

FREQUENCY AND RISK FACTORS FOR TOXOCARIASIS IN CHILDREN FROM A PEDIATRIC OUTPATIENT CENTER IN SOUTHEASTERN BRAZIL

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SUMMARY

To estimate the frequency of anti-*Toxocara* sp. antibodies, and evaluate factors associated with this infection, sera from 242 male and female children, aged from one to fifteen years old, attended at the Hospital of the Federal University of Uberlândia, Minas Gerais State, Brazil, were analyzed by ELISA. Information on the patients was collected and registered using an investigative questionnaire, and details on possible clinical alterations were obtained from the medical charts of 187 patients. Of a total of 242 samples, 21 (8.7%) were positive for anti-*Toxocara* sp. antibodies. The presence of dogs and cats and the school variable (place of contact), appeared to be significantly associated ($p < 0.05$) with a positive serology. Respiratory symptoms and eosinophil counts greater than 20% also showed a positive statistical correlation with a positive serology for *Toxocara* sp.. Factors such as sex and age, and symptoms like headache, stomach ache, convulsive crises and anemia were not associated with toxocariasis.

KEYWORDS: *Toxocara canis*; Toxocariasis; Risk factors.

INTRODUCTION

BEAVER *et al.*⁷ defined Visceral Larva Migrans syndrome (VLM) as a prolonged migration of helminth larvae within the organism of unusual hosts. *Toxocara canis* and *T. cati* are common ascarid parasites of dogs and cats, and are considered to be the principal etiological agents of the syndrome⁴. Humans become infected after the ingestion of either embryonated ascarid eggs or of tissues from infected paratenic hosts²⁴.

Human infection by *Toxocara* sp. is distributed worldwide and appears in variable frequencies, depending on local factors such as close contact with soil contaminated by dog feces, and low income levels of the exposed community^{1,33}. The rate of infection and occurrence of symptoms are more frequent in young children up to the age of 12 years old, mainly in rural areas; however, adults, are also infected^{4,17}. The most common signs and symptoms of infection include fever, respiratory complaints and hepatomegaly. However, in most cases, there are no clinical manifestations, and the disease develops in an asymptomatic manner³⁶. With regard to the clinical spectrum of toxocariasis, the principal finding from the last 25 years is that the disease can exhibit a distinct clinical picture^{25,39}, known as covert toxocariasis. The current generally accepted classification of human toxocariasis includes covert toxocariasis, ocular toxocariasis and neurological toxocariasis.

Various studies have investigated human infection by *Toxocara* sp. in Brazil, usually reporting high frequencies^{1,4,11,12,33}.

The laboratory diagnosis of human *Toxocara* infection relies mainly on a *T. canis* larval excretory-secretory (TcES) antigen based enzyme-linked immunosorbent assay (ELISA), which is said to show 78% sensitivity and 92% specificity²³.

The present study aims to establish the frequency of anti-*Toxocara* antibodies in children attended at the pediatric center of the Hospital of the Universidade Federal de Uberlândia, Minas Gerais State, Brazil. In addition, we attempt to identify certain risk factors associated with the infection.

MATERIAL AND METHODS

The pediatric center of the Hospital of the Universidade Federal de Uberlândia (HC-UFU) is a children's reference center for the Uberlândia municipal district in Minas Gerais State, Brazil, and attends almost all pediatric cases related to public health.

Blood was collected from 242 children, male and female, aged between one and fifteen years old, randomly selected and attended at the pediatric center of HC-UFU, during the period between February and September, 2004. Children infected with different diseases, without

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specific toxocariasis manifestations, were included in the study. A semi-structured questionnaire was given to the study participants to register patient history (sex, age, and origin) environmental and behavioral data (geophagy, contact with sand, contact site and presence of dogs and cats). Information on possible specific clinical signs (respiratory complications, headache, stomach ache, convulsive crises, anemia, eosinophilia) was obtained from the patients' medical charts. Only 187 of the 242 patients completed the questionnaire.

A total IgG anti-*Toxocara* sp. antibody test was performed using the ELISA immuno-enzymatic method, employing secretory-excretory antigens from second stage larvae (L₂) cultivated *in vitro* in accordance with the technique of de SAVIGNY¹⁴. All sera were absorbed with total *Ascaris suum* antigenic extract to avoid cross-reaction between the *Toxocara* sp. antigens and *Ascaris* sp. antibodies^{6,14,15}. A goat anti-human IgG (Gamma-Chain) specific peroxidase conjugate was used. Absorbance was read at 492 nm to establish optical density; sera with titers higher than or approximating 1:160 were considered positive¹¹.

Epi Info 6.04 software was used for statistical analysis. A descriptive univariate analysis was performed to evaluate the frequency of the variables (risk factors) and anti-*Toxocara* sp. antibodies, estimated using an Odds ratio. The relative proportions were calculated with a confidence interval of 95%. Possible associations were identified using the Chi-squared and Fisher's exact statistical tests at a significance level of 5%.

The present protocol was evaluated and approved with no ethical restrictions by the Ethics Committee of the Universidade Federal de Uberlândia (n°107/2002 - CEP/UFU-07/02/2003).

RESULTS

Of the 242 children's serum samples, 21 (8.7%) were positive for anti-*Toxocara* antibodies. Of these, 14 were from males (66.7%) and 7

from (33.3%) females, whose average age was 5.8 years, with a standard deviation of 3.04 years. Of the positive patients investigated, 19 (90.5%) lived in urban areas of Uberlândia, and 2 (9.8%) were from nearby towns. Sex, age, origin and origin site variables showed no significant relationship ($p \geq 0.05$) with positive serology for anti-*Toxocara* sp. antibodies.

The presence of animals and the place of contact with sand showed a significant association ($p \leq 0.05$) with positive anti-*Toxocara* sp. antibodies in the sera analyzed. The presence of dogs or cats was not significantly associated with seropositivity. However when both dogs and cats were present at the residence there was a 3.57-fold greater risk of a positive serology for toxocariasis compared to residents without these animals. This association was statistically significant ($p \leq 0.05$). The school variable (place of contact) was significantly associated with ($p \leq 0.05$) the presence of anti-*Toxocara* sp. antibodies. There was no statistical association among seropositivity, sand contact and geophagy ($p \geq 0.05$) (Table 1).

Analysis of the clinical variables showed that breathing alterations correlated significantly ($p \leq 0.05$) with a positive serology for toxocariasis. Stomach ache, headache, convulsive crises, anemia and eosinophilia were not statistically associated ($p \geq 0.05$) with the presence of anti-*Toxocara* sp. antibodies in the sera investigated.

The number of eosinophils was counted in 144 of 187 patients (77%), and showed a statistically significant association with seropositivity to *Toxocara* sp. antibodies ($p \geq 0.05$) (Table 2).

DISCUSSION

A frequency of 8.7% for anti-*Toxocara* sp. antibodies suggests that the children in the study population were in contact with the parasite, and developed an immune response with the production of specific antibodies. There are no reports on serological surveys for anti-*Toxocara*

Table 1

Distribution of anti-*Toxocara* sp. antibody frequencies (%) and Odds Ratios according to environmental and behavioral variables in children attended at a pediatric center of the Hospital of the Federal University of Uberlândia, Brazil

Variable		Sample (n)		Anti- <i>Toxocara</i> sp. antibodies (%)	OR	CI	p
		Absolute frequency	Relative frequency (%)				
Presence of animal	Dog	128.0	52.9	7.0	1.07	(0.33-3.8)	0.89
	Cat	6.0	2.5	33.3	7.08	(0.52-60.6)	0.07
	Dog/Cat	17.0	7.0	23.5	3.57	(1.13-11.32)	0.04*
	None	91.0	37.6	6.6	1.0		
Contact with sand	No	136.0	56.2	5.9	1.0		
	Yes	106.0	43.8	12.3	2.23	(0.89-5.61)	0.08
Sand contact site	House	85.0	35.1	9.4	1.65	(0.54-5.08)	0.33
	School	12.0	5.0	33.3	7.94	(1.40-37.7)	0.009*
	House/School	10.0	4.1	10.0	1.76	(0.04-15.92)	0.48
	None	135.0	55.8	5.9	1.0		
Geophagy	No	213	88	7.5	1.0		
	Yes	29	12	17.2	2.56	(0.67-8.1)	0.08

OD - Odds Ratio; CI - Confidence Interval; * - Significant values $p \leq 0.05$

Table 2

Distribution of clinical variables with regard to positive serology for toxocariasis in accordance with the analysis of 187 medical charts for children attended at the Hospital of the Federal University of Uberlândia, Brazil

Variables		Anti- <i>Toxocara</i> sp. antibodies				Total	p	
		Positive		Negative				
		No.	%	No.	%	No.	%	
Breathing problems	Yes	6.0	15.8	32.0	84.2	38.0	100	0.01*
	No	7.0	4.7	142.0	95.3	149.0	100	
Stomach ache	Yes	2.0	10.5	17.0	89.5	19.0	100	0.62
	No	11.0	6.5	157.0	93.5	168.0	100	
Headache	Yes	1.0	10.0	9.0	90.0	10.0	100	0.9
	No	12.0	6.9	161.0	93.1	173.0	100	
Anemia	Yes	1.0	10.0	9.0	90.0	10.0	100	0.52
	No	12.0	6.7	165.0	93.3	177.0	100	
Epilepsy	Yes	2.0	16.6	10.0	83.4	12.0	100	0.19
	No	11.0	6.3	164.0	93.7	175.0	100	
Eosinophilia	Yes	11.0	7.6	133.0	92.4	144.0	100	0.73
	No	2.0	4.6	41.0	95.4	43.0	100	
Eosinophilia	> 20%	5.0	55.5	4.0	44.4	9.0	100	0.0001*
	≤ 20%	6.0	4.4	129.0	95.6	135.0	100	

* - Significant values; $p \leq 0.05$

sp. antibodies in Uberlândia, Minas Gerais, and the known studies concern the prevalence of eggs and larvae in public parks¹³.

The lower seropositivity for anti-*Toxocara* sp. antibodies found in this study differs from other reports in Brazil^{2,4,31,33} and in other countries^{21,32}. Interpretation of seroprevalence data is still problematic owing to different cut-off titers used by the various investigators and difficulty in evaluating the relationship between antibody titers, infection and clinical findings for the disease. Prevalence data based on hospitalized patients' serum, blood donors and high risk subgroups tend to introduce bias and do not provide accurate population data¹.

Observations from this study suggest the lack of a significant statistical association between sex, age or origin of the child and anti-*Toxocara* antibodies. Some areas with a high prevalence of human *Toxocara* infection do not show significant differences according to age². In the present study, the mean age of the seropositive children was 5.8 years old (standard deviation of 3.8 years). Most serological surveys have shown a predominance of seropositivity in children aged between two and 15 years old, considered to be the classic age group for susceptibility to toxocariasis^{1,3}. BUNDY *et al.*⁹ suggested that the prevalence rates of infection may provide some indication of an age-infection relationship, and that the rates found for the under five years old age group probably constitute a true reflection of infection acquisition rate, since this population is not infected at birth. MAGNAVAL *et al.*²⁹ and THOMPSON *et al.*⁴⁰, studying two tropical areas of intense transmission, demonstrated a progressive increase in seroprevalence up to 25-30 years of age, and occasionally over this limit, suggesting that toxocariasis may no longer be considered an exclusively pediatric zoonosis. Gender does not seem to be an important factor regarding *T. canis* infection among healthy children in this study, although a higher frequency was observed in males. Several studies have shown a higher frequency of *Toxocara* infection in male

individuals, which may derive from differences in the play and social behaviors of boys, resulting in increased exposure to the eggs of *Toxocara* sp.^{24,35}. However, EHRHARD & KERNHAUM¹⁶ noted a predominance of women among adult patients, and FENOY *et al.*¹⁹ and LJUNGSTROME & KNAPEN²⁷ observed a greater frequency in rural communities. The high prevalence for *Toxocara* serology may result from various factors, the most important of which concerns living conditions associated with urban infrastructure. Individuals residing in houses whose walls directly delimit the garden, in addition to those who filter their drinking water, had significantly lower frequencies of *Toxocara* infection⁵.

Some environmental and behavioral variables were significantly associated with the positive anti-*Toxocara* antibody responses. Regarding the presence of animals, there is controversy as to the importance of contact with dogs as a risk factor for human toxocariasis. CHIEFFI *et al.*¹⁰ and SCHANTZ *et al.*³⁷, reported a higher frequency of infection in individuals who were in contact with dogs, however, other studies found no association between ownership or professional contact with dogs and *Toxocara* infection^{22,26}. FAN *et al.*¹⁸ observed high seropositive rates in 79.4% of dog owners, and 67.9% in non-owners, suggesting that these two groups are equally at risk of infection. These results are in line with those of WOODRUFF *et al.*⁴² who observed that 50% of patients with clinical toxocariasis owned dogs. In the current study, the presence of cats tended to increase the risk of infection, although not statistically significant, probably owing to the small sample size. However, the presence of both cats and dogs together significantly increased risk; since the data do not show an increased risk among those who own dogs alone, presumably the contact with cats influenced the results. MUNDIM *et al.*³⁴ report that in addition to *T. cati*, cats may also be infected with *T. canis*, and although *T. canis* accounts for the greater part of larva migrans in human beings, *T. cati* is apparently responsible for ocular and visceral changes²⁰.

In this study, geophagy and contact with soil did not contribute to the risk of infection by toxocaríasis, contradicting findings by AJAYI *et al.*² showing highly significant association between seropositivity and geophagy. Contact with soil tended to be associated with risk of infection by the parasite; however, the association was not statistically significant (Odds ratio = 2.23/CI = 0.89 - 5.61).

The school variable is a risk factor for children playing in such areas. WORLEY *et al.*⁴³ showed that positive titers for anti-*Toxocara* sp. antibodies are not related to the fact that children attend school, but rather to a group of factors linked to the hygienic and behavioral habits of the children. The school may offer a greater risk of infection owing to the presence of small areas covered with sand where the children play, which may be contaminated.

We found a statistical correlation between the presence of anti-*Toxocara* sp. antibodies and breathing difficulties. This positive correlation also has been mentioned by SNYDER³⁸ who considered it to be a toxocaríasis specific disorder. MACHADO & ACHKAR²⁸, emphasized that toxocaríasis is associated with multiple clinical characteristics and may be confused with other diseases.

Hematological alterations have been described in clinical cases of toxocaríasis^{30,41} and BEAVER *et al.*⁷ reported a close relationship between eosinophilia and visceral larva migrans. In the present study, anemia or eosinophilia were not statistically correlated with the presence of anti-*Toxocara* sp. antibodies, which agrees with findings by ANARUMA-FILHO *et al.*⁵. However, considering those children with high eosinophil counts, eosinophilia 20% higher than the statistically significant limit may be associated with *Toxocara* sp. infection (Table 2).

The current study confirms the presence of anti-*Toxocara* sp. antibodies in children from the municipality of Uberlândia, Minas Gerais, a situation that represents a considerable health problem for this population, particularly children. Studies employing a large sample size are necessary to evaluate the real situation, and thus to implement measures aiming to reduce infection risk factors.

RESUMO

Freqüência e fatores de risco associados à toxocaríase em crianças de ambulatório pediátrico na região sudeste do Brasil

Para determinar a freqüência de anticorpos anti-*Toxocara* sp. e os fatores de risco associados à infecção, soros de 242 crianças de ambos os sexos, com idade entre um a 15 anos, atendidas no ambulatório do Hospital da Universidade Federal de Uberlândia, Minas Gerais, Brasil, foram analisados pelo ELISA. Questionários foram aplicados, para obter informações sobre os pacientes. As informações sobre as alterações clínicas, de 187 pacientes, foram obtidas no prontuário médico. Do total das 242 amostras, 21 (8,7%) foram positivas para anticorpos anti-*Toxocara* sp.. A presença de cães e gatos e a variável escola (local de contato) sugerem associação significativa ($p < 0.05$) com a sorologia positiva. Sintomas respiratórios e elevada contagem de eosinófilos ($> 20\%$) mostram correlação positiva com a soropositividade para *Toxocara* sp.. A idade, sexo, sintomas como dor de cabeça, dor estomacal, crises convulsivas e anemia não apresentaram associação com toxocaríase.

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Received: 12 April 2006

Accepted: 17 May 2006