

Larva migrans in Votuporanga, São Paulo, Brazil: Where does the danger hide?

Larva migrans em Votuporanga, SP, Brazil: Onde se esconde o perigo?

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Abstract

Soil samples collected near municipal schools (public/EMEI and private/EPEI schools), clubs (CLB), public squares (PS) and residential condominiums (CND) and samples of animal faeces from the Zoonosis Control Centre (CCZ) of the municipality of Votuporanga/SP were analysed using the Baermann method for the detection of zoonotic helminth larvae. The prevalence rates of the nematode genera identified were determined, and the results were compared using Fisher's exact and chi-square frequency tests. Information about cases of larvae migrans in the population were collected from the Family Health Units and the private health plans. All sites were positive for *Ancylostoma* spp. and, with the exception of EPEIs and dog faeces, for *Strongyloides* spp. The prevalence of *Ancylostoma* spp. was 87.5% for CND samples, 74.29% for EMEIs, 63.64% for CLB, 61.76% for PS and 64.29% for dog's and 42.86% for cats at CCZ. The prevalence of *Strongyloides* spp. ranged from 14.29% (cats/CCZ) to 41.18% (PS). Cases of cutaneous larva migrans were reported during interviews. Thus, from the public health perspective, the risk of individuals that frequent recreational areas in the municipality, especially children, to be infected by helminth larvae is noteworthy, indicating the need to develop policies aimed at controlling this important zoonosis.

Keywords: Larva migrans, *Ancylostoma*, *Strongyloides*, soil.

Resumo

Amostras de solo colhidas em escolas municipais (ensino público/EMEI e privado/EPEI), clubes (CLB), praças públicas (PP), condomínios residenciais (CND) e de fezes de animais do Centro de Controle de Zoonoses (CCZ) do município de Votuporanga/SP, foram submetidas ao método Baermann para detecção de larvas de helmintos zoonóticos. Foram determinadas as taxas de prevalência dos nematódeos identificados, e os resultados confrontados pelos testes Exato de Fisher e Qui-quadrado. Atendimento de casos de larvas migrans na população foram levantados em entrevistas realizadas em Unidades de Saúde da Família e em planos de saúde privados. Todos os locais apresentaram positividade para *Ancylostoma* spp. e, com exceção de EPEIs e de fezes caninas, para *Strongyloides* spp. Prevalência de *Ancylostoma* spp. foi verificada em amostras de CNDs (87,50%), EMEIs (74,29%), CLBs (63,64%), PPs (61,76%) e em fezes de cães e gatos do CCZ (64,29% e 42,86%, respectivamente). As prevalências de *Strongyloides* spp. variaram de 14,29% (gatos/CCZ) a 41,18% (PP). Atendimento de casos de larva migrans cutânea foram relatados nas entrevistas. Portanto, ressalta-se o risco de frequentadores de áreas de lazer do município, especialmente crianças, de adquirirem infecção por larvas de helmintos, indicando a necessidade da elaboração de propostas de políticas voltadas ao controle dessa importante zoonose.

Palavras-chave: Larva migrans, *Ancylostoma*, *Strongyloides*, solo.

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Introduction

The nematodes *Ancylostoma* spp., *Toxocara* spp. and *Strongyloides stercoralis*, parasites of dogs and cats, are considered important zoonotic agents. They represent a serious problem for public health because humans are an accidental host, who become infected by direct contact with infected animals either by active penetration of the larvae through the skin or by ingesting eggs present on soil, fomites, hands, food and water (Peruca et al., 2009; Ganesh & Cruz, 2011; Dantas-Torres & Otranto, 2014; Chen et al., 2018).

Larvae of *Ancylostoma braziliensis*, the main species responsible for the “cutaneous larva migrans” pathology, popularly known in Brazil as “bicho geográfico”, penetrate and migrate through the skin in an irregular pattern, causing pruritic, erythematous and serpiginous dermal lesions (Robles & Habashy, 2018). In turn, when ingested, *Toxocara* spp. eggs can cause “visceral”, “ocular”, “neurological” or “asymptomatic” larva migrans, which can affect various organs, such as the liver, heart, lungs, kidneys, muscles, eyes and brain, leading to a set of clinical symptoms with serious health consequences (Carvalho & Rocha, 2011; Chen et al., 2018;). Infection by *Strongyloides stercoralis*, which have a cardiopulmonary development cycle, occurs through skin penetration of infective larvae present in soil, faeces or water.

Cases of larva migrans in humans have been reported in several countries, including Brazil (Jackson et al., 2006; Del Giudice et al., 2019; Reis et al., 2019; Rodrigues et al., 2019). The infection of dogs and cats by the nematodes *Ancylostoma* spp., *Toxocara* spp. and *Strongyloides* spp. has also been reported (Bresciani et al., 2008; Ferreira et al., 2016; Oliveira-Arbex et al., 2017).

The growing number of domestic, peridomestic and stray dogs, combined with the easy access of these animals to recreational sites, can significantly increase soil contamination by potentially zoonotic helminths, constituting a potential risk for individuals frequenting such sites, especially for children, as reported by Chen et al. (2018) and Evaristo et al. (2019).

Although there has been an increase in the number of studies on this topic in the last two decades, according to Chen et al. (2018), toxocariasis remains a public health challenge worldwide, leading to the need for continuous surveillance.

The detection of eggs in the external environment is internationally recognized as an important indicator of zoonotic contamination (Błaszowska et al., 2015). In this sense, the presence of eggs and larvae of the nematodes *Ancylostoma* spp., *Toxocara* spp. and *Strongyloides* spp. in sands or soils of recreational areas located in children’s playgrounds, public parks and schools is a risk to which the population is subjected to in different regions of Brazil (Nunes et al., 2000; Cassenote et al., 2011; Chen et al., 2018; Gonçalves & Paludo, 2018; Monteiro et al., 2018; Robles & Habashy, 2018; Padilha et al., 2019; Rocha et al., 2019).

Given the zoonotic importance of helminth parasites of dogs and cats and the relevance of the diagnosis of the situation in the country, seeking to support the development of control programmes and the implementation of effective public health measures, the present study aimed to evaluate soil samples (sand/soil) from recreational areas frequented by children and samples of faeces of dogs and cats from Zoonotic Control Centre, in the municipality of Votuporanga, state of São Paulo, Brazil, for the presence of *Ancylostoma* spp. and *Strongyloides* spp larvae. Information about cases of larval migrans in the municipality were also obtained.

Materials and methods

Study Site

The study was conducted in the municipality of Votuporanga (20°25’06” south latitude and 49°58’39” west longitude, with smooth relief and altitude of 525m), in the northwest region of the state of São Paulo, Brazil, with an estimated population of 94,547 inhabitants in 2019 (IBGE, 2019). According to Koeppen, this region has an aw climate, with a rainy season in the summer, followed by a mild and dry winter; the average annual rainfall is approximately 1,500 mm.

Samples

A total of 123 sand/soil samples were collected from recreational areas frequented by children, located near schools, clubs, condominiums and parks in the city, and 21 faecal samples from dogs and cats housed in the Zoonosis Control Centre (CCZ, for its acronym in Portuguese). Through the Department of Education and Urban Development of the municipality, nine municipal preschools (EMEIs = 35 samples) and nine public squares (PSS = 34 samples)

were selected that had recreational areas on their premises. Three private preschools (EPEIs = 24 samples), two member-only recreational clubs, one of them a country club (CLBs = 22 samples), and the playground of two private residential condominiums (CNDs = 08 samples) were also included in the study.

Sand and/or soil samples were collected using a PVC pipe at approximately eight centimetres depth relative to the surface, preferably in moist and shaded areas (01 sample/5m²). Faecal samples from dogs (14 samples) and cats (07 samples) housed in the CCZ of the municipality were collected in the morning before cleaning the area, where the animals were kept in individual or collective pens.

All samples were duly stored, labelled and transported to the Laboratory of Animal Parasitology of Votuporanga at the Biological Institute/APTA.

Parasitological procedures

All samples were screened for nematode larvae using the Baermann method and optical microscopy was used to identify the larvae according to Ueno & Gonçalves (1998). The prevalence rates of the identified nematode genera were calculated (Wagner, 1998). Helminth eggs and protozoan oocysts were also investigated in the faecal samples of the animals from the Zoonosis Control Center using the technique of Gordon & Whitlock (1939).

Survey of the Occurrence of larva migrans in the municipality

A survey of cases of cutaneous and visceral larva migrans was conducted through interviews in nine Family Health Units (USFs, for its acronym in Portuguese), representative of the districts of the municipality, according to the Department of Urban Development of the municipality and in two private health plans. The interviews were conducted in person when the responsible for the USF or for the health plans was asked about registers of larva migrans cases in the retrospective period of one year (yes or no). Nine pediatricians and/or dermatologists were also interviewed in their private offices, about medical care of larva migrans cases.

Statistical analysis

The results were compared using Fisher's exact test and chi-square at the 95% confidence level (StatSoft, Inc., 2014). The study was approved by the Ethics Committee of the Centro Universitário de Votuporanga - UNIFEV/SP (Process Number 1,443,306) and conducted within the applicable ethical standards.

Results

All sampled sites were positive for *Ancylostoma* spp. larvae and, except for EPEIs and dog faeces, for *Strongyloides* spp. larvae. The prevalence of *Ancylostoma* spp. (61.11%) was significantly higher ($P < 0.05$) than that of *Strongyloides* spp. (22.92%) (Table 1).

Table 1. Results of the faecal parasitology tests performed with samples collected in the municipality of Votuporanga, SP, Brazil. Multiple comparisons of prevalence at different sites.

Local/Samples		Helminths (larvae) / Prevalence(%)*	
		<i>Ancylostoma</i> spp.	<i>Strongyloides</i> spp.
Municipal Preschools I (EMEI)	35	74.29 Aa	28.57 Ab
Private Preschools (EPEI)	24	33.33 Ba	0.00 Cb
Public Squares (PP)	34	61.76 Aa	41.18 Aa
Private Residential Condominiums (CND)	8	87.50 Aa	25.00 ABCb
Member-only Recreational Clubs (CLB)	22	63.64 Aa	27.27 ABb
Zoonosis Control Centre (CCZ-dog feces)	14	64.29 Aa	0.00 Cb
Zoonosis Control Centre (CCZ-cats feces)	7	42.86 ABa	14.29 ABCa
TOTAL	144	61.11 a	22.92 b

*: Values followed by the same letters, uppercase in the column and lowercase in the row, do not differ by Fisher's Exact test (Frequencies less than 5) or Chi-Square (Frequencies equal to or greater than 5) ($p \geq 0.05$)

There was no positivity for *Strongyloides* spp. in soil samples of EPs or in fecal samples of dog from the CCZ. The prevalence rates in soil samples of PP, EMEIs, CLBs, CNDs and in cat faeces (41.18%, 28.57%, 27.27% and 25.00%, respectively) was not statistically different ($P > 0.05$) (Table 1).

Regarding the sites sampled, the highest prevalence rates of *Ancylostoma* spp. were observed in samples from CNDs (87.50%) and EMEIs (74.29%), followed by CLBs (63.64%), PSs (61, 76%) and the CCZ (dogs: 64.29% and cats 42.86%), with no significant differences ($P > 0.05$) between these sites; however, the prevalence observed in samples from EPEIs (33.33%) was significantly lower ($P < 0.05$) than the other sites, except for the cat faeces prevalence (Table 1).

EMEIs had the second highest prevalence rate of *Ancylostoma* sp. (74.29%), with 88.89% of schools exhibiting positive results. A child with lesions characteristic of cutaneous larva migrans was identified in one of the EMEIs during sampling (Figure 1). *Strongyloides* spp. presented prevalence rates ranging from 14.29% (cat faeces/CCZ) to 41.18% (PSs), with no significant differences ($P > 0.05$) between the results of sites positive for this nematode (Table 1). Although positive, the values for CNDs (25.00%) and cat faeces/CCZ (14.29%) were also not significantly different ($P > 0.05$) from the sites that did not show contamination (EPEIs and dog faeces/CCZ). Samples from the two studied clubs showed a predominance of *Ancylostoma* spp. (63.64%) and *Strongyloides* spp. (27.27%), and the highest number of positive samples was observed in the country club, which has a larger open area that animals can easily access.



Figure 1. Characteristics lesions of cutaneous larva migrans observed on a student attending a municipal preschool during the study. Votuporanga, SP, Brazil.

Tests of faecal samples from animals housed in the CCZ revealed *Ancylostoma* spp. larvae in dog and cat faeces (64.29% and 42.86%, respectively), without significant differences ($P > 0.05$) between them. Only cats were parasitized by *Strongyloides* spp., but no significant differences ($P > 0.05$) relative to the other sites positive for the nematode or even relative to the sites negative for the nematode were observed.

Cases of cutaneous larva migrans were reported in all of the interviewed USFs (Table 2). Only one private health plan reported cases of such pathology. Fifty-nine cases of cutaneous larva migrans were reported by the doctors interviewed in their private offices.

No cases of visceral larva migrans were reported by the USFs, by the private health plans or by interviewed doctors.

Toxocara spp eggs and protozoan oocysts were diagnosed in the faecal samples of dogs and cats from Zoonosis Control Center.

Table 2. Report of cases of cutaneous larva migrans (CLM) registered at the Family Health Units (USFs) and at private health plans of Votuporanga, SP, Brazil.

Family Health Units (USF) - Private Health Plans (PSP) - Doctors	Reported cases of cutaneous larva migrans	Coverage
USF 1	yes	EMEI
USF 2	yes	EMEI, PP, CR
USF 3	yes	EMEI; PP
USF 4	yes	EMEI; PP
USF 5	yes	EMEI; EPEI; PP; CR
USF 6	yes	EMEI - V
USF 7	yes	EMEI - III
USF 8	yes	EMEI; PP
USF 9	yes	EMEI; EPEI; PP
PSP 1	yes	-----
PSP 2	no	
PEDIATRICIANS AND/OR DERMATOLOGISTS	59 cases	-----

Discussion

The positivity for *Ancylostoma* spp. larvae at all sites sampled and for *Strongyloides* sp. larvae at most sites highlights the challenge to public health and the need for continuous surveillance of zoonoses (Peruca et al., 2009; Chen et al., 2018).

Although the prevalence of *Strongyloides* spp. (22.92%) has been significantly lower ($P < 0.05$) than the *Ancylostoma* spp (61.11%), attention should be paid to its potential as a zoonotic agent, as well as to its presence in the municipality, which has already been reported by Malta (2005), who diagnosed *S. stercoralis* in children attending day care centers of the city of Votuporanga/SP.

The higher prevalence of *Ancylostoma* spp. (87.50%) found in samples from the CND playgrounds was unexpected because these areas are delimited by walls and have controlled entries, which should contribute to this zoonosis prevention, as mentioned by Rocha et al. (2019). Moreover, because CNDs represent medium- and high-standard residences, it is expected that the pets that frequent the internal areas have been dewormed and/or have regularly seen a veterinarian. These findings emphasize the need of health education to the population about zoonoses transmitted by pets and other owned animals (Domingues et al., 2015).

The number of municipal schools that were positive for *Ancylostoma* spp. (88.89% of the EMEIs) and the high prevalence found (74.29%) draw attention to the danger of children contracting cutaneous larva migrans from their environment, a risk that was confirmed when a student with lesions characteristic of cutaneous larva migrans was identified during sample collection at one of the schools. These results were higher than those found by Nunes et al. (2000), in sand samples from municipal preschools in Araçatuba, also located at the northwest region of the state SP (46.4% of schools with positivity), and by Cassenote et al. (2011), in Fernandópolis, where 6.1% positivity for geohelminth eggs was observed in municipal preschool sandboxes. However, results close to those found in the present study were observed in the city of São Paulo, where 84.3% of samples from preschool recreational areas were positive for *Toxocara* spp. and/or *Ancylostoma* spp. (Maeda et al., 2010).

The fact that a greater number of positive samples was observed in the country club, which has a large open area and stray cats and dogs in the area, indicates that the easy access of peridomestic and stray dogs to recreational areas can significantly increase soil contamination by potentially zoonotic helminths (Cassenote et al., 2011). In addition, reports on social networks of the occurrence of cases of cutaneous larva migrans in children attending the site were also found during the study. Although the club’s board had already changed the park’s sand, the environment became re-infested in a short period of time. Thus, in addition to replacing the sand, it was

recommended to prevent the entry of dogs and cats into the area. However, this measure will only be effective with the implementation of a public policy focused on the control of stray animals in the city, together with society's awareness regarding the responsible ownership of animals, as previously mentioned.

In the case of open sites, the possibility of the population being contaminated by *Ancylostoma* spp. in public squares was also recorded in Rio Grande do Sul by Evaristo et al. (2019), where a prevalence of 61.11% was observed, a result similar to that shown in the present study for PS samples (61.76%).

The high prevalence of *Ancylostoma* spp. in dogs housed in the CCZ (64.29%) was also reported by Bresciani et al. (2008) in dogs surrendered to the CCZ in the same region of the state (75.38%).

Considering that the dogs and cats housed in the CCZ are stray animals which, before being captured, circulated freely through the city, with access to recreational areas of parks, clubs and even schools, the positivity of parasitism in faeces from these animals is consistent with the results obtained for samples collected elsewhere.

The results reveal, from the public health perspective, the risk of people, especially children, frequenting recreational areas in the municipality of Votuporanga, SP, Brazil, being infected by *Ancylostoma* spp. and *Strongyloides* spp., emphasizing the need to develop public policies aimed at controlling this important zoonosis.

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