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ENVIRONMENTAL CONTAMINATION BY *Toxocara* sp. EGGS IN RIBEIRÃO PRETO, SÃO PAULO STATE, BRAZIL

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SUMMARY

Toxocariasis is a zoonosis mainly caused by *Toxocara canis,* an intestinal nematode of dogs. Man acquires the infection through accidental ingestion of viable eggs, and the toxocariasis clinical manifestations may vary from an asymptomatic infection up to the Visceral Larva *Migrans* syndrome. Seventy eight public squares of Ribeirão Preto, São Paulo, Brazil, including Bonfim Paulista district were visited aiming to evaluate the soil contamination by *Toxocara* eggs. The squares were divided in five different areas corresponding to the Sanitary Districts of the city. From May to December 2003, soil samples weighting about 250 g each were collected from five distinct sites of each public square. The laboratorial analysis was done by centrifugal-flotation techniques in magnesium sulphate solutions with 5% of potassium iodide (d = 1.33) and zinc sulphate (d = 1.20), and by the sedimentation-flotation in conic chalices with zinc sulphate (d = 1.20). *Toxocara* sp. eggs were found on 16 (20.5%) squares, with the lowest prevalence (12%) at the central area. From these results, it is expected that the legal authority will adopt protection measures for the city public areas, reducing thus the contamination risk by *Toxocara* sp. eggs.

KEYWORDS: *Toxocara* sp.; Soil contamination; Zoonosis.

INTRODUCTION

Human toxocariasis, a zoonosis caused by intestinal nematodes of dogs and cats, is an important public health issue in developed and developing countries[21,22]. Human infection occurs by the accidental ingestion of infective eggs, usually from *Toxocara canis*, present in the soil, vegetables or other contaminated surfaces. Children are the most susceptible group, due to contact with the soil while playing. The clinical manifestations of toxocariasis may vary from an asymptomatic infection till the most severe one, the Visceral Larva *Migrans* (VLM)[16,21,22], caused by the inflammatory response to the larvae migration through different vital organs and tissues of the body, including the Central Nervous System (CNS). Ocular Larva *Migrans* (OLM) can lead to partial or total loss of vision[21].

It is assumed that VML cases are underestimated because the clinical signs are frequently nonspecific. Nevertheless, serological assays performed in several Brazilian municipalities and in different population groups have demonstrated that this zoonosis is widespread, with prevalence from 3 to 39%[1,3,4,5,9,10,18]. Several studies performed in Brazil[2,7,11,12,13,20] and in other countries[6,14,16,17,19,23,24,25], have evidenced soil contamination of public areas by *Toxocara* sp. eggs, varying from 5.71 to 92%, thus calling attention to the risk of human contamination.

The present work aimed to evaluate the soil contamination of public areas in the city of Ribeirão Preto, by *Toxocara* sp. eggs.

MATERIAL AND METHODS

Sample choice: There are 207 public squares in the city of Ribeirão Preto, including Bonfim Paulista district. According to the city Parks and Gardens Sector, 160 of them are partly or fully urbanized. The study sample was made of 78 squares, 67 of which where randomly chosen and 11 included in the research for having children's playground. The sample size was calculated, considering a confidence level of 95%, normal distribution, prevalence of 25% and worst acceptable of 7.5%. The distribution of the squares corresponded to the areas comprised by the five Health Districts of the city: southwest, northwest, north, southeast and central, with boundaries established by the Municipal Health Secretariat.

Period and collection of the samples: From May to December, 2003, soil samples from five equidistant points (four at the sides and one central) were collected from each public square, weekly and in the morning, and stocked in brand new plastic bags. After eliminating the soil superficial dirt, about 250 g of soil were removed down to 5 cm deep. As a precaution, a minimum distance of two meters was established for the soil collection, whenever feces were visible.

Processing of the soil samples: The samples were kept under refrigeration and processed within a maximum period of 24 hours from the collection. After careful homogenization, 30 g were taken from each soil sample, divided into three aliquots of 10 g each. Two aliquots

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were placed into 50 mL tubes, washed with a 1.0% Tween 80 solution, and left to rest for 20 minutes. After that time, the sediments were resuspended, and the tubes were filled up with saturated magnesium sulphate solution, added with 5% potassium iodide (d = 1.33) and zinc sulphate (d = 1.20). After centrifugation, the supernatant material of each tube was removed onto microscopic slides, covered by coverslips and examined for *Toxocara* eggs. The remaining 10 g aliquot was placed into a sedimentation chalice, washed with a 0.5% solution of sodium hypochloride, and after 10 minute rest, the sediment was resuspended in a zinc sulphate saturated solution (d = 1.20). After 30 minutes, surface samples were analyzed under light microscope.

Statistical analysis: Student's "t" test for ratios was used to evaluate the differences in square contamination prevalence between city areas, using a confidence interval of 95%. The analysis of the performance of the diagnostic techniques employed was done using the Chi-square test.

RESULTS

Eggs of *Toxocara* sp. were found in 16 (20.5%) of the 78 public squares researched, by any of the laboratorial technique. Table 1 shows the distribution of contamination prevalence on public squares, according to the different city areas.

Among the soil samples analyzed individually, the number of *Toxocara* sp. eggs recovered varied from one to a maximum of three. Regarding the evolutionary stage of the eggs, they were embryonated in five (31.2%) of the contaminated public squares, being either in the morula (60%) or in the larva phase (30%). The presence of strayed dogs was observed in 22 (28%) squares, at the moment of the research.

Table 1
Distribution of public squares contaminated by *Toxocara* sp. eggs, according to the geographic areas of Ribeirão Preto city, São Paulo, Brazil

	Public squares				
	Existent	Researched	Contaminated		
Area	N	N	N	%	(CI = 95%)
Southwest	13	07	02	28.5	29.5 (0 - 58.0)
Northwest	17	10	03	30.0	25.1 (4.9 - 55.1)
North	22	10	03	30.0	25.1 (4.9 - 55.1)
Southeast	53	26	05	19.2	13.3 (5.9 - 32.5)
Central	55	25	03	12.0	11.2 (0.8 - 23.2)
Total	160	78	16	20.5	

Table 2 shows the presence of *Toxocara* sp. eggs at the 16 public squares investigated, according to the diagnostic technique used.

DISCUSSION

The positivity of 20.5% observed in this study was similar to those reported by COSTA-CRUZ *et al*.[12] in Uberlândia, MG (23.07%) and by ALCÂNTARA *et al*.[2] in Salvador, BA (24.8%), and a slightly higher than that obtained by SANTARÉM *et al*.[20] in Botucatu, SP (17.5%). However, it was lower than those reported by CHIEFFI & MULLER[7] in Londrina, PR (60%), COELHO *et al*.[11] in Sorocaba, SP (53.3%) and FERREIRA *et al*.[13], in Rio de Janeiro, RJ (41.6%). Several factors may have contributed to this inconsistency, such as climatic and environmental conditions, texture of the soil analyzed, diagnostic methodology employed, presence of dogs, etc. In our study it was possible to observe the presence of different environmental conditions among the squares, because we researched not only squares with plenty of vegetation, favoring shadowing and soil humidity preservation, but also squares with poor vegetation and a dry soil, due to prolonged exposure to the sun, which causes quick disintegration of the *Toxocara* eggs. Another factor observed for keeping the soil humidity in some squares was the presence of a gardener responsible for its maintenance. The presence of strayed dogs in 28% of the squares may have contributed to the soil contamination, not only by *Toxocara canis*, but by several other parasites with zoonotic potential, as these dogs had not been dewormed.

Table 2
Presence of *Toxocara* sp. eggs in the soil of 16 public squares in the city of Ribeirão Preto, SP, Brazil, according to the diagnostic technique used

		Diagnostic technique		
		Centrifugation		Flotation
Area	Square number*	$MgSO_4$	$ZnSO_4$	$ZnSO_4$
Southwest	135	+	+	(-)
	205	+	(-)	+
	1	+	+	+
Northwest	10	(-)	+	(-)
	67	+	(-)	(-)
	25	+	+	+
North	108	(-)	+	(-)
	149	+	+	+
	3	+	(-)	(-)
	103	+	+	(-)
Southeast	122	+	(-)	(-)
	158	+	+	+
	183	+	+	+
	56	+	+	+
Central	61	+	(-)	(-)
	173	+	(-)	(-)
Total	Positive	14 (87.5%)	10 (62.5%)	07 (43.7%)
	Negative	02 (12.5%)	06 (37.5%)	09 (56.3%)

+ : presence (-): absence; * Source: Cadastre of the Public Parks Sector, Municipality of Ribeirão Preto.

The results obtained in this study allowed us to draw the profile of soil contamination distribution among the different city areas. The lowest prevalence in the central area (12%), represented by the city center, neighboring regions and also Bonfim Paulista district, is probably related to the fact that its inhabitants have a better socioeconomic and cultural level, thus facilitating veterinary assistance and routine anthelmintic treatments of the dogs. In addition, a lot of them live in buildings, where dogs are not allowed.

Although this was a study performed mostly along the dry season (May to September), the fact of embryonated eggs have been found in

31.2% of the contaminated squares, indicates that there were favorable environmental conditions to their development, representing thus a public health risk. The presence of viable eggs was also observed by several authors in studies performed in Japan[23], Peru[15], Mexico[24] and Cuba[19]. In Brazil, CHIEFFI & MULLER[8] analyzing soil samples in the city of Londrina for 15 months, found *Toxocara* sp. eggs every month, but the presence of viable eggs was only observed from May to June and from September to December, leading to the assumption that the rain season did not have a big influence in the eggs viability. Regarding the diagnostic techniques employed in this study, the flotation method in magnesium sulphate with 5% of potassium iodide proved to be the most efficient in the recovery of *Toxocara* eggs, detecting the presence of eggs in 87.5% of the contaminated squares ($\chi^2 = 6.740$; $p = 0.0344$)

This study represents the first written report on the contamination of *Toxocara* eggs in public areas of the city of Ribeirão Preto. We hope that on the basis of these data prevention and control measures will be taken by the local health authorities, such as making citizens aware of the importance of preventing dog defecation in public areas, controlling strayed dogs and promoting laws to protect public areas, mainly children's playgrounds.

RESUMO

Contaminação ambiental por ovos de *Toxocara* sp. no município de Ribeirão Preto, Estado de São Paulo, Brasil

A toxocaríase é uma zoonose causada principalmente pelo *Toxocara canis*, nematóide intestinal de cães. O homem adquire a infecção através da ingestão acidental de ovos viáveis, sendo que as manifestações clínicas da toxocaríase podem variar desde uma infecção assintomática à síndrome da Larva *Migrans* Visceral. Com o objetivo de avaliar a contaminação do solo por ovos de *Toxocara* sp, foram visitadas 78 praças públicas de Ribeirão Preto, incluindo o distrito de Bonfim Paulista. As praças foram distribuídas em cinco áreas diferentes correspondentes as mesmas dos Distritos Sanitários do município. Entre maio a dezembro de 2003 foram coletadas de cinco pontos de cada praça amostras de solo de aproximadamente 250 gramas. A análise laboratorial foi realizada pelas técnicas da centrífugo-flutuação em soluções de sulfato de magnésio com 5% de iodeto de potássio (d = 1.33) e de sulfato de zinco (d = 1.20) e da flotação-sedimentação em cálice cônico com sulfato de zinco (d = 1.20). Foram encontrados ovos de *Toxocara* sp. em 16 (20,5%) praças, sendo que a região central apresentou a mais baixa prevalência (12,0%). Espera-se que a partir destes resultados as autoridades competentes adotem medidas de proteção das áreas públicas do município, reduzindo o risco da contaminação por ovos de *Toxocara* sp.

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