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HIGH PREVALENCE OF PARASITES WITH ZOONOTIC POTENTIAL IN SOILS OF PUBLIC SQUARES AND PARKS IN THE METROPOLITAN REGION OF RECIFE-PE, BRAZIL

ALTA PREVALÊNCIA DE PARASITA COM POTENCIAL ZOONÓTICO EM SOLOS DE PRAÇAS E PARQUES PÚBLICOS DA REGIÃO METROPOLITANA DE RECIFE-PE, BRASIL

ALTA PREVALENCIA DE PARÁSITO CON POTENCIAL ZOONÓTICO EN SUELOS DE PLAZAS Y PARQUES PÚBLICOS EN LA REGIÓN METROPOLITANA DE RECIFE-PE, BRASIL

Igor Marley Pereira de Andrade[1]
Ana Júlia Santos[2]
Yasmin Vitoria Moura de Sena[3]
Isabela Marques de Lima[4]
Kamylle Cynnara Tavares da Silva[5]
Rutyelle Moreira de Melo Sousa[6]
Rodrigo Ferreira da Silva[7]
Caroline Paiva da Silva[8]
Nayara dos Santos Araújo[9]
Mariana Santana Queires[10]
Ingrid da Silva Florêncio Freire[11]
Doralice Conceição da Paz Neta[12]
André de Lima Aire[13]

ABSTRACT

Several species of helminths and protozoa use soil as a medium for development and infection and are responsible for neglected tropical diseases, including zoonoses. The soil of public areas such as squares and parks represents a transmission risk for humans and other animals. Thus, the aim of the present study was to assess the frequency of soil-isolated parasites in squares and public parks in the city of Recife, Pernambuco - Brazil. Soil samples were collected from six squares or parks located in the metropolitan region of the municipality. The material analysis was performed using the Rugai technique adapted for soil, allowing the identification of evolutionary stages of parasites through optical microscopy and taxonomic classification. All soil samples collected showed contamination by at least two genera of parasites, with up to six different parasites highlighted in Parques Dona Lindu, 13 de Maio, and Praça do Derby. Larvae of *Ancylostoma* spp. and *Strongyloides* spp. were found in all collection sites, with a frequency of 100% in Parques Dona Lindu and Lagoa do Araçá, and 90% in Parque 13 de Maio. In Parque da Jaqueira, only larvae of *Ancylostoma* spp., with a frequency of 50%, and of *Strongyloides* spp., with 70%. Eggs of *Ascaris lumbricoides* were detected only in the soil of Praça do Derby, with a frequency of 10%. The data obtained from the studied sites showed high soil contamination by geohelminths, highlighting their relevance for both public health and veterinary and human medicine.

KEYWORDS

Environmental contamination; Human health; Zoonotic.

RESUMO

Várias espécies de helmintos e protozoários usam o solo como meio de desenvolvimento e infecção e são responsáveis por doenças tropicais negligenciadas, incluindo zoonoses. O solo de áreas públicas como praças e parques representa risco de transmissão para o homem e outros animais. Dessa forma, o objetivo do presente estudo foi avaliar a frequência de parasitos isolados do solo em praças e parques públicos da cidade do Recife, Pernambuco - Brasil. Amostras de solo foram coletadas de seis praças ou parques localizados na Região metropolitana do Recife-PE. De cada praça ou o parque 10 amostras de solo foram coletadas e processadas segundo a técnica de Rugai adaptada para solo, permitindo a identificação de estágios evolutivos de parasitos por meio de microscopia óptica e posterior classificação taxonômica. Todas as amostras de solo coletadas apresentaram contaminação por pelo menos dois gêneros de parasitos, com destaque para até seis diferentes parasitos nos Parques Dona Lindu, 13 de Maio e na Praça do Derby. Larvas de *Ancylostoma* spp. e *Strongyloides* spp. foram encontradas em todos os locais de coleta, com destaque para a frequência de 100% nos parques Dona Lindu e Lagoa do Araçá, e de 90% no parque 13 de Maio. No Parque da Jaqueira, foram identificadas apenas larvas de *Ancylostoma* spp., com uma frequência de 50%, e de *Strongyloides* spp., com 70%. Ovos de *Ascaris lumbricoides* foram detectados apenas no solo da Praça do Derby, com uma frequência de 10%. Os resultados destacam elevada contaminação do solo por larvas de geohelmintos, além de ovos e cistos, destacando relevância tanto para a saúde pública na medicina veterinária e humana e na contaminação ambiental.

PALAVRAS-CHAVE

Contaminação Ambiental. Saúde Humana. Zoonoses.

RESUMEN

Varias especies de helmintos y protozoos utilizan el suelo como medio para su desarrollo e infección y son responsables de enfermedades tropicales desatendidas, incluidas las zoonosis. El suelo de áreas públicas como plazas y parques representa un riesgo de transmisión para el hombre y otros animales. Por lo tanto, el objetivo del presente estudio fue evaluar la frecuencia de parásitos aislados del suelo en plazas y parques públicos de la ciudad de Recife, Pernambuco - Brasil. Se recolectaron muestras de suelo de seis plazas o parques ubicados en la Región metropolitana del municipio. El análisis del material se realizó utilizando la técnica de Rugai adaptada para suelo, lo que permitió la identificación de estadios evolutivos de parásitos mediante microscopía óptica y clasificación taxonómica. Todas las muestras de suelo recolectadas presentaron contaminación por al menos dos géneros de parási-

tos, destacándose hasta seis diferentes parásitos en los Parques Dona Lindu, 13 de Mayo y en la Plaza do Derby. Se encontraron larvas de *Ancylostoma* spp. y *Strongyloides* spp. en todos los lugares de recolección, destacándose una frecuencia del 100% en los parques Dona Lindu y Lagoa do Araçá, y del 90% en el parque 13 de Mayo. En el Parque da Jaqueira, solo se identificaron larvas de *Ancylostoma* spp., con una frecuencia del 50%, y de *Strongyloides* spp. con el 70%. Los huevos de *A. lumbricoides* se detectaron solo en el suelo de la Plaza do Derby, con una frecuencia del 10%. Los datos obtenidos de los lugares estudiados mostraron una alta contaminación del suelo por geohelmintos, destacando su relevancia tanto para la salud pública como para la medicina veterinaria y humana.

PALABRAS CLAVE

Contaminación ambiental; Salud humana; Zoonosis.

1 INTRODUCTION

In a public health context, One Health is particularly applied to investigate, understand, control, and prevent infectious and parasitic diseases, with special attention to zoonoses (STEFFAN *et al.*, 2018; ELLWANGER; CAVALLERO 2023). Parasitic infections have high prevalence and incidence among populations with low economic and political visibility and voice, who live under social vulnerability, including poor or lacking leisure, education, health services, basic sanitation, potable water, and inadequate housing (IDRIS *et al.*, 2019; MONTRESOR *et al.*, 2020). Regarding parasitic infections caused by soil-transmitted helminths, it is estimated that 1.5 billion people are infected, another 5.3 billion are at risk of infection, and they are included in the World Health Organization (WHO) list of neglected tropical diseases (JOURDAN *et al.*, 2018).

Several species of nematodes, cestodes, trematodes and protozoa use the soil as a means of development and/or of infection of a new host (STEFFAN *et al.*, 2018). Contaminated soil is an important route for the maintenance of infection, including ascariasis, trichuriasis, hookworm infection, strongyloidiasis, visceral larva migrans, and cutaneous larva migrans, due to the need for etiological agents to use the soil as a step to complete their biological cycle (GILL *et al.*, 2020; ALMEIDA *et al.*, 2024; BRADBURY; STREIT, 2024).

Warm temperatures and adequately moist soils promote the survival, reproduction, and spread of geohelminths, while poverty and inadequate sanitation and hygiene are the main risk factors for infection (MPAKA-MBATHA *et al.*, 2023; NG'ETICH *et al.*, 2024). Additionally, the growing number of domestic, semi-domestic, or stray dogs and cats represents an imminent risk of soil contamination and the transmission of parasites with zoonotic potential, especially in public spaces such as squares and parks where the feces of these animals, as well as human fecal material, are commonly found (GORGÔNIO *et al.*, 2021; OLIVEIRA *et al.*, 2021; MATEO *et al.*, 2023; BRADBURY; STREIT, 2024).

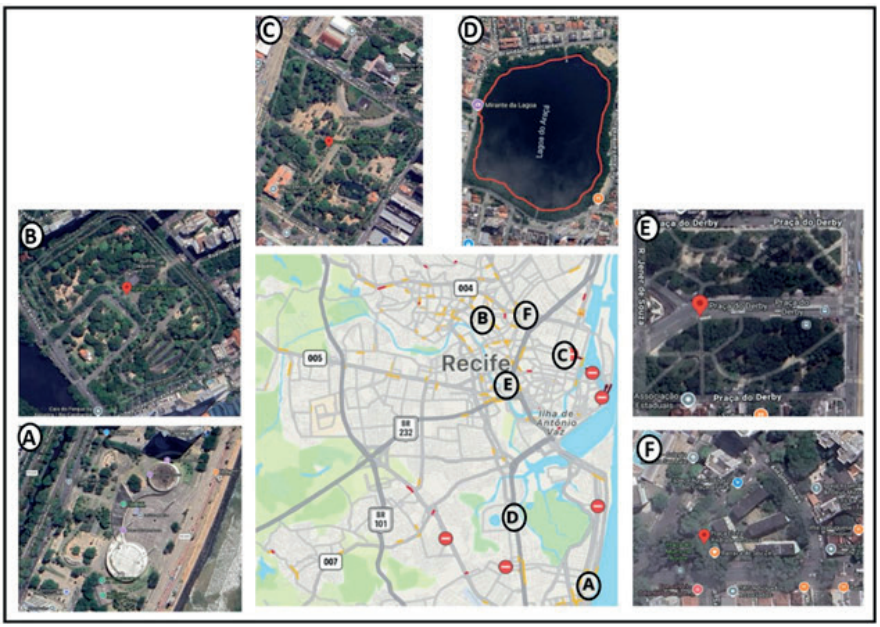
The presence of infective stages of parasites in the soils of public squares and parks is a significant sanitary, environmental, and public health problem (IDRIS *et al.*, 2019; OLIVEIRA *et al.*, 2021; GORGÔNIO *et al.*, 2021). Geohelminth infection can occur passively through the ingestion of eggs in contaminated water and/or food, or actively through the penetration of infective larvae (L3), especially from the family Ancylostomatidae, *Strongyloides* spp., *Toxocara canis* and *T. cati* species (GREAVES *et al.*, 2013; IDRIS *et al.*, 2019; BRADBURY; STREIT 2024).

Given the scarcity of publications on the frequency of parasites in public environments in the city of Recife, the present research aimed, through parasitological examinations, to evaluate the frequency of parasites isolated from the soil in public squares and parks in the city of Recife, Pernambuco, Brazil. The purpose was to aid in the development of preventive measures and to alert users and relevant public health authorities in human, animal, and environmental health about the risks of transmission of parasitic infections.

2 METHODS

The study was conducted between January and March 2023. Figure 1 shows the identification, location and geographic coordinates of the squares and parks where soil samples were collected; namely Dona Lindu Park (A), Jaqueira Park (B), 13 de Maio Park (C), Lagoa do Araçá Park (D), Derby Square (E), and Elvira Andrade de Souza Square (F). All the squares and parks are public and located in the Metropolitan Region of Recife, PE, Brazil. Soil collections were performed every 7 or 14 days, depending on the availability of transportation and the processing and analysis of previously collected samples.

Figure 1 – Geographic location and satellite images of the parks and squares in the study, in sequence: (A) Dona Lindu Park (9° 8' south latitude and 34° 54' west longitude), (B) Jaqueira Park (8° 2' south latitude and 34° 54' west longitude), (C) 13 de Maio Park (8° 3' south latitude and 34° 52' west longitude), (D) Lagoa do Araçá Park (8° 5' south latitude and 34° 54' west longitude), (E) Derby Square (8° 3' south latitude and 34° 53' west longitude), and (F) Elvira Andrade de Souza Square (8° 2' south latitude and 34° 54' west longitude). All the squares and parks are public and located in the Metropolitan Region of Recife, PE, Brazil



Source: Google Earth

From each square or park, soil samples (n = 10) were strategically collected from different locations. The collection was carried out in places where children and/or adults practice recreational and/or sports activities, maintaining a minimum equidistance of 3 and a maximum of 6 meters between each sample. With the aid of a sterile, disposable plastic spoon to avoid cross-contamination, the superficial soil layer was removed by scraping to a depth of approximately 6 cm, and the soil was then transferred to a sterile Nasco Whirl-Pak® plastic bag. The samples were individually identified and placed in a thermal box for transport. The time between sample collection and processing was less than one hour to ensure the viability of the investigated developmental stages. All samples from each park or square were collected on the same day.

The processing and analysis of the soil were carried out at the Parasitological Examination Laboratory of the Parasitology Department in the Academic Area of Tropical Medicine at the Federal University of Pernambuco (UFPE). The samples were individually processed using the Rugai technique and collaborators adapted for soil, as proposed by Carvalho *et al.* (2005). Briefly, two hun-

dred grams of soil were equally divided into five parts and wrapped in gauze packets (30 cm x 30 cm), submerged in sedimentation cups (125 mL) containing water at 45 ± 2ºC, and left to rest for at least one hour. The water temperature allows the isolation of helminth larvae due to their thermohydrotropism. Additionally, eggs and cysts, when present, settle by gravity while the larger soil components remain trapped in the gauze packets. Subsequently, the gauze was removed, and the material was left to sediment for another hour.

From the sedimented material, approximately 5 mL was drawn from the bottom of the sedimentation cups using disposable, sterile Pasteur pipettes and transferred to Wasserman tubes, then centrifuged at 2,000 rpm for 2 minutes. Finally, five histological slides were prepared with the sediment from the centrifuge, stained with 2% Lugol, and covered with cover slips (22 mm x 22 mm). The slides were analyzed under an optical microscope at 100x and 400x magnification. A sample was considered positive when it presented at least one developmental stage of any parasite. For the analysis, the morphology and taxonomy of larvae, eggs and cysts were considered according to Cimerman and Franco (2012), Monteiro (2017), and ESCCAP (2022).

3 RESULTS AND DISCUSSION

According to the results shown in Table 1, there was a high level of soil contamination by parasites of importance in veterinary and human public health. Furthermore, Figure 2 shows the parasites isolated from the soil of parks and squares. All soil samples collected showed contamination with at least two genera of parasites in Jaqueira Park and up to six different parasites in Dona Lindu Park, 13 de Maio Park, and Derby Square. *Ancylostoma* spp. and *Strongyloides* spp. larvae were isolated in all collection sites, with a notable frequency of 100% and 90% in Dona Lindu Park and Lagoa do Araçá Park, and in 13 de Maio Park, respectively (Table 1, Figure 2). In the Derby square, the frequency of *Ancylostoma* spp. and *Strongyloides* spp. larvae was 100% and 70%, respectively. In Jaqueira park, only the larval of *Ancylostoma* spp and *Strongyloides* spp. was found, with a frequency of 50% and 70%, respectively. Moreover, with the exception of Jaqueira Park, helminth eggs were isolated in samples from all other locations.

Figure 2 shows the evolutionary stages of parasites isolated from soil samples in parks and public squares. The presence of eggs indicates the existence of favorable environmental conditions (temperature and humidity) for embryonic development and corroborates the high frequency of *Ancylostoma* spp. and *Strongyloides* spp. larvae resulting from the hatching of these eggs and the release of the larvae into the environment before collection. Eggs from the Ancylostomidae family and *T. trichiura* were isolated in samples from Dona Lindu and 13 de Maio parks.

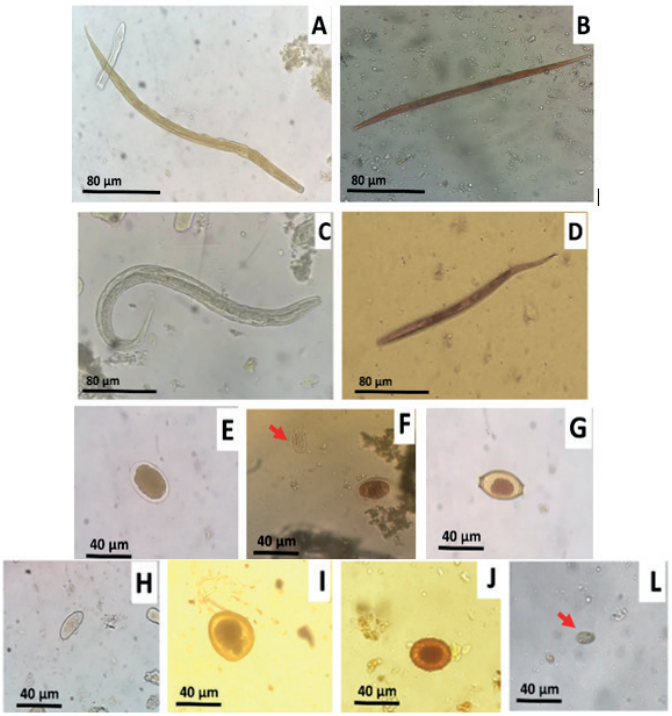
Additionally, in Lagoa do Araçá Park, there was a 30% positivity rate for Ancylostomidae family eggs and a 20% rate for *T. trichiura* eggs in Derby square. *A. lumbricoides* eggs (10%) were found only in the soil of Derby square. In Derby square and Dona Lindu park, there was a 20% frequency of *E. vermicularis* eggs. There were frequencies of 40%, 20% and 30% for *Toxocara* spp. eggs in the parks Lagoa do Araçá and 13 de Maio, and Square Elvira A. de Souza, respectively.

Table 1 – Frequency (%) of evolutionary phases of parasites isolated from soil samples in public parks and squares in Recife-PE, Brazil, 2023

Sample collection área*	Evolutionary stages of parasites								Total Frequency (%)
	Larvae		Eggs					Cyst	
	Ancylostoma spp.	*Strongyloides* spp.	Ancylostomidae	*Trichuris trichiura*	*Ascaris lumbricoides*	*Enterobius vermicularis*	*Toxocara* spp.	*Giardia duodenalis*	
Parks									
Dona Lindu	100	100	20	10	-	20	-	30	100
Lagoa do Araçá	100	100	30	-	-	-	40	10	100
Jaqueira	50	70	-		-	-	-	-	70
13 de maio	90	90	10	20	-	-	20	5	100
Square									
Elvira A. de Souza	60	40	-	-	-	-	30	-	100
Derby	100	70	-	20	10	20	-	20	100

*Ten samples from each square or park.
Source: Research data.

Figure 2 – Evolutionary stages of parasites isolated from soil samples in public parks and squares in Recife-PE, Brazil, 2023. *Ancylostoma* spp. larva (A and B) and *Strongyloides* spp (C and D) larva, Ancylostomidae family eggs (E and F), *Giardia duodenalis* cyst (red arrow in F and L), *Trichuris trichiura* egg (G), *Enterobius vermicularis* egg (H), *Toxocara* spp. egg (I), and *Ascaris lumbricoides* egg (J)



Source: Research data.

Soil-transmitted helminths are enteric parasites with a biological cycle that includes at least one mandatory developmental stage in the soil. They are capable of infecting humans and non-human animals through contact with contaminated soil (ZILIOTTO *et al.*, 2022). This study highlights the high contamination levels of helminth larvae and eggs, as well as protozoan cysts, in the soil of public squares and parks in the metropolitan area of Recife-PE, Brazil. It also raises awareness among visitors, especially parents and/or guardians of children and domestic dogs and cats, about the risk of infection.

Parasitic contamination in public spaces intended for recreational and sports activities has been the subject of studies. In this context, some studies contribute to the discussion on infections caused by parasites with zoonotic and anthropozoonotic potential (IDRIS *et al.*, 2019; ZILIOTTO *et al.*, 2022; MATEO *et al.*, 2023), with particular emphasis on those reporting the high prevalence of enteroparasites and the pathophysiological changes caused by parasitism (ELLWANGER *et al.*, 2022).

Harmful anthropogenic activities to the environment and animals facilitate the transmission of various zoonotic parasites (ELLWANGER; CAVALLERO, 2023). In addition to being in direct contact with contaminated soil, humans can be infected via the ingestion of poorly washed/undercooked food and water that has been contaminated with infectious parasitic forms, common habits in areas with inadequate sanitary infrastructure (STEFFAN *et al.*, 2018). Studies report the association of parasitism with environmental contamination, socioeconomic profile, and basic sanitation in different regions worldwide, including Brazil (MONTRESOR *et al.*, 2020; GORGÔNIO *et al.*, 2021; OLIVEIRA *et al.*, 2021; ZILIOTTO *et al.*, 2022; ALMEIDA *et al.*, 2024).

Public squares and parks are essential places for leisure, sports, social interaction, and socialization of the population. Thus, the selection of public squares and parks in the present study considered the high daily flow of users and the presence of areas for recreational and sports activities for children and adults. According to the World Health Organization (WHO), soil is considered one of the main means of parasite transmission to the human population and other animals, including domestic dogs and cats (MONTRESOR *et al.*, 2020). In this context, the soil harbors evolutionary stages with fertilized and/or larvated eggs and free larvae in immature stages, providing the necessary conditions for embryogenesis and the development of infective stages, and keeping them viable for months or even years. Furthermore, it is important to highlight the easy dissemination of larvae, eggs, and cysts by rain, wind, or coprophilic insects (ALMEIDA *et al.*, 2024).

Studies investigating soil contamination by parasites report a high frequency of larvae from the *Ancylostoma* spp. and *Strongyloides* spp. genera and eggs from Ancylostomatidae and *Toxocara* spp. (IDRIS *et al.*, 2019; ROCHA *et al.*, 2019; OLIVEIRA *et al.*, 2021; ALMEIDA *et al.*, 2024; BRADBURY; STREIT, 2024). Our results corroborate this statement as *Ancylostoma* spp. and *Strongyloides* spp. larvae were found in all researched locations, and in three out of the five locations, we found the presence of Ancylostomatidae (Figure 2 E, F) and *Toxocara* spp. eggs (Figure 2 I). Our findings align with those of Ziliotto *et al.* (2022), who studied the soil from 20 different collection points on the university campus belonging to Universidade Federal do Rio Grande do Sul, located in Porto Alegre, in the far south of Brazil. In this study, all collection sites were positive for larvae, and about 50% were positive for helminth eggs, particularly Ancylostomatidae eggs. The presence of these parasites was

associated with the presence of dogs and the absence or scarcity of local sanitation (OLIVEIRA *et al*., 2021; GORGÔNIO *et al*., 2021; ZILIOTTO *et al*., 2022; ALMEIDA *et al*., 2024; BRADBURY; STREIT, 2024).

Bortolatto *et al*. (2017) highlight the importance of basic education and sanitation for owners who walk their dogs, as dog feces, when not collected and disposed of properly, contribute to soil contamination and consequently the transmission of zoonoses. Conversely, in our study, we observed environmental contamination with dog feces in all researched locations except Jaqueira park. In our study, soil samples from Jaqueira park were positive only for *Ancylostoma* spp. larvae (50%) and *Strongyloides* spp. larvae (70%). This finding may be associated with the fact that it is the only park with restricted access for public use, entry and exit control, surrounded by a fence, limiting dog access, and has a public restroom.

Our results and hypotheses for the low frequency of *Ancylostoma* spp. and *Strongyloides* spp. are in agreement with the studies by Sprenger *et al*. (2014), who evaluated geohelminth contamination in public areas with epidemiological risk in Curitiba, Brazil, and Rocha *et al*. (2019), who studied the prevalence of larva migrans in public park soils in the city of Redenção, state of Pará, Brazil. On the other hand, in squares and parks where dogs are allowed access, there was a high frequency of helminth larvae and eggs, a result similar to the studies by Gorgônio *et al*. (2021), Oliveira *et al*. (2021), Ziliotto *et al.* (2022), and Almeida *et al*. (2024).

Dogs and cats are the definitive hosts of *T. canis* and *T. catis*, which lodges in the intestinal lumen of these ani mals and is excreted in the form of eggs, larvae, and adults in the faeces (MATEO *et al*., 2023). Feces of dogs and cats, when contaminated, can contain parasites of significant public health importance, such as *T. canis* and *T. cati* responsible for toxocariasis or visceral larva migrans, and *A. braziliense* and *A. caninum*, responsible for cutaneous larva migrans (GREAVES *et al*., 2013; ROCHA *et al*.,2019; ALMEIDA *et al*., 2024; CDC, 2024).

Unlike our study, Ziliotto *et al*. (2022) report a frequency of less than 10% for *A. lumbricoides* and *T. trichiura* eggs. The presence of *A. lumbricoides* (Figure 2 J) and *E. vermicularis* eggs (Figure 2 H) suggests human fecal contamination, as these are stenoxenous parasite species. Thus, it is possible to suggest that soil contamination may have occurred through the dissemination of human fecal material via direct excretion and/or inadequate sanitation conditions. We highlighted contamination by *A. lumbricoides* and *E. vermicularis* eggs in Derby Square, a location without public restrooms and with a high number of homeless individuals.

Moreover, homeless people live in precarious hygiene and social exclusion conditions, directly exposed to infectious agents through direct contact with the soil by walking barefoot and/or lying in squares and parks. Regarding defecation, they most often use public spaces, including the soil of squares and parks (FILGUEIRAS, 2019; ANTUNES *et al*., 2020). Inadequate water supply and sanitation, overcrowded living conditions, lack of access to healthcare, and low education levels make poorer people more susceptible to infections and diseases. Meanwhile, research on soil-transmitted helminths occupies less than 1% of global health research funding (WEXLER; VALENTINE, 2017; NG'ETICH *et al*., 2024).

Other soil-borne parasites, such as protozoa *Giardia duodenalis* are capable of infecting most mammals in addition to having a zoonotic potential (BONATTI; FRANCO, 2016). *G. duodenalis* is one of the most prevalent enteric parasites globally, with a high prevalence in both developing and deve-

loped countries (CAI *et al*., 2021; MATEO *et al*., 2023). In Brazil, the frequency of *G. duodenalis* cysts isolated in soil samples from squares and parks is directly related to the high incidence of giardiasis, corroborating the results of Colli *et al*. (2015), which reported a frequency of 9.2% of human infection in an urban area in southern Brazil.

Except for Jaqueira park and Elvira A. de Souza square, *G. duodenalis* cysts were found in the other sampling locations, with percentage variation from 5% (Parque 13 de Maio) to 30% (Parque Dona Lindu), (Figure 2 E, L). A frequency of 20.83% for *Giardia duodenalis* cysts was reported by Farias *et al*. (2021) while studying the soil of public parks in Espírito Santo. Ferraz *et al*. (2020) highlight concerns about the maintenance and chronicity of giardiasis in the dog/cats/human cycle and its transmission through contaminated soil. Mateo *et al*. (2023) reported high frequency of *G. duodenalis* in feces of dogs (40.9%) and cats (14.3%). Giardiasis is often asymptomatic, which makes diagnosis difficult and renders these animals significant disseminators of cysts in the environment, potentially contaminating humans and other animals (ANTUNES *et al*., 2020).

4 CONCLUSION

The results demonstrate high soil contamination by helminths and protozoa of public health importance for both veterinary and human medicine in squares and public parks in Recife, Pernambuco. Our findings will certainly contribute to a better understanding of the infection dynamics and serve as a warning for planning infection control measures. Improving the sanitary quality of squares and parks depends on strategies for One Health actions, considered unique and composed of three inseparable areas: human, animal, and environmental. This movement is growing as a strategy for better understanding and resolving contemporary health problems arising from the convergence of human, animal, and environmental factors, hence the need for interdisciplinary collaboration aimed at improving human and animal health.

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1 Acadêmico do curso de Farmácia, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0003-0761-5673. E-mail: igor.marley@ufpe.br

2 Acadêmica do curso de Enfermagem, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0008-7069-1093. E-mail: julia.santos2@ufpe.br

3 Acadêmica do curso de Enfermagem, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0000-0002-7142-1860. E-mail: yasmin.sena@ufpe.br

4 Acadêmica do curso de Farmácia, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0000-5806-2173. E-mail: isabela.mlima@ufpe.br

5 Acadêmica do curso de Biomedicina, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0001-5044-4182. E-mail: kamylle.cynnara@ufpe.br

6 Acadêmica do curso de Biomedicina, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0002-8090-7069. E-mail: rutyelle.sousa@ufpe.br

7 Acadêmico do curso de Enfermagem, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0008-8922-6626. E-mail: rodrigo.rfs@ufpe.br

8 Acadêmica do curso de Enfermagem, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0005-6025-1586. E-mail: caroline.paiva@ufpe.br

9 Acadêmica do curso de Biomedicina, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0008-2287-9753. E-mail: nayara.santosaraujo@ufpe.br

10 Acadêmica do curso de Biomedicina, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0009-0005-0644-0749. E-mail: mariana.queires@ufpe.br

11 Acadêmica do curso de Enfermagem, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0000-0003-3513-0166. E-mail: ingrid.ffreire@ufpe.br

12 Acadêmica do curso de Biomedicina, Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0000-0001-5057-6460. Email: doralice.paz@ufpe.br

13 Doutor em Medicina Tropical. Biólogo. Tutor do Programa de Educação Tutorial em Parasitologia da Universidade Federal de Pernambuco (UFPE), Recife – PE. Brasil. ORCID 0000-0001-9283-1466. E-mail: andre.laires@ufpe.br

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