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FREQUENCY OF *Toxocara* INFECTION IN CHILDREN ATTENDED BY THE HEALTH PUBLIC SERVICE OF MARINGÁ, SOUTH BRAZIL

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SUMMARY

The lack of specific laboratorial diagnosis methods and precise symptoms makes the toxocaríasis a neglected disease in Public Health Services. This study aims to determine the frequency of *Toxocara* spp. infection in children attended by the Health Public Service of Hospital Municipal de Maringá, South Brazil. To evaluate the association of epidemiological and clinical data, an observational and cross-section study was carried out. From 14,690 attended children/year aged from seven month to 12 years old, 450 serum samples were randomly collected from September/2004 to September/2005. A questionnaire was used to evaluate epidemiological, clinical and hematological data. An ELISA using *Toxocara canis* larval excretory-secretory products as antigen detected 130 (28.8%) positive sera, mainly between children from seven month to five years old ($p = 0.0016$). Significant correlation was observed between positive serology for *Toxocara*, and frequent playing in sandbox at school or daycare center ($p = 0.011$) and the presence of a cat at home ($p = 0.056$). From the families, 50% were dog owners which exposed soil backyards. Eosinophilia ($p = 0.776$), and signs and symptoms analyzed (fever $p = 0.992$, pneumonia $p = 0.289$, cold-like symptoms $p = 0.277$, cough $p = 0.783$, gastrointestinal problems $p = 0.877$, migraine $p = 0.979$, abdominal pain $p = 0.965$, joint pain $p = 0.686$ and skin rash $p = 0.105$) could not be related to the presence of anti-*Toxocara* antibodies. Therefore, two asthmatics children showed titles of 1:10,240 and accentuated eosinophilia ($p = 0.0001$). The authors emphasize the needs of prevention activities.

KEYWORDS: Seroprevalence; *Toxocara* spp.; Children; Public service; Risk factors.

INTRODUCTION

Human toxocaríasis is a zoonosis spread worldwide. It shows different prevalences^{1,2,3,5,6,10,11,16,28,31} depending on innumerable factors, such as poverty, human contact with soil contaminated by dog feces, and residence in rural areas. *Toxocara canis* and *Toxocara cati*, helminths belonging to the family Ascarididae, are common intestinal parasites of dogs and cats^{8,32}. *Toxocara* is the main genus responsible for the Visceral Larva Migrans (VLM) syndrome or toxocaríasis in humans, *T. canis* being the primary causal agent of this parasitosis³³.

Human infection occurs through accidental ingestion of embryonated eggs of *Toxocara* spp. found in soil and sand contaminated by animal feces²⁵. The migration and persistence of nematode larvae in unusual host organs cause the VLM syndrome^{1,17}. The larvae cause mechanical injury as they migrate through the viscera, inducing the host to develop different degrees of inflammatory response²⁹. Children are most likely to be infected, probably because of the association of their undeveloped immune system, the amount of eggs ingested¹⁹, and the frequency of reinfections³⁰. Most patients who are seropositive for *Toxocara* spp. show no clinical signs⁷.

Human toxocaríasis is classified in four forms: Systemic (classical and incomplete), Compartmentalized (ocular-OLM and neurological-NLM), covert, and asymptomatic^{24,30}. The classical form of VLM is characterized by high eosinophilia, fever, hepatosplenomegaly, hypergammaglobulinemia, and lung involvement, and affects mainly children^{9,30}. In the visceral incomplete form, mainly hepatomegaly and high eosinophilia occurs. The NLM form is usually asymptomatic, although there may be symptoms ranging from minor neurological deficiencies to eosinophilic meningoencephalitis¹². In OLM, low titers of anti-*Toxocara* spp. antibodies and discrete eosinophilia have been observed⁷. This is a complex disease, for which the factors involved in its development and ocular compromise are still unknown⁴. The covert form is characterized by non-specific symptoms due to constant stimulus of parasite antigens on the host immune system; there may also be pulmonary involvement, such as asthma, acute bronchitis, dermatological disorders, myositis, lymphadenopathy, and pseudorheumatic syndromes such as arthralgia³⁰.

The diagnosis of human toxocaríasis is essentially based on immunological tests, because of the difficulty of locating larvae of *Toxocara* spp. in biopsy tissue. The prime diagnostic method is the

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ELISA (Enzyme-Linked Immunosorbent Assay) test using the excretory-secretory antigens of infective larvae of *Toxocara canis*¹³.

Studies in Brazil have reported prevalences from 2% to 54%^{2,5,10,16,25}. Toxocariasis is usually neglected by the Brazilian Public Health Service, and is often not mentioned in medical courses. Cases of toxocariasis involving the eyes, liver, lungs, or brain may be detected by the use of immunological tests in seroprevalence assays²⁸.

The lack of epidemiological data for toxocariasis in the state of Paraná, as well as the high frequency of eggs of *Toxocara* spp. found in public squares and lawns in the urban area of the city of Maringá³⁴ justified the present assessment. The objective of this investigation was to determine the frequency of *Toxocara* spp. infection in child patients attended by the ambulatory service of the Maringá Municipal Hospital (MMH), and to associate them with epidemiological and clinical data.

MATERIAL AND METHODS

An observable cross-section study with incomplete selection was carried out. The study was approved by the institutional Research Ethics Committee (COPEP/UEM-305/2004), and by the MMH administrators. The individuals were invited to participate and informed in detail about the research, and voluntary written consent was obtained from the subjects enrolled.

Sampling: Based on the survey of the 2003-2004 annual statistics of 14,690 children attended by the Maringá Municipal Hospital, Paraná State, aged seven months to twelve years, 450 samples of blood were randomly collected from male and female patients, from September 2004 to September 2005. The size of the sample was calculated from an expected prevalence of 25%, with a confidence interval (CI) of 95%. This hospital serves exclusively users of the Brazilian Public Health System.

Serology: Blood samples were collected at the MMH ambulatory clinic, from patients referred for laboratory examinations (hematological and biochemical tests). The serum was preserved with buffered glycerin (1:1) and stored at -20 °C until it was analyzed. IgG anti-*Toxocara* spp. antibodies were investigated by the ELISA method, by means of a *Toxocara* excretory-secretory antigen (TES) from second-stage *T. canis* larvae obtained according to DE SAVIGNY *et al.*¹³, modified by ELEFANT *et al.*¹⁵. Reactive serum, non-reactive serum, and limiar reactive serum (LRS) were used in the entire survey. The ELISA cut-off was determined each time, using human positive and negative sera. The serologic results were considered reactive when the dilution of the serum showed an optical density (OD) of absorbance equal or higher than the cut-off. The results were expressed as the Index of Reactivity (IR) = optical density of the sample/optical density of LRS. Reacting samples were considered as those which showed IR ≥ 1 . All samples were tested in duplicate. This assay showed 78% sensitivity and 92% specificity.

Hemogram: The hemogram was done at the MMH Laboratory of Clinical Analyses, and the data for eosinophilia and hypochromia were compiled.

Clinical and epidemiological data: In order to evaluate possible risk factors for toxocariasis, the participants completed a questionnaire to obtain information about epidemiological data including: onicophagy habit; cats or dogs at the home; peridomestic exposed soil, grass, and sandbox; place where the child usually played (school or daycare center); whether the child played often in the public square with a sandbox, grass, or exposed soil; whether the child played in other locations with a sandbox, grass, or exposed soil; family income; age; sex; location of the home. The questionnaire also requested clinical data such as bronchitis, asthma, fever, pneumonia, cold, cough, diarrhea and vomiting, cephalia, pruritus or widespread eczema, or pain in the lower limbs.

Statistical analysis: A database was established using Microsoft Excel® 2000 and Statistics Version 6.0. A nonparametric Chi-square (χ^2) test was performed to compare the frequency of infected or uninfected children with each epidemiological, clinical, and hematological parameter investigated, using $p \leq 0.05$ as the level of significance.

RESULTS

A total of 130 children (28.8%) were positive for *Toxocara* spp. There was no significant difference between sexes ($p = 0.297$). Most seropositive children were from seven months to five years old (Table 1).

Table 1

Frequency of *Toxocara* spp. infection among children aged seven months to 12 years old attended by the Maringá Municipal Hospital (MMH), Paraná

	Examined (%)	Positive (%)	p*
Age group (years)			
7m - 5y	294 (65.3)	83 (28.2)	0.0016
>5y - 12y	156 (34.7)	47 (30.1)	
Total	450	130	
Sex			
Male	223 (49.6)	66 (50.8)	0.9013
Female	227 (50.4)	64 (49.2)	

M = month; y = years * (Chi-square test, $p \leq 0.05$).

There was an important significant correlation between positive serology for toxocariasis, and frequent playing in a sandbox at the school or daycare center (OR = 1.933) and the presence of a cat at home (OR = 1.957) (Table 2).

The children came from different urban neighborhoods of Maringá. Most of the families (92%) had an income from one to three minimum wages.

The signs and symptoms revealed upon examination by MMH pediatricians could not be correlated with positive serology for *Toxocara* spp. (Table 3). Nor could eosinophilia and hypochromia except for hypochromia at ages from seven months to two years ($p = 0.0001$). For two children who presented bronchopneumonia, one two years old and the other one year old, the antibodies of both showed high titers

Table 2
Possible risk factors observed in children positive for *Toxocara* IgG antibody attended by the ambulatory service of the Maringá Municipal Hospital, Paraná, 2004 - 2005

Variable	Group	Seropositive (%)	Seronegative (%)	Odds Ratio	p*	IC
Onicophagy	Yes	57 (43.8)	131 (40.9)	1.127	0.570	(0.746; 1.701)
	No	73 (56.2)	189 (59.1)			
Dog	Yes	78 (60.0)	192 (60.0)	1.000	1.000	(0.660; 1.516)
	No	52 (40.0)	128 (40.0)			
Cat	Yes	15 (11.5)	20 (6.2)	1.957	0.056	(0.968; 3.952)
	No	115 (88.5)	300 (93.8)			
Sand ¹	Yes	15 (11.5)	39 (12.2)	0.940	0.840	(0.499; 1.771)
	No	115 (88.5)	281 (87.8)			
Soil ¹	Yes	76 (58.5)	182 (56.9)	1.067	0.757	(0.706; 1.613)
	No	54 (41.5)	138 (43.1)			
Grass ¹	Yes	47 (36.2)	90 (28.1)	1.447	0.093	(0.939; 2.231)
	No	83 (63.8)	230 (71.9)			
Sand ²	Yes	16 (12.3)	37 (11.6)	1.073	0.824	(0.574; 2.007)
	No	114 (87.7)	283 (88.4)			
Soil ²	Yes	45 (34.6)	111 (34.7)	0.997	0.988	(0.650; 1.530)
	No	85 (65.4)	209 (65.3)			
Grass ²	Yes	25 (19.2)	54 (16.9)	1.173	0.550	(0.694; 1.983)
	No	105 (80.8)	266 (83.1)			
Sand ³	Yes	30 (23.1)	43 (13.4)	1.933	0.011	(1.150; 3.249)
	No	100 (76.9)	277 (86.6)			
Soil ³	Yes	22 (16.9)	36 (11.3)	1.607	0.103	(0.904; 2.855)
	No	108 (83.1)	284 (88.8)			
Grass ³	Yes	31 (23.8)	72 (22.5)	1.079	0.758	(0.666; 1.745)
	No	99 (76.2)	248 (77.5)			

¹ Present in backyards; ² In other areas where the child usually plays; ³ Present at the school; * (Chi-square test, p ≤ 0.05).

(1:10,240). Both children played on exposed soil and owned more than one dog. The hemogram of the 2-year-old indicated eosinophilia of 24,000 eosinophils/mm³ and that of the 1-year-old revealed eosinophilia of 2,755 eosinophils/mm³. Treatment with thiabendazole caused reversion of the clinical picture in one child. The other child was not located after the initial examination.

DISCUSSION

The seroprevalence observed in children who were attended at the MMH ambulatory clinic was similar to that shown by children at public hospitals in other Brazilian cities such as Campo Grande (Mato Grosso do Sul State) and Brasília (Federal District) with 35.5% and 21.8% seropositivity, respectively^{10,25}. The MMH provides only public-health services for children from the districts and the city of Maringá. A higher prevalence (54.8%) was observed by FIGUEIREDO *et al.*¹⁶ in 208 children from one to 14 years old who were attended at the Pediatric, Immunology and Pneumology ambulatory clinics of the University of Santo Amaro in the city of São Paulo. This finding differed from ours because the latter clinic provides specialized care to ambulatory patients; the children are from the periphery (mostly shantytowns) of São Paulo, and half of them were asthmatic. The seroprevalence of toxocariasis is closely related to the hygienic conditions found among people from different sociocultural and economic classes¹¹. As in the reports of ALDERETE *et al.*², ALONSO *et al.*³, ALTCHER *et al.*⁴, FIGUEIREDO *et al.*¹⁶ and MAGNAVAL *et al.*²³ there was no statistical

difference in seroprevalence of this parasitosis between the genders.

Of 130 seropositive children, 64% were from seven months to five years old. The result of this assay may have been influenced by the high number of children of this age who were attended at the MMH ambulatory clinic. During their early years, children seem to be more often exposed to numerous infections because of the frequent contact with contaminated soil or sand by animal feces^{3,19,33}. It was observed a higher prevalence of anti-*Toxocara* spp. antibodies in children until five years old, and ALDERETE *et al.*² reported the highest positivity rate in four-year-old children. On the other hand, MURADIAN *et al.*²⁶ observed only 14% of positivity in children from one to five years old, and higher percentages in older children.

In Brazil, the test for toxocariasis is unavailable through the public-health services, although the ELISA method is inexpensive. This method has been recommended as a first-line test to detect toxocariasis, despite its 78% sensitivity. The possibility of a cross-reaction with *Ascaris lumbricoides* was minimized in our study, because the sera from all the patients were adsorbed with *A. suum* antigen before the test. This procedure avoided the use of other tests such as the Western blot concomitantly with the ELISA²¹.

Playing in a sandbox at the school or daycare center was the epidemiological factor most closely related to the prevalence of toxocariasis in Maringá, in children of all ages. We believe that this

Table 3

Signs and symptoms diagnosed in children positive for *Toxocara* IgG antibody attended by the ambulatory service of Maringá Municipal Hospital, 2004 – 2005

	Seropositive (%) (n =118)	Odds Ratio	IC	p*
Fever				
Yes	62 (52.5)	1.026	(0.669; 1.575)	0.992
No	56 (47.5)			
Pneumonia				
Yes	7 (5.9)	1.976	(0.718; 5.435)	0.289
No	111 (94.1)			
Cold-like symptoms				
Yes	15 (12.7)	1.549	(0.785; 3.056)	0.277
No	103 (87.3)			
Cough				
Yes	23 (19.5)	0.894	(0.524; 1.527)	0.783
No	95 (80.5)			
Gastrointestinal problems				
Yes	49 (41.5)	0.943	(0.611; 1.454)	0.877
No	69 (58.5)			
Migraine				
Yes	15 (12.7)	0.941	(0.497; 1.781)	0.979
No	103 (87.3)			
Abdominal pain				
Yes	10 (8.5)	0.943	(0.440; 2.023)	0.965
No	108 (91.5)			
Joint pain				
Yes	2 (1.7)	0.9862	(0.188; 5.156)	0.686
No	116 (98.3)			
Skin rash				
Yes	8 (6.8)	2.573	(0.942; 7.024)	0.105
No	110 (93.2)			

*Chi-square test (p ≤ 0.05)

correlation is due to the fact that the sandboxes frequently remain uncovered, allowing stray animals to use them when classes are not in session. A quantitative study of sand in public squares of this city, in winter and summer, found that *Toxocara* spp. eggs were widely distributed, comprising 50% of the parasite structures found³⁴. ALDERETE *et al.*² observed that eggs of *Toxocara* spp. were found in at least one sample of soil from each of five schools investigated. ZUNINO *et al.*³⁶ reported that these structures were those most often diagnosed in fecal material collected from urban public parks in Chubut, Argentina. In Rio de Janeiro, other factors such as the presence of puppies in the home and contact with soil contributed to seropositivity in children attended by a hospital ambulatory clinic¹⁶.

Other factors, such as the presence of cats in domiciles and contact with grass in the backyard during play periods, also contributed to the increase in the toxocariasis rate in the present investigation. Some epidemiological studies have attempted to identify risk factors for acquiring toxocariasis^{5,19,21}. These factors range from the male gender²¹ to residence in a shantytown and a residence with a dirty backyard². WOLFE & WRIGHT³⁵ reported that embryonated *T. canis* eggs were

present in 4.2% of hair samples collected from 60 dogs, showing that direct contact with infected animals could transmit toxocariasis. However, contact with soil contaminated by cat and dog feces may be the main cause of the observed seropositivity. It has been reported that the risk factor for toxocariasis is contact with soil contaminated with cat or dog feces, rather than owning a cat or dog at home^{1,27}.

Regarding the symptoms and signs, two children showed positive serology for *Toxocara* spp., presenting both bronchopneumonia and high eosinophilia. There is a strong association between positive serology for *T. canis* and eosinophilia and IgE^{18, 26}. JACOB *et al.*²⁰ described an association between the VLM syndrome and bronchial spasms, pneumonia, and respiratory insufficiency. We know that clinical manifestations are variable and that they depend on the number of eggs ingested, the tissue involved, and the allergic response by the host^{17,26}. Most of the time, toxocariasis can occur without symptoms^{14,24,30}, being related to low ELISA titers²². Because there were few symptomatic children in this study, caution should be employed when interpreting positive results through population screening. For the most part, these could represent past exposure to the parasite rather than recent infection²⁴. It is necessary to remember that human toxocariasis is a chronic infection, which may last for a number of years, and reactivated larvae may migrate into the eye or the brain at any time³⁰.

This study established that toxocariasis is an infection that occurs in South Brazil, and is related to environmental contamination, mainly of sandboxes of schools and daycare centers by animal feces. The prevalence of this parasitosis could be investigated if *T. canis* antigen were available in the health care services to perform toxocariasis diagnosis as early as possible, thus aiding the medical professionals in the ready recognition and treatment of symptomatic cases. It has also emphasized the need to develop educational campaigns in the education and health services, to improve measures for the sanitary control of pets and stray dogs and cats, and cleanliness and monitoring of recreational areas.

RESUMO

Frequência de infecção por *Toxocara* em crianças atendidas em serviço público de Maringá, sul do Brasil

A falta de métodos de diagnóstico laboratorial específico e sintomas específicos fazem da toxocaríase uma doença negligenciada nos serviços públicos de saúde. Este estudo teve por objetivo determinar a frequência de infecção por *Toxocara* spp. em crianças atendidas no serviço público do Hospital Municipal de Maringá, sul do Brasil, e avaliar a associação com dados epidemiológicos e clínicos, em estudo observacional e transversal. De 14.690 crianças/ano atendidas, com idade entre sete meses a 12 anos, foram coletados 450 soros de setembro/2004 a setembro/2005. Um questionário foi utilizado para avaliar dados epidemiológicos, clínicos e hematológicos. Pelo teste ELISA, com antígeno de excreção/secreção de larvas de *Toxocara canis*, detectou-se 130 (28,8%) soros positivos, principalmente em crianças entre sete meses e cinco anos (p = 0,0016). Houve significativa correlação entre sorologia positiva para *Toxocara* e freqüente recreação das crianças em caixas de areia da escola ou pré escola (p = 0,011) e presença do gato no domicílio (p = 0,056). Das famílias dessas crianças,

50% possuíam cachorros e o quintal com solo exposto. Eosinofilia ($p = 0,776$), sinais e sintomas (febre $p = 0,992$, pneumonia $p = 0,289$, resfriado $p = 0,277$, tosse $p = 0,783$, problema gastrointestinal $p = 0,877$, dor de cabeça $p = 0,979$, dor abdominal $p = 0,965$, dores articulares $p = 0,686$, urticária $p = 0,105$) não se correlacionaram com a soropositividade. Todavia, duas crianças asmáticas apresentaram títulos de 1:10.240 ($\geq 1:320$) e acentuada eosinofilia ($p = 0.0001$). Os autores enfatizam a necessidade de atividades preventivas.

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