Article/Artigo

Anti-*Toxocara* antibodies detected in children attending elementary school in Vitoria, State of *Espírito Santo*, Brazil: prevalence and associated factors

Anticorpos anti-*Toxocara* em crianças admitidas na escola fundamental em Vitória, Estado do Espírito Santo: prevalência e fatores associados

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ABSTRACT

Introduction: The aim of this study was to evaluate the frequency of anti-*Toxocara* antibodies in serum from 7-year-old children attending elementary school in Vitória-ES, Brazil and to correlate these antibodies with socio-demographic factors, the presence of intestinal helminths, blood eosinophil numbers, past history of allergy or asthma, and clinical manifestations of helminth infections. **Methods**: The detection of anti-*Toxocara* antibodies was performed using an ELISA (Cellabs Pty Ltd) on serum from 391 children who had already been examined by fecal examination and blood cell counts. Data from clinical and physical examinations were obtained for all children. **Results**: The prevalence of anti-*Toxocara* antibodies was 51.6%, with no gender differences. No significant differences were observed between positive serology and the presence or absence of intestinal worms (60.3 and 51.7%, respectively; p = 0.286). The only variables significantly related to positive serology were onycophagy and the use of unfiltered water. Although eosinophilia (blood eosinophil count higher than 600/mm^3) was significantly related to the presence of a positive ELISA result, this significance disappeared when we considered only children without worms or without a past history of allergy or asthma. No clinical symptoms related to *Toxocara* infection were observed. **Conclusions:** There is a high prevalence of anti-*Toxocara* antibodies in children attending elementary schools in Vitória, which may be partially related to cross-reactivity with intestinal helminths or to a high frequency of infection with a small number of *Toxocara* eggs.

Keywords: *Toxocara canis.* Toxocariasis. *Toxocara* infection. Visceral larva migrans.

RESUMO

Introdução: O objetivo desse estudo foi avaliar a prevalência de anticorpos anti-*Toxocara* em crianças admitidas no primeiro ano de escola fundamental em Vitória e correlacionar com variáveis sociodemográficas, helmintos intestinais, eosinófilos no sangue, geofagia, onicofagia, história de asma e alergia cutânea e manifestações clínicas. **Métodos**: A pesquisa de anticorpos anti-*Toxocara*, utilizando um teste de ELISA (Cellabs), foi realizada em 391 crianças nas quais foram realizados exames parasitológicos de fezes e hemograma completo. Todas as crianças foram submetidas a exame clínico e físico. **Resultados**: A prevalência de reação positiva foi de 51,6%, sem diferença entre os sexos. Não foram observadas diferenças significativas na prevalência de reação positiva em crianças com ou sem helmintos intestinais (60,3 e 51,7%, respectivamente; p = 0.286). Ainda que a frequência de eosinófilos acima de 600/mm^3 tenha sido significativamente maior em crianças com sorologia positiva, a significância desapareceu quando se considerou as crianças sem helmintos intestinais ou história pregressa de asma ou alergia cutânea. As únicas variáveis significativamente correlacionadas, de modo independente, com a presença de sorologia positiva foram onicofagia e hábito de beber água não filtrada. Nenhuma criança apresentou manifestação clínica relacionada com a presença de anticorpos anti-*Toxocara*. **Conclusões:** A prevalência de anticorpos anti-*Toxocara* em crianças admitidas nas escolas elementares em Vitória é alta, a qual pode estar, em parte, relacionada à reação cruzada com antígenos de helmintos intestinais ou devida a frequente exposição a baixas quantidades de ovos do *Toxocara*.

Palavras-chaves: *Toxocara canis.* Toxocarase. Infecção com *Toxocara.* Larva migrans visceral.

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INTRODUCTION

The prevalence of *Toxocara* infection as measured by evaluating anti-*Toxocara* antibodies in serum is variable, with higher prevalence observed in developing countries[1-4]. Some studies have demonstrated a positive correlation between the presence of anti-*Toxocara* antibodies and geophagy, onycophagy, a poor hygiene profile and contact with dogs[4-7].

A highly variable prevalence has been reported in Brazil, largely based on descriptive studies using different samples (**Table 1** summarizes the studies reporting the prevalence of anti-*Toxocara* antibodies in Brazil published from 1990 to 2009[8-22]).The only population-based study was published by Chieffi et al[8], and this study reported a prevalence of 2.8-15.5% in children up to 15 years old from four different cities in the state of São Paulo.

A prevalence of 33.3-39% was reported in random samples of children at a Children's Reference Hospital in *Vitória*[11, 18]. In this hospital, a significant association between staphylococcal infection and the presence of anti-*Toxocara* antibodies was reported[23]. In addition, larva migrans granulomas were observed in 3.2% of livers from routine autopsies at the same hospital[24]. Therefore, *Toxocara* infection appears to be prevalent in children from Brazil.

The Schoolchildren's Health Program of the municipality of *Vitoria* performs clinical examinations on all children in the first year of public elementary schools. A stool examination, a complete blood cell count and a hemoglobin evaluation are also performed. The aim of the present investigation was to assess the presence of anti-*Toxocara* antibodies in a representative number of these schoolchildren and to correlate these antibodies with clinical manifestations of infection, socio-economic patterns, hygiene profiles, eosinophil counts and the presence of intestinal helminths.

TABLE 1 - **Frequency of anti-*Toxocara* antibodies in children from different Brazilian cities**.

City	Sample	Frequency (%)	Reference
*Campinas (SP)**	< 15 years (n = 139)	2.8	-
*Marília e Presidente Prudente (SP)**	< 15 years (n = 237)	5.5	-
*Santos (SP)**	< 15 years (n = 107)	13.1	*Chieffi et al.[8]
Recife (PE)	children/eosinophilia (n = 54)	40.0	Virginia et al.[9]
Campo Grande (MS)	children/eosinophilia (n = 45)	35.5	Matos et al.[10]
Vitoria (ES)	children > 1 years at a Children's Hospital (n = 100)	39.0	Moreira-Silva et al.[11]
Campinas (SP)	children and adults (n = 138)	23.9	Anaruma-Filho et al.[12]
São Paulo (Butantã)	school children 7-16 years (n = 399)	38.8	Alderete et al.[7]
Brasilia	children 1-12 years (n = 302)	21.8	Campos Jr et al.[13]
Recife	children/adolescents (n = 386)	39.4	Aguiar-Santos et al.[14]
Sorocaba (SP)	schoolchildren (n = 180)	38.3	Coelho et al.[15]
Santo Amaro (SP)	children 1-14 years (n = 208)	54.8	Figueiredo et al.[16]
São Paulo (São Remo)	children 1-15 years (n = 338)	26.9	Muradian et al.[17]
Vitória (ES)	children1-15 years (n = 255)	33.4	Musso et al.[18]
Uberlândia (MG)	children 1-15 years (n = 242)	8.7	Teixeira et al.[19]
Comunidade do Rio Uatumã (AM)	children and adults 0-76 years (n = 100)	52.0	Damian et al.[20]
Maringá (PR)	children 7months -12 years (n = 450)	28.8	Paludo et al.[21]
Ramal do Granada (AC)	children and adults 5-90 years (n = 403)	26.8	Rubinsky-Elefant et al.[22]

SP: *São Paulo*, **PE:** *Pernambuco*, **MS:** *Mato Grosso do Sul*, **ES:** *Espírito Santo*, **MG:** *Minas Gerais*, **AM:** *Amazonas*, **PR:** *Paraná*, **AC:** *Acre*.

*This reference includes data from Campinas, Marília and Presidente Prudente

METHODS

Serum samples were collected from 391 schoolchildren in the first year at eight elementary schools randomly selected from 39 schools located in low-income neighborhoods of *Vitória*. Socio-demographic data including age, gender, family income, parents' schooling, occurrence of geophagy and onycophagy, use of treated water and sewage facilities, and contact with dogs were collected in interviews with parents that agreed to participate in this investigation.

Stool examinations performed using the sedimentation method, blood cells counts and hemoglobin measurements were performed in the Central Laboratory of the municipality of *Vitória*. The blood cell counts and hemoglobin measurements were performed using automated instruments, with differential counts checked by Giemsa-stained smears. Aliquots of blood were collected for hematologic studies and were then centrifuged, and the sera were stored at -20ºC until use for anti-*Toxocara* antibody detection.

Anti-*Toxocara* antibodies were detected using a commercial test (*Toxocara* IgG ELISA, Cellabs Pty Ltd, Brookvale, Australia) that uses secretory/excretory antigens of the second stage larvae of *Toxocara canis*. The tests were performed according to the manufacturer's instructions. Samples with optical densities (OD) ≤ 0.500 were considered negative, and samples with OD > 0.500 were considered positive.

All children were submitted to physical examination and their history of allergy or other disease was determined during an interview with their parents.

Statistical analysis was performed using SPSS 9.0 for Windows. The frequencies of the variables were calculated with 95% confidence intervals. Following statistical tests, p values < 0.05 were considered significant.

Ethical considerations

The research was approved by the ethics committee *of Centro de Ciências da Saúde, Universidade Federal do Espírito Santo*.

RESULTS

The detection of anti-*Toxocara* antibodies was performed on serum from 391 children. Information pertaining to socio-economic status and sanitary conditions was obtained for approximately 340 of these children. All children were 7 years old, the age for admission to elementary schools in *Vitória*.

The results of the ELISA to measure anti-*Toxocara* antibodies are shown in **Table 2**, classified by gender and OD values in the ELISA. The overall prevalence of positive results (OD > 0.500) was 51.6%, without significant gender differences.

A fecal examination was performed with 308 children, and 88 (28.5%) children had at least one intestinal helminth. *Ascaris lumbricoides* was the most prevalent helminth in this study (53/88, 60.2% of intestinal helminths identified). Other identified helminths were *Trichuris trichiura* (13/82,14.8%), *Enterobius vermicularis* (11/82, 12.5%) and *Hymenolepis nana* (11/88, 12.5%).

Table 3 shows the ELISA results for anti-*Toxocara* antibodies in children with negative results upon fecal examination, positive results for at least one intestinal nematode and positive or negative results for *Ascaris lumbricoides*. Although the frequencies of positive antibody tests with different OD were higher in children with *Ascaris* infection, the differences were not statistically significant. The frequency of positive serology in children without helminth infections was 51.7% (116/224), similar to the overall frequency observed. As the parasite prevalence rates may be underestimated because a single stool sample per child was examined in this study, we also considered the children's past history of worm infection. When considering only children without worms and with no past history of worm infection, the prevalence of positive antibody test results was lower (51/109 or 46.7%) but not significantly different from the overall prevalence observed (46.7 versus 51.7%; p= 0.428).

TABLE 2 - Results of ELISA tests to detect anti-*Toxocara* antibodies in 391 seven-year-old school children in public elementary schools from the urban periphery of *Vitória*, State of *Espírito Santo*, Brazil.

	All cases (n = 391)			Male (n = 206)			Female (n = 185)		
ELISA test (OD)	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Negative tests*									
(≤ 0.500)	189	48.4	(44.8-52.0)	96	46.6	(41.6-51.6)	93	40.3	(36.1-45.4)
Positive tests*									
(0.501-1.00)	92	23.5	(19.6-27.9)	55	26.6	(20.6-32.6)	37	40.3	(33.3-47.3)
(> 1.00)	110	28.1	(23.9-32.7)	55	26.7	(20.6-32.8)	55	29.7	(23.6-36.6)
Total	**202**	**51.6**	**(46.7-56.5)**	**110**	**53.4**	**(46.6-60.2)**	**92**	**49.7**	**(42.6-56.8)**

OD: optical density.

*negative or positive tests defined according the manufacturer´s instructions. Chi square test: p = 0.431.

TABLE 3 -Results of ELISA tests for the detection of anti-*Toxocara* antibodies in 305 seven-year-old schoolchildren attending public elementary schools from the urban periphery of *Vitória*, State of *Espírito Santo*, Brazil, separated based on the presence or absence of intestinal helminths.

ELISA test	Intestinal helminths			*Ascaris lunmbricoides*		
(optical density)	negative	positive	p*	negative	positive	p*
Negative						
(≤ 0.500)	108	36	1.00[a]	123	21	0.611[a]
Positive						
(0.501 - 1.00)	56	16		60	12	
(> 1.00)	60	29	0.200[a]	69	20	0.477[a]
All positive cases	116	45	0.651[b]	129	32	0.286[b]

[a]comparison taking in account different optical density levels in negative and positive cases, [b]comparison of all positive and negative cases. **ELISA:** enzyme-linked immunosorbent assay.

*χ_2 test

As shown in **Table 4**, eosinophilia was frequent in the individuals studied (120 of 245 children had an eosinophil count higher than 600cells/mm^3)and was detected significantly more often in children with positive serology for *Toxocara*. However, the presence of other intestinal helminths, skin allergy and asthma are important causes of eosinophilia in children. To verify whether a positive test for anti-*Toxocara* antibodies is a factor that influences peripheral blood eosinophil counts, the proportions of positive and negative antibody results were compared with the frequency of eosinophilia in children with and without intestinal helminths or a past history of skin allergy or asthma. There was no significant difference in eosinophil counts higher than 600cells/mm^3 between the children with positive or negative anti-*Toxocara* antibody tests. Similarly, the presence of positive antibody test results did not influence the prevalence of eosinophilia in children with intestinal helminths or with a past history of allergy or asthma, thus confirming that the presence of anti-*Toxocara* antibodies in the serum was not associated with increased blood eosinophils in this sample.

The frequencies of positive anti-*Toxocara* antibody tests relative to socio-economic status, sanitary conditions, onycophagy, geophagy and contact with dogs are summarized in **Table 5**. Low family income, onycophagy, presence of dogs in household and drinking non-filtered water was significantly associated with positive antibody tests. A multivariate analysis using a logistic regression demonstrated that drinking non-filtered water and onycophagy were independently associated with positive serology for *Toxocara*. Abnormalities upon physical examination were not found in any children in this study.

A past history of asthma, skin allergy and abdominal pain did not correlate with antibodies against *Toxocara*. Among 84 children with a past history of asthma and 85 children with a past history of skin allergy, anti-*Toxocara* antibodies were detected in 46 (54.7%) and 42 (49.4%), frequencies similar to those observed in children without a past history of asthma or skin allergy (86/171, 50.3%; Chi-square test, p=0.475 and 0.825 versus asthma and skin allergy).

TABLE 4 -Eosinophil counts in seven-year-old children from elementary schools in *Vitoria*, State of *Espírito Santo*, Brazil, with or without intestinal helminths or a past history of cutaneous allergy or asthma, according the presence or absence of a positive antibody test for *Toxocara canis*.

	Blood eosinophils/mm^3		
Groups	< 600	> 600	p*
All cases (n =336)			
anti-*Toxocara* (+)	93	87	-
anti-*Toxocara* (-)	122	33	0.000
Without intestinal helminths or past history of skin allergy or asthma (n = 115)			
anti-*Toxocara* (+)	37	20	-
anti-*Toxocara* (-)	44	14	0.114
With intestinal helminths or past history of skin allergy or asthma (n = 130)			
anti-*Toxocara* (+)	78	52	-
anti-*Toxocara* (-)	74	61	0.403

*chi-square test with Yates correction.

TABLE 5 - Gender, sanitary conditions and familial socio-economic status associated with the presence of a positive result in ELISA test for anti-*Toxocara* antibodies in serum samples from seven-year-old schoolchildren attending elementary school in of *Vitória*, State of *Espírito Santo*, Brazil.

Variables	ELISA test		OR (95% CI)	P*
	positive	negative		
Gender				
male	110	96		
female	92	93	1.16 (0.76-1.76)	0.533
Mother's schooling				
elementary	61	58		
first degree	73	63		
secondary	32	36		
university	1	3	not applied	0.420
Father's schooling				
elementary	60	60		
first degree	69	62		
secondary	39	28		
university	1	4	not applied	0.385
Family income				
< U$300	131	100		
≥ U$300	44	63	1.88 (1.15-3.07)	0.008
Geophagy				
yes	12	14		
no	163	149	0.78 (0.33-1.87)	0.684
Onycophagy				
yes	115	83		
no	60	80	1.85 (1.16-2.93)	0.008
Water facilities				
yes	173	160		
no	2	3	1.62 (0.22-14.05)	0.675
Unfiltered water				
yes	51	13		
no	124	150	4.75 (2.37-9.64)	0.001
Sewage facilities				
yes	174	162		
no	1	1	1.07 (0.98-1.02)	0,733
Dog in the household				
yes	78	54		
no	97	109	1.62 (1.02 - 2.06)	0.031

*multivariate analysis by logistic regression. **ELISA:** enzyme-linked immunosorbent assay.

DISCUSSION

These results demonstrate a high prevalence of anti-*Toxocara* antibodies in seven-year-old children living in the urban periphery of *Vitória*. This prevalence was higher than that reported in other Brazilian studies but similar to that observed in *Santo Amaro*, SP[16]. Even excluding children with intestinal helminths, the frequency of positive serology was still high. Moreover, 110 children had ELISA-positive results with OD >1.00, considered by the assay manufacturer to be indicative of more recent infection.

Cross-reactivity against other helminth antigens may be responsible for the high frequency of positive ELISA tests for anti-*Toxocara* antibodies. This cross-reactivity does exist, mainly against A. *lumbricoides*[25,26], and we did not adsorb the serum samples with other nematode antigens before performing the ELISA in this study. However, because the frequency of positive ELISA tests in children without worms was 51.7%, similar to the overall frequency of positive test results, we believe that the high frequency of positive serology observed in this study is not due to cross-reactivity. Moreover, no differences were observed in the frequency of positive results between the individuals with and without intestinal worms or with and without *A. lumbricoides* (**Table 3**). This observation confirms that although cross-reactivity exists, the frequency of positive serology in children without intestinal worms is high. Although only one fecal sample per child was examined, our conclusion is strengthened by the observation of an increased percentage of positive antibody tests when we considered both children with parasites and children with a past history of worm infection. The cross-reactivity may be responsible for the frequent detection of ELISA-positive samples with high OD in asymptomatic children.

The high prevalence of anti-*Toxocara* antibodies in seven-year-old children is consistent with other studies performed in a children's hospital in *Vitória*. These studies reported prevalences from 30 to 39% of admitted children with positive antibody tests for *Toxocara*[11,18], and 3.2% of livers obtained from routine autopsies had granulomas containing *Toxocara* antigens at the same hospital[24].

In contrast to other reports[7,11,12,16,21], we did not observe a significant difference for gender regarding the prevalence of positive tests for anti-*Toxocara* antibodies. These results are consistent with other studies showing no association between gender and risk of *Toxocara* infection[22,27-29]. However, positive correlations were observed between positive ELISA tests for *Toxocara* and low family income, the presence of dogs at home and onycophagia. These results are consistent with the results reported by other authors using samples from different Brazilian cities[7,13-15]. A multivariate analysis demonstrated that onycophagia and the use of unfiltered water were associated with positive antibody tests for *Toxocara*. An independent association between onycophagia and the presence of anti-*Toxocara* antibodies was also reported for children in *São Paulo*[7]. An association between the presence of anti-*Toxocara* antibodies and the use of unfiltered water has rarely been investigated. However, an investigation in the Amazonia did not reveal a correlation between positive serology for *Toxocara* and drinking unfiltered water[22]. Some authors have suggested the hypothesis that water contaminated with *Toxocara* eggs may have a role in *Toxocara* infection in humans[30]. It is possible that eggs transmitted by neighborhood dust from neighborhoods may contaminate water within the home. In accordance with this possibility, the presence of dog stool in areas near the dwellings was frequently observed during the visits to the children's homes.

Eosinophilia was observed in the samples studied and was more frequent in patientswith positive antibody test results for *Toxocara*. However, when we evaluated eosinophil counts in children without other causes of eosinophilia, such as intestinal worms, allergy or asthma, this difference disappeared. In accordance with this observation, there was no significant association between positive anti-helminth antibodies and eosinophil counts in children bearing intestinal worms or allergy. Thus, the results presented in this study demonstrate that the presence of anti-*Toxocara* antibodies in asymptomatic children is not an isolated factor that induces eosinophilia; the increased eosinophil counts in these cases are associated with the presence of other worms and or allergies.

We did not find a correlation between positive antibody tests for *Toxocara* and geophagy, as reported in other studies[3,5,31-34].

Although controversial, an association between allergic disorders and *Toxocara* infection has been reported[7,34-37]. In our sample, no significant association was found between the presence of positive serology for *Toxocara* and a past history of asthma or other allergic manifestations.

Clinical examination of all children did not demonstrate signs or symptoms that could be correlated with *Toxocara* infection. The absence of clinical manifestations in children with anti-*Toxocara* antibodies is consistent with the observation that the great majority of children that acquire *Toxocara* larvae develop silent infections with only rare manifestations of visceral or ocular larva migrans[38,39].

In conclusion, the results reported here revealed high prevalence of anti-*Toxocara* antibodies in seven-year-old children living in the urban periphery of *Vitória* as detected by the ELISA using *Toxocara canis* secretory/excretory antigens, and this finding suggests that the rate of infection with this nematode in this population is high. Cross-reactivity with other helminth infections may be partially responsible for the observed results, but the prevalence of positive antibody tests was high in children without intestinal worms or a past history of worm infection. Clinical manifestations related to *Toxocara* infection were not observed. The high prevalence of *Toxocara* antibodies reported here may be associated with past infections with small number of larvae or with memory responses after recurrent infections resulting in low larval load.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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