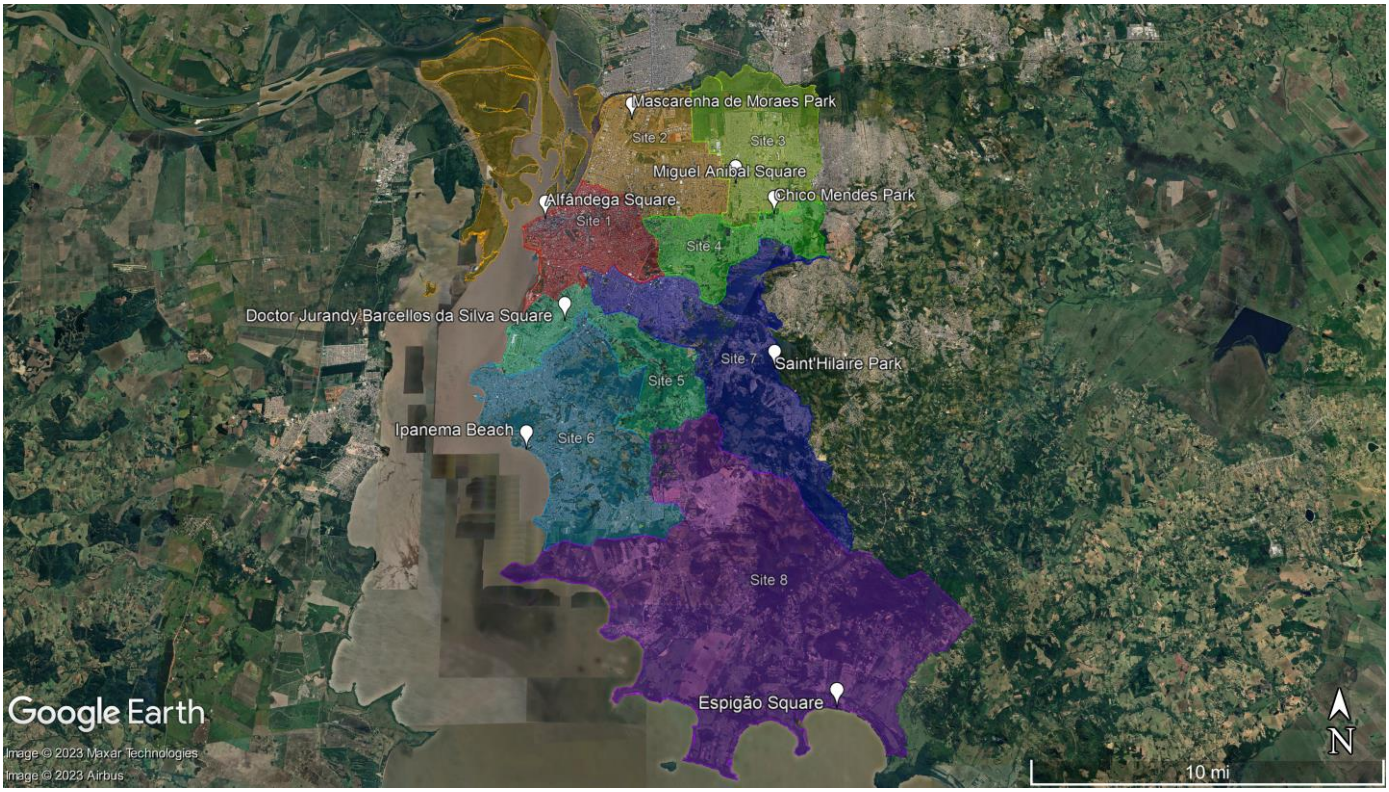

Figure 1. Study area showing the eight planning regions of Porto Alegre city. Each region is highlighted in a different color. Sampling sites (detailed in Table 1) are indicated by white markers.

Samples were collected twice at each site: once in the winter of 2022 (July to September) and once in the summer of 2023 (January to March). For each season and from each study site, ten points were selected for soil sampling, considering places with indicatives of human and animal circulation. At each point, 150 g of topsoil was collected according to our pilot study (see Ziliotto et al. [52] for detailed methods). The samples were placed in sterile plastic tubes, and packed in a Styrofoam box, and then transported to the laboratory for microscopic and molecular analyses. Eighty samples were collected in winter and 80 in summer (ten samples from each sampling site per season), for a total of 160 soil samples.

Finally, to record data on biotic and abiotic factors that influence the presence/absence of soil nematodes, STH, and other parasites, we applied a previously described form to assess of socio-environmental and anthropic activity at each sampling site [52].

4.3. Microscopy Analysis of Soil Samples

We recognize that there are a variety of effective techniques for detecting parasites [46–49,53]. In this study, microscopic analysis was used for the detection of STH larvae and eggs as it is considered the gold standard technique for STH studies [54]. In brief, soil samples were subjected to spontaneous sedimentation, stained with 2% Lugol, and analyzed under a microscope at 100× and 400× magnification, according to the Rugai's method [55] adapted for soil samples [31]. There analyses were performed by two trained microscopists. The methodological details used in this study were described by Ziliotto et al. [52]. The references used for the identification of the parasites were Neves [56], De Carli [57], Mariano et al. [58], and CDC [59]. As mentioned above, a total of 160 soil samples were analyzed. The samples were analyzed in duplicate (two slides for each sample, one slide for each microscopist), totaling 320 slides analyzed during the study.

4.4. DNA Extraction and Molecular Analysis

For each sampling site, 50 g of topsoil from each of the ten sampling points was pooled. A small fraction of this pooled sample was subjected to DNA extraction using the Invitrogen™ PureLink™ Microbiome DNA Purification Kit for soil samples, following the manufacturer's recommendations. DNA extraction was performed in quadruplicate for each of the eight sampling sites (with each replicate corresponding to the pooled ten samples of the respective site, as previously mentioned), This resulted in 32 DNA samples from winter samplings and 32 DNA samples from summer samplings, for a total of 64 DNA samples.

Using the resulting DNA samples, we performed molecular analysis (polymerase chain reaction—PCR) to verify the presence of *Toxoplasma gondii* DNA in the samples. Conventional PCR targeting a non-coding fragment of 529 base pairs repeated in *T. gondii* genome was carried out according to the protocol of the Brazilian Ministry of Health for the investigation of *T. gondii* in environmental and food samples [60]. The primers used were: Tox4 5′-GCTGCAGGGAGGAAGACGAAAGTTG-3′ and Tox5 5′-CGCTGCAGACACAGTGCATCTGGATT-3′ [61]. According to the protocol, this technique showed 89% sensitivity and 91% of specificity. The PCR products were analyzed on a 1.5% agarose gel under UV light. The presence of a 529 base-pair band represents a positive result for *T. gondii* DNA [60].

4.5. Statistical Analysis

The proportions of samples positive for non-pathogenic nematode larvae and pathogenic parasite structures between summer and winter were compared using Fisher's exact test. Tests were performed using WinPepi version 11.6 [62], and a p-value ≤ 0.05 was considered statistically significant.

5. Conclusions

This study described updated data on the presence/absence of various pathogenic parasites and non-pathogenic nematodes in soil samples from parks and public squares in Porto Alegre city, Brazil. These results highlight the importance of monitoring the prevalence of soil-transmitted parasites in places subjected to high human and animal circulation, which increases the risk of infection. Improvements in socio-environmental conditions, especially in the sanitation infrastructure of Porto Alegre are mandatory. Finally, our findings also suggest that climate change may affect soil biodiversity, favoring some groups of pathogenic parasites (e.g., STH), that may be more resilient to environmental changes and favored by higher temperatures.

Author Contributions: Conceptualization, M.Z. and J.H.E.; Methodology, M.Z. and J.H.E.; Investigation, M.Z., J.H.E. and J.A.B.C.; Validation, M.Z. and J.H.E.; Formal analysis, M.Z. and J.H.E.; Writing—Original Draft, M.Z.; Writing—Review & Editing, M.Z., J.H.E. and J.A.B.C.; Visualization, M.Z. and J.H.E.; Supervision, J.H.E. and J.A.B.C.; Resources, J.A.B.C.; Funding acquisition, J.A.B.C. All authors have read and agreed to the published version of the manuscript.

Funding: Marina Ziliotto receives a doctoral fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). Joel Henrique Ellwanger receives a postdoctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Programa Nacional de Pós-Doutorado—PNPD/CAPES, Brazil, Finance Code 001). José Artur Bogo Chies receives a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (Bolsa de Produtividade em Pesquisa—Nível 1A, CNPq, Brazil) and has research funded by CAPES (CAPES AUXPE 686/2020; Brazil).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We thank to the team of Parque Natural Municipal Saint'Hilarie for the support during soil samplings, and the team at Laboratório de Protozoologia (Faculty of Veterinary Medicine—UFRGS) and Parasitology Sector (UFRGS) for providing us positive controls. Graphical abstract was created with the aid of Microsoft 365, and Figure 1 with the aid of Google Maps.

Conflicts of Interest: The authors declare no conflict of interest.

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