

HUMAN TOXOCARIASIS: FREQUENCY OF ANTI-*Toxocara* ANTIBODIES IN CHILDREN AND ADOLESCENTS FROM AN OUTPATIENT CLINIC FOR LYMPHATIC FILARIASIS IN RECIFE, NORTHEAST BRAZIL

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SUMMARY

In a transversal study on a sample of 386 children and adolescents from an outpatient clinic for filariasis in Recife, Northeast Brazil, the frequency of anti-*Toxocara* antibodies and its relation to age, gender, number of peripheral eosinophils, *Wuchereria bancrofti* microfilariae and intestinal helminths was determined. The total anti-*Toxocara* IgG antibody frequency was 39.4%, by ELISA technique. The difference in frequency between males (40.1%) and females (37.6%) was not statistically significant. The 6 to 10-year-old subset presented the highest frequency of anti-*Toxocara* antibodies (60%), and within this age group there was a statistically significant male bias. There was also a significant association between the number of eosinophils and the presence of anti-*Toxocara* antibodies. Intestinal parasite frequency was 52.1%, but no association was found between this data and the presence of anti-*Toxocara* antibodies. In the present sample, 42.2% of the patients were *Wuchereria bancrofti* carriers, however, again this was not associated with the presence of anti-*Toxocara* antibodies. In conclusion, anti-*Toxocara* antibodies were highly prevalent in this sample. The present data show that there is no cross correlation between anti-*Toxocara* IgG antibody and the presence of intestinal helminths and filariasis.

KEYWORDS: Toxocariasis; Visceral larva migrans; Anti-*Toxocara* antibodies; *Toxocara canis*; Eosinophilia; *Wuchereria bancrofti*.

INTRODUCTION

BEAVER *et al.* (1952) described Visceral Larva Migrans syndrome (VLMS) as a prolonged migration of helminth larvae in the organism of unusual hosts. *Toxocara canis* is the agent most commonly implicated in the etiology of this illness among human populations³. Infection originates from soil contaminated with parasite eggs eliminated in the feces of common host species, such as dogs and cats. In humans, the parasite completes a somatic-pulmonary biological cycle, which may manifest itself in one of three clinical forms¹³: VLMS (originating from the larval invasion of human tissues); ocular toxocariasis (specific signs and symptoms at the ocular globe); and asymptomatic toxocariasis.

Toxocariasis diagnosis used to be based on clinical data or the occasional finding of larvae within tissues. An important improvement occurred with the development of Enzyme Linked Immunosorbent Assay (ELISA) using *Toxocara canis* excretion-secretion antigens that allowed broader epidemiological studies to be carried out. It has been found that the parasitic infection prevalence varies widely in the globe and even within a single country, due to its epidemiological characteristics^{4,14,18,21,24,25,31}.

In many Brazilian regions, particularly in the Northeast countryside,

one should expect to find a higher prevalence than those presently reported in the U.S. or Europe. Despite the favorable epidemiological conditions for mass occurrence of human infection, such as the large number of *T. canis* infected street dogs²⁰, there is a surprisingly low frequency of VLMS cases diagnosed in the Brazilian Northeast. However, few epidemiological studies have been carried out on this subject, thus preventing an accurate estimation of the prevalence of toxocariasis for the region. Consequently, these conditions may signify one of the largest unrecognized parasitic infections in Recife and in view of the potential morbidity further investigation is warranted.

The present study was done in order to determine the frequency of anti-*Toxocara* antibodies in the serum of children and adolescents exposed to other endemic parasitosis in Recife, as well as to verify the possible role of these infections on the anti-*Toxocara* serology.

MATERIAL AND METHODS

A transversal study was carried out on children and adolescents attended at Centro de Pesquisas Aggeu Magalhães (CPqAM/Fiocruz) outpatient clinic, a national reference center for lymphatic filariasis, in Recife city (Brazilian Northeast) from 1987 to 1996.

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The sample studied consisted of 500 individuals, all less than 19 years old. Taking into account an 11%^{5,6} expected prevalence and 95% confidence interval, a minimum of 326 individuals was estimated. In fact, a total of 386 patients were selected and examined, of which 269 were male (69.7%) and 117 (30.3%) were female. Individuals without serological samples available were excluded.

The following routine laboratory exams were performed: sera anti-*Toxocara* antibodies; eosinophils absolute count and tests for intestinal parasites and *Wuchereria bancrofti* microfilariae in the blood sample.

Eosinophils absolute count was obtained by direct inspection in a Neubauer chamber²⁷, with a reference value⁷ of 500 eosinophils/mm³. For larvae, eggs and cysts study of the intestinal parasites, the Lutz method as well as the Baerman-Moraes method were applied, using one or more stool samples for diagnosis. Protozoan species were not identified.

Membrane filtration technique was used in filariasis study, as described by DENNIS & KEAN¹⁰. Venous blood samples were collected between 11:00 p.m. and 1:00 a.m., according to the circadian periodicity of microfilariae¹¹.

Total IgG anti-*Toxocara* antibodies study was carried out by ELISA immuno-enzymatic method, using secretory and excretory antigens from second stage larvae (L₂) cultivated *in vitro*^{8,9}. All sera were adsorbed with total *Ascaris suum* antigenic extract to avoid cross reactions between *Toxocara* antigens and *Ascaris* antibodies^{1,22}. The cutoff density point in serological assays varied from 0.520 to 0.720 and was determined for every test using the mean of the negative controls plus two standard deviations (2 SD).

Epi Info 6.04 software was used for statistical analysis. A descriptive univariate analysis was carried out to verify the frequency of the variables and the seroprevalence of anti-*Toxocara* antibodies in the total sample, pooled according to gender and age. The relative proportions were calculated with a confidence interval of 95%. To identify possible associations between dependent variable (seropositivity for *Toxocara*) and independent variables Pearson chi-square test with Yates correction was used. The significance level was set at 5%.

The protocol design as well as the use of biological fluid samples stocked at our Institution had the approval of the local ethical committee.

RESULTS

General characterization of population study: Eosinophil count was carried out on 303 (78.5%) of the 386 individuals enrolled. Of these, 67.7% were eosinophilic (values above 500 eosinophils/mm³), whereas 35.9% presented hypereosinophilia, i.e. values above 1000 eosinophils/mm³.

Intestinal helminths were present in 52.1% of individuals that underwent coprological examination. Table 1 describes the intestinal parasites found and summarizes the frequency, as well as other characteristics of the sample population.

Wuchereria bancrofti microfilariae (mf) study was carried out on

Table 1

Distribution according to age, gender, number of peripheral eosinophils, presence of intestinal parasites and *Wuchereria bancrofti* microfilariae in children and adolescents from an outpatient clinic in Recife, Northeast Brazil

Variables	N	%	CI 95%
Age (years)	386	100	
≤ 5	28	7.2	4.9-10.3
6 - 10	45	11.7	8.6-15.3
11 - 15	110	28.5	24.0-33.3
16 - 18	203	52.6	47.5-57.7
Sex	386	100	
Male	269	69.7	64.8-74.2
Female	117	30.3	25.8-35.2
Eosinophils (mm ³) ¹	303	100	
≤ 500	98	32.3	27.1-37.9
501 - 1000	96	31.7	26.5-37.2
1001 - 3000	78	25.7	20.9-31.0
> 3000	31	10.2	7.1-14.2
<i>Wuchereria bancrofti</i> ²	353	100	
Positive	149	42.2	37.0-47.5
Negative	204	57.8	52.4-63.0
Intestinal parasites ³	382	100	
Total negative	183	47.9	42.8-53.0
Total positive ⁴	199	52.1	46.9-57.2
<i>Strongyloides stercoralis</i>	30	7.9	5.4-11.0
<i>Ascaris lumbricoides</i>	102	26.7	22.3-31.4
Ancylostomatidae	50	13.1	9.9-16.9
<i>Trichuris trichiura</i>	105	27.5	23.1-32.3
<i>Schistosoma mansoni</i>	13	3.4	1.8-5.7
<i>Enterobius vermicularis</i>	4	1.0	0.3-2.7
<i>Taenia sp.</i>	4	1.0	0.3-2.7
Protozoa	78	20.4	16.5-24.8

¹ Lack of information from 83 individuals (21.5%); ² Lack of information from 33 individuals (8.5%); ³ Lack of information from 04 individuals (1.0%); ⁴ Some individuals were polyparasitic.

353 individuals (91.4% of overall sample), and 42.2% were diagnosed as carriers. The mean density was 410 mf/ml (range 1 to 12.650 mf/ml).

Frequency of anti-Toxocara antibodies in relation to gender, age, the presence of intestinal parasites or W. bancrofti microfilariae: Anti-*Toxocara* antibodies frequency was 39.4% (152/386). The antibody frequency among male individuals was 40.1% (108/269) while in females it was 37.6% (44/117). This difference was not statistically significant (Table 2). However, when a stratified analysis by age group was carried out, a 88.9% antibodies frequency was found in males aged 6 to 10, compared to 40.7% in age paired females. Thus, there was a statistically significant greater risk (2.1 times) of being seropositive for males in this age group.

The highest seropositivity frequency was found in the 6 to 10-year-old subgroup, representing 60% (27/45) of positive individuals. This was followed by individuals aged 11 to 15 years; less than or equal to 5 years; and 16 to 18 years with 46.4% (51/110), 32.1% (9/28) and 32% (65/203) seropositivity, respectively. All of these subgroups presented statistically significant differences (Table 2).

Table 2

Distribution of serology for *Toxocara* according to gender, age, *Wuchereria bancrofti* microfilariae, number of peripheral eosinophils, and intestinal parasitic infection in children and adolescents from an out-patient clinic in Recife, Northeast Brazil

Variables	Serology for <i>Toxocara</i>						Statistics	
	Positive		Negative		Total		CI 95%	
	N	%	N	%	N	%		
Sex								
Male	108	40.1	161	59.9	269	100.0	64.8-74.2	$\chi^2 = 0.13$ (Y) p = 0.72
Female	44	37.6	73	62.4	117	100.0	25.8-35.2	
Total	152	39.4	234	60.6	386	100.0		
Age group								
≤ 5 years	9	32.1	19	67.9	28	100.0	4.9-10.3	$\chi^2 = 15.48$ (Y) p = 0.001
6 – 10 years	27	60.0	18	40.0	45	100.0	8.6-15.3	
11 – 15 years	51	46.4	59	53.6	110	100.0	24.0-33.3	
16 – 18 years	65	32.0	138	68.0	203	100.0	47.5-57.7	
Total	152	39.4	234	60.6	386	100.0		
<i>Wuchereria bancrofti</i>								
Positive	67	45.0	82	55.0	149	100.0	37.0-47.6	$\chi^2 = 2.08$ (Y) p = 0.15
Negative	75	36.8	129	63.2	204	100.0	52.5-63.0	
Total	142	40.2	211	59.8	353	100.0		
Number of eosinophils								
≤ 500	14	14.3	84	85.7	98	100.0	27.1-37.9	$\chi^2 = 41.99$ (Y) p < 0.001
501-1000	40	41.7	56	58.3	96	100.0	26.5-37.2	
1001-3000	44	56.4	34	43.6	78	100.0	20.9-31.0	
> 3000	19	61.3	12	38.7	31	100.0	7.1-14.2	
Total	117	38.6	186	61.4	303	100.0		
Parasite infection								
Positive	77	38.7	122	61.3	199	100.0	47.0-57.2	$\chi^2 = 0.00$ (Y) p = 0.98
Negative	72	39.3	111	60.7	183	100.0	42.8-53.0	
Total	149	39.0	233	61.0	382	100.0		

Considering only those individuals with positive serology that also underwent studies for *Wuchereria bancrofti*, 52.8% (75/142) were not carriers while 47.2% (67/142) were carriers. This difference was not statistically significant.

Around 51.7% of seropositive children and adolescents presented some type of intestinal parasite whereas no parasite was detected in 48.3%. This difference was not statistically significant.

In relation to seropositive individuals with an eosinophil count, 12% (14/117) presented normal eosinophil values, whereas 53.8% (63/117) had hypereosinophilia. Individuals with higher eosinophil counts (> 3000 cells/mm³) also presented a greater frequency of anti-*Toxocara* antibodies (61.3%) (Fig. 1). Only 35 individuals had hypereosinophilia without associated intestinal parasitosis or filariasis and among these, 16 presented anti-*Toxocara* antibodies.

The relation between the number of eosinophils and seropositivity frequencies was statistically significant, with a 4.29 times greater seropositivity risk in individuals with values above 3000 eosinophils/mm³ when compared to those with less than or equal to 500 eosinophils/mm³ (Fig. 1).

DISCUSSION

We are aware that the anti-*Toxocara* antibodies frequency in the

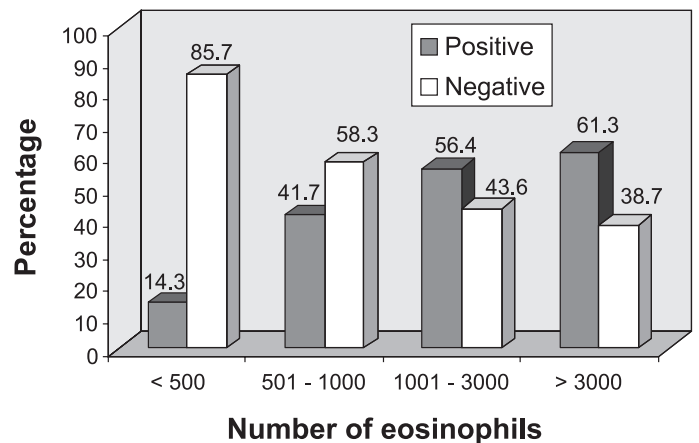


Fig. 1 – Frequency of positive and negative serology for *Toxocara* in children and adolescents at an outpatient clinic in Recife, Northeast Brazil, according to the number of eosinophils

current study may not reflect its real prevalence in the population of the Recife metropolitan region. Nevertheless, the present data are relevant as can figure out the *Toxocara* prevalence in a population sample exposed to other intestinal parasitosis, especially *Wuchereria bancrofti*.

Our Institution’s outpatient clinic attends a large number of individuals with peripheral eosinophilia. This eventual laboratory finding

is the major reason for referral to our ambulatory and leads to a higher overall hypereosinophilic patient profile in relation to the general population. Since the presence of peripheral eosinophilia is one of the laboratorial criteria considered for a presumed *Toxocara* infection, it should be stated that the present sample population had a greater chance of presenting anti-*Toxocara* antibodies.

The high frequency of anti-*Toxocara* antibodies found in this study was similar to reports in Brazil^{23,26,33} and other countries^{17,21} but nevertheless higher than that described by some authors^{4,14,24,25}. However, the comparison between the present results and these reports is difficult due to several factors, such as: seroepidemiological studies based on a small number of children; heterogeneity of individuals studied (i.e. blood donors, hospitalized patients or high risk subgroups for toxocariasis); non-standardized laboratory procedures; and different cutoff points in the various serodiagnostic techniques.

Intestinal parasite infection was detected in a high percentage of individuals, resulting in a significant frequency of geohelminths between the detected parasites. They are well known agents for the onset of peripheral eosinophilia²⁸. The high frequency of intestinal helminths observed in the present study suggests that other risk factors described for *Toxocara* infection^{14,21,31}, such as poor hygiene habits and inadequate sanitation, must have contributed^{14,21,31}.

The frequency analysis of anti-*Toxocara* antibodies in a population survey from a tropical area with serious sanitary and socioeconomic deficiency is further complicated by potential cross reactivity between a large variety of other parasites to which a given individual may have been exposed. This could lead to false-positive results²¹.

Recife city is considered to be an endemic area of bancroftian filariasis³⁴. Thus, the microfilariae examination of serum samples could be important to determine whether a cross reactivity of *Toxocara* with other nematodes is present in individuals with positive serology. However, present data discarded a correlation between the presence of *W. bancrofti* microfilariae and anti-*Toxocara* antibodies.

No significant difference was observed between the frequency of intestinal parasites and anti-*Toxocara* antibodies. Thus, one should be aware of the possibility that these infections overlap in the population.

We found a significant gender difference in antibody frequency only in 6 to 10-year-old subgroup, with a male bias. Our data corroborates other reports that indicate greater frequency of *Toxocara* infection among males in certain age groups^{9,12,16,19,29}. Taking into account the Brazilian culture, this difference may be due to more restricted outdoor activities for female children.

The peak antibody frequency found in the present study (6 to 10-year-old subgroup) was similar to that described by CHIEFFI *et al.* in a population study in Santos city, São Paulo State, in southeastern Brazil⁶. They reported a 13.1% seroprevalence in individuals less than 15 years of age, but the prevalence among those in the 5 to 10-year-old group reached 19.0%.

A high frequency of anti-*Toxocara* antibodies among pediatric patients seems to be unquestionable^{9,16,35}, but there has been a growing

number of reports indicating the presence of infection in adults^{14,30}. This is especially relevant to regions considered to be endemic areas for toxocariasis (even for adults), such as the southeast of France¹⁵. Therefore, while 2 to 5-year-old children have always been considered the classic age group for susceptibility to VLMS, one should not make the error of considering that toxocariasis is an exclusively childhood illness.

BEAVER² reported a close relation between eosinophilia and visceral larva migrans. Prior to the development of ELISA technique, this criterion for eosinophilia (with mainly extreme values) was adopted as an important laboratorial indicator of toxocariasis. Even today, it is considered a classic criterion of visceral larva migrans. However, the absence of an elevated number of peripheral eosinophils does not exclude the possibility of toxocariasis³⁰.

The literature also indicates that eosinophilia in *Toxocara* seropositive individuals seems to be age related, as it is more common in children than in adults³².

Analyzing the present data, we were able to identify other probable causes for eosinophilia besides a possible *Toxocara* infection, such as the presence of intestinal parasites and filarial infection. The latter appears to be particularly probable in patients with the clinical form of Tropical Pulmonary Eosinophilia (TPE), with elevated values of peripheral eosinophils.

The initial objective of this work was to use anti-*Toxocara* antibody ELISA to assess the toxocariasis infection at an outpatient clinic in Recife, a recognized endemic area of filariasis and geohelminthiasis. Although the present data does not allow a more profound analysis of all risk factors for *Toxocara* infection, it strongly suggests that the Brazilian Northeast has a high human toxocariasis prevalence, which has so far been underestimated and, consequently, under-diagnosed. On the other hand, in the present population sample the presence of filariasis apparently does not alter the ELISA test with secretion and excretion L2 antigen, which strengthens the relevance of this nosology within the region.

RESUMO

Toxocaríase humana: frequência de anticorpos anti-*Toxocara* em crianças e adolescentes atendidos em um ambulatório especializado de filariose linfática em Recife, Nordeste do Brasil

Através de estudo do tipo transversal com amostra constituída por 386 crianças e adolescentes atendidos em um ambulatório especializado de filariose, do Recife, nordeste do Brasil, determinou-se a frequência de anticorpos anti-*Toxocara* e sua relação com faixa etária, sexo, número de eosinófilos periféricos, microfílarias de *Wuchereria bancrofti* e parasitos intestinais. A frequência encontrada de anticorpos IgG total anti-*Toxocara*, realizada através da técnica de ELISA, foi de 39,4%, com 40,1% no sexo masculino e 37,6% no feminino, diferença esta sem significância estatística. O grupo com maior frequência de anticorpos anti-*Toxocara* foi o de 6-10 anos (60%) e, apenas nessa faixa etária, encontrou-se uma diferença estatisticamente significativa quanto ao sexo, com predomínio do masculino. Observou-se associação estatisticamente significativa entre o número de eosinófilos e a presença de anticorpos anti-*Toxocara*. A frequência de parasitos intestinais foi de 52,1%, porém sem associação entre este achado e a presença de anticorpos anti-*Toxocara*. Na presente análise, 42,2% dos

pacientes eram portadores de microfilárias de *Wuchereria bancrofti*, porém esta infecção não esteve associada à presença de anticorpos anti-*Toxocara* o que sugere que não houve cruzamento do ELISA com a presença de parasitoses intestinais e filariose.

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