Human Toxocariasis: a Seroepidemiological Survey in Schoolchildren of Sorocaba, Brazil

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*A seroepidemiological survey for toxocariasis, among 180 schoolchildren of the public schools of Sorocaba City, state of São Paulo, Brazil, was carried out from August 2000 to July 2001. ELISA test was performed using excretory and secretory antigens for the detection of IgG anti-*Toxocara *antibodies. Information regarding the children was obtained from the parents or legal guardians. The results showed that the mean age was 5.4 ± 1.4 years, the infection coefficient (IC) was 38.3 and the infection risk was higher among the children living in the city outskirts (IC = 47.4) where the socioeconomic conditions were worse than in the central region of the city (IC = 11.1). There was an association between higher frequency of seroreactivity in the ELISA test and the condition of living in a house with a yard and/or unpaved street. The same was observed in relation to a history of enteroparasitism. There was also an association between a seronegative ELISA test and previous treatment of pet dogs and/or cats with vermifuge. Based on these results, the authors propose that public health programs should include anthelmintic for dogs and cats during the antirabies vaccination campaigns, in order to diminish environmental contamination with* Toxocara *spp. eggs and consequently human infection.*

Key words: *Toxocara* sp. - *Toxocara canis* - toxocariasis - helminthiasis

Visceral larva migrans syndrome (VLM) was described by Beaver et al. (1952). This pathology, also denominated toxocariasis or toxocarosis is caused by the prolonged migration of the nematode larva, of *Ascaridida* order that are parasites of animal and human tissue.

Humans acquire VLM by ingesting infective eggs of helminth parasites, mainly *Toxocara canis*, from animals. In human tissues development of the larva is interrupted and they do not reach the adult stage. It has been reported that the larva can persist viable in the tissues of animal models, such as the *rhesus* monkey, for up to 10 years (Beaver 1966).

In humans, the larva can cause two syndromes: VLM, involving various organs, and ocular larva migrans that is restricted to the eye and optic nerve (Despommier 2003).

The usual hosts of *Toxocara* sp. are dogs and cats, however direct contact with these animals is not a potential risk (WHO 1990). The main source of human infection is considered to be environmental contamination with *Toxocara* sp. eggs, principally in public areas of large urban centers, such as parks and gardens that are frequented by dogs and cats as well as by humans. This presents a great risk particularly to children who have more contact with the sand and soil (Overgaauw 1997). Various investigators have realized environmental research in order to verify the presence of eggs from this parasite in such public places: Alcantara et al. (1989) found *Toxocara* sp. eggs in canine excrement and soil samples from 23 districts of Salvador City, state of Bahia, Brazil; Campos et al. (1987), reported 66.6% positivity in Goiania, state of Goiás, Brazil; Chieffi and Müller (1976), observed 60% in public places of Londrina City, state of Paraná, Brazil; Costa-Cruz et al. (1994), detected 23.07% in public places of Uberlândia City, Minas Gerais, Brazil; Ferreira et al. (1976), found 41.6% in the soil of public squares of Rio de Janeiro City, Rio de Janeiro, Brazil; Silva (1984) found eggs of this parasite on the ground of three public squares also in Rio de Janeiro City; and Lescano et al. (1998), found 80% positivity in soil samples obtained from public squares in Lima City, Peru.

Specifically regarding Sorocaba City, São Paulo, Brazil, Coelho et al. (2001), investigated the presence of *Toxocara* sp. eggs in soil samples collected from public squares in 1999 and found eggs in 53.3% of the locations studied.

According to the Brazilian Institute of Geography and Statistics (IBGE 2000), the city of Sorocaba had 435,000 inhabitants in 1999 and during a municipal campaign against rabies, a total of 85,000 dogs were vaccinated, this number was equivalent to 20% of the human population. The World Health Organization (WHO 1990) recommends that the canine population of each locality should correspond to at the most 10% of the human population. Since only a part of the canine population was actually vaccinated it can be deduced that the number of dogs was even greater.

In view of the above and results obtained by Coelho et al. (2001), regarding the canine population existent in Sorocaba City, the objective of the present study was to perform a seroepidemiological survey in order to detect

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anti-toxocara antibodies among schoolchildren attending public schools of the city. The respective infection coefficients (IC) were then compared in relation to factors such as: whether the children resided in the city center or suburbs; whether their house had a garden; whether the street was paved, the presence of cats and dogs; and if these had been treated for worms.

CASUISTRY AND METHODS

The research project was approved by the Research Ethics Committee of the Medical and Biological Sciences Center at the Pontifical Catholic University of the state of São Paulo. The children's parents or legal guardians signed a free and informed term of consent prior to participation in the study.

The sample population was determined by mathematical model, in order to ensure the representativity of schoolchildren at the public schools. In all, 180 children participated in this study, with age ranging from three to seven years, selected at random, from August 2000 to July 2001.

Firstly a questionnaire was completed by the parents or legal guardians, to identify and obtain epidemiological information regarding toxocariasis and financial status of the families. Then blood samples were collected from each child, the serum was separated and maintained at negative 20°C, until use. The serums were previously treated with antigen obtained from *Ascaris suum* and later used in the ELISA test for detection of anti-*Toxocara* IgG antibodies. This test was realized at the Central Laboratory of the Adolfo Lutz Institute, Health Secretary of the State of São Paulo State, using standardized technique described by Camargo et al. (1992). The excretory and secretory antigens were obtained from *Toxocara* sp. third-stage larva.

The serologic results were considered reactive when the dilution of the serum presented an optical density (OD) of absorbance equal or higher than the cut off that was set at 0.375.

The results were submitted to statistical analysis, utilizing the chi-square, Fisher and Mann-Whitney tests, for contingency tables, Siegel (1975), with the objective of comparing data from the questionnaire with the results of the survey for serum anti-toxocara antibodies.

RESULTS

Out of 180 children that participated, 92 (51.1%) were male and 88 (48.9%) female; 135 (75%) lived and went to school in the suburbs and 45 (25%) in the central region of the city, these figures were reflected in the number and location of public schools with a ratio of 3:1 schools in the outskirts and city center, respectively.

Afterwards a possible sex bias was investigated in the results of the ELISA and as none was found, this variable was disregarded in the present study.

When the children were grouped according to age the distribution was: 2 years old, 4 (2.2%); 3 years old, 11 (6.1%); 4 years old, 34 (18.9%); 5 years old, 37 (20.6%); 6 years old, 50 (27.8%); 7 years old, 33 (18.3%), and 8 years old 11 (6.1%). The mean age was 5.4 ± 1.4 years.

Regarding housing, 174 (96.7%) children lived in a unifamily dwelling; 136 (75.5%) own house; 178 (98,9%) in a masonry house; 79 (43.9%) in a house with a yard; 55 (30.5%) houses were on unpaved streets. The mean number of rooms per residence was 4.3 and the mean number of residents per house was 4.5 (Table I).

Almost all the children lived on roads with street lighting 175 (97.2%); the houses of 173 were connected to the central sewage system; 179 (99.4%) had piped potable water from the city water supply; and 180 (100%) had trash collection (Table II).

TABLE I

Dwelling conditions of schoolchildren whose serum samples were reactive in ELISA test for toxocariasis in Sorocaba City, state of São Paulo, from August 2000 to July 2001

Dwelling	ELISA test reactive/total number of children						Central vs outskirts
	Central region			Outskirts			
	Reactive	Total	%	Reactive	Total	%	
Unifamily	5	45	11.1	61	129	47.3	$p < 0.001$
Own home	5	35	14.3	45	101	44.6	$p < 0.01$
Masonry	5	45	11.1	62	133	46.6	$p < 0.001$
Unpaved yard	0	12	-	37	67	55.2	$p < 0.001$
Unpaved road	0	2	-	33	53	62.3	$p < 0.001$

TABLE II

Public services at the residence place and schoolchildren reactive sera, ELISA test for toxocariasis, in Sorocaba City, state of São Paulo, during August 2000 to July 2001

Public service	ELISA test reactive/total number of children						Central vs outskirts
	Central region			Outskirts			
	Reactive	Total	%	Reactive	Total	%	
Electric light	5	45	11.1	61	130	46.9	$p < 0.001$
Sewerage system	5	44	11.4	60	129	46.5	$p < 0.001$
Potable water	5	45	11.1	64	134	47.8	$p < 0.001$
Trash collection	5	45	11.1	64	135	47.4	$p < 0.001$

It was found that 130 (72.2%) of the children kept dogs and/or cats at home; 30 (16.7%) of those interviewed said that the animal lived inside the house; 89 (49.4%) children reported playing with the animals and 4 (2.2%) of them slept with their pets. Animal excrements were collected and disposed of in trash receptacles by 116 (64.4%) of those interviewed and 75 (41.7%) reported that the dogs had received prior treatment for worms (Table III).

In relation to the habits of these children, 172 (95.5%) played in sand-boxes and 6 (3.3%) reported geophagy. As seen in the personal morbid antecedents, 91 (50.5%) children had had internal parasites, 63 (35%) respiratory problems, 69 (38.3%) allergies, and 18 (10%) visual disturbances (Table IV); 59 (32.8%) children had a medical assistance plan.

The socioeconomic stratification was established by the Brazilian Economic Classification Standard, that permits an estimation of the purchasing power. This enabled the children to be stratified into social categories as well as to estimate the respective family mean monthly purchase (FMMP). The children were grouped into six social classes, the highest denominated A2 and the lowest E (Table V).

The ELISA test for detection of anti-*Toxocara* anti-

TABLE III

Contact of the schoolchildren with domestic dogs and/or cats, reactivity results with serum samples, in the ELISA test for toxocariasis, in Sorocaba City, state of São Paulo, from August 2000 to July 2001

Contact with domestic dogs/cats	ELISA test reactive/total number of children						Central vs outskirts
	Central region			Outskirts			
	Reactive	Total	%	Reactive	Total	%	
Present at home	4	32	12.5	49	98	50.0	$p < 0.001$
Live inside home	0	5	-	9	25	36.0	$p > 0.05$
Play with animal	2	22	9.1	34	67	50.7	$p < 0.001$
Sleep with animal	0	1	-	2	3	66.7	$p > 0.05$
Excrement in trash	3	29	10.3	43	87	49.4	$p < 0.001$
Previous treatment of dogs	1	24	4.2	25	51	49.0	$p < 0.001$

TABLE IV

Behavior of schoolchildren, personal morbid antecedents and reactivity results with serum samples, in the ELISA test for toxocariasis, in Sorocaba City, state of São Paulo, during August 2000 to July 2001

Behavior and morbid antecedents	ELISA test reactive/total number of children						Central vs outskirts
	Central region			Outskirts			
	Reactive	Total	%	Reactive	Total	%	
Play in sand-boxes	5	45	11.1	61	127	48.0	$p < 0.001$
Geophagy	0	2	-	3	4	75.0	$p > 0.05$
Enteroparasitosis	2	13	15.4	41	78	52.6	$p < 0.02$
Respiratory symptoms	3	20	15.0	18	43	41.9	$p < 0.05$
Symptoms of allergy	3	19	15.8	16	50	32.0	$p > 0.05$
Visual symptoms	0	1	-	8	17	47.1	$p > 0.05$
Medical assistance plan	2	31	6.5	11	28	39.3	$p < 0.01$

TABLE V

Socioeconomic stratification of the schoolchildren families and reactivity results with serum samples, in the ELISA test for toxocariasis, in Sorocaba City, State of São Paulo, from August 2000 to July 2001

Social class (FMMP)	ELISA test reactive/total number of children						Central vs outskirts
	Central region			Outskirts			
	Reactive	Total	%	Reactive	Total	%	
A2 R$5894.00	0	3	-	-	-	-	-
B1 R$3742.00	0	11	-	-	-	-	-
B2 R$1614.00	1	12	8.3	2	9	22.2	$p > 0.05$
C R$ 844.00	4	17	23.5	29	62	46.8	$p > 0.05$
D R$ 435.00	0	2	-	32	62	51.6	$p > 0.05$
E R$ 229.00	-	-	-	1	2	50.0	-

FMMP = family mean monthly purchase

bodies was reactive in the serum samples of 69 children (IC = 38.3) of which 37 were males (IC = 40.2) and 32 females (IC = 36.4) ($p > 0.05$). The IC was calculated using the reactive ELISA test results per 100 children that participated in this study. From the 45 children that lived in the central region of the city, five (11.1%) were reactive in the ELISA test. While 64/135 (47.4%) of those that lived in the periphery were reactive ($p < 0.001$).

The results that presented statistical significance ($p < 0.05$) were: a greater IC among the children that lived in the periphery; and those who lived in houses with yards and on unpaved streets presented greater serologic reactivity in the ELISA test. There was also a statistically significant association between serologic reactivity in the ELISA test and personal morbid antecedents of enteroparasitosis.

Other statistically significant associations were nonreactive ELISA results and information regarding previous treatment of dogs and personal morbid antecedents of allergy.

DISCUSSION

Seroepidemiological surveys are an important research tool, especially in Public Health. They enable the determination of the infection coefficient for a given pathology in a population, both in longitudinal and epidemiologic studies. In the various population groups, the seroepidemiological studies to detect anti-toxocara antibodies have shown that the seroprevalence is higher among children. Consequently, it is important to test for these antibodies (Chieffi et al. 1990) and (Jacob et al. 1987), mainly among those who have peripheral eosinophilia ≥ 400 eosinophils/ml of blood (Ljungström & Van Knapen 1989, Woodruff 1970).

The present study determined that IC by *Toxocara* sp., in schoolchildren of the public schools of the Sorocaba City, was 38.3 during the period of the study and there was no significant difference between the male and female schoolchildren. This fact was expected, since the infection source is environmental, with an equal exposure, as described by Chieffi et al. (1990), in São Paulo City, Anaruma Filho et al. (2002), in Campinas City, Brazil, Alonso et al. (2000), in Argentina; Ljungström et al. (1989), in Sweden, and Moreira-Silva et al. (1998), in Vitória City, Espirito Santo, Brazil.

The IC of the schoolchildren was higher among those that lived in the periphery of the city compared to those living in the Central region. This fact is probably due to the better socioeconomic conditions of the families that reside in the central region. In the city center, 57.8% of the children belonged to social classes A2, B1, and B2; 37.8% to class C; 4.4% to class D, and none to class E. On the other hand, regarding the children that lived in the peripheral region, 93.3% were from families of social classes C, D, or E; 6.7% were from B2 and none from classes A2 or B1. There was an association between less favorable socioeconomic conditions and unpaved yards and streets.

Another important finding was the association of infection by *Toxocara* sp. and the socioeconomic conditions of these families, as can be observed on comparing the IC among schoolchildren that lived in houses with unpaved yards and streets (Table III). This can be explained in part by the life cycle of *Toxocara* sp. that requires the egg shed in animal feces to evolve in the soil and reach the infective phase with third stage larva (Pessoa & Martins 1982) and (Woodruff 1970). Under these conditions, infection can occur when the children play and are in contact with the soil. It is important to underscore that 99.4% of these children lived in houses that had piped drinking water.

The association between personal morbid antecedents and the IC for toxocariasis was higher in the group that reported previous internal parasites. This fact leads to the conclusion that there was a correlation between worse social economic conditions and the higher IC for toxocariasis in Sorocaba City. This association was also found by Alderete et al. (1999), in Butantã, São Paulo City, São Paulo, Brazil, and by Agudelo et al. (1990), in Bogotá, Colombia.

Dogs and or cats frequenting the interior the houses did not show an association with *Toxocara* sp. infection in the groups of children studied, which differs from the observations of Chieffi et al. (1988). This is probably due to the fact that the eggs need soil to develop and the houses and streets are paved (Woodruff 1970) thus interrupting the life cycle.

Moreira-Silva et al. (1998), in Vitória City, Espírito Santo, in a seroepidemiological survey, among hospitalized children with a mean age of 6.6 ± 4.1 years and with various pathologies, found 39% seropositivity for toxocariasis. Triviño et al. (1999), in Chile, using somatic antigen in the ELISA test for toxocariasis, found 33.3% reactivity with serum of children that were under 15 years of age. These authors obtained results close to those found in the present study, realized in Sorocaba City, São Paulo.

In this survey there was no association between geophagy and ELISA seropositivity, differing from the results obtained by Alonso et al. (2000), in Argentina. This could be explained by reluctance on behalf of the parents or legal guardians to admit such a behavior due to embarrassment when completing the questionnaire.

Another intriguing occurrence, in this study, was the lack of association between the children playing in sandboxes and seropositivity in the ELISA test, since it is on the contrary to the correlation between unpaved yards and streets. This finding will be the subject of a future study to determine the infective capacity and viability of *Toxocara* sp. eggs shed on sand compared to those in soil.

The association between seronegative ELISA test and previous treatment of domestic animals for worms suggests that this should be considered in Public Health programs among populations with a high seroprevalence. Thus, the authors propose mass antihelminthic treatment of dogs and cats should be included in the public campaigns to vaccinate animals against rabies that are realized on a yearly basis. Such a measure would reduce the rate of infection by *Toxocara* sp. among these animals and thereby diminish environmental contamination with infective eggs and consequently human infection.

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