

## CLINICAL AND SEROLOGICAL EVIDENCE OF *Toxocara* INFECTION IN SCHOOL CHILDREN FROM MORROPE DISTRICT, LAMBAYEQUE, PERU

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### SUMMARY

The aim of this study was to estimate the frequency of human toxocariasis in a child population from Morrope district, Lambayeque, Peru. From October to December 2005, 182 school children (96 male and 86 female) were studied. Blood samples were collected for *Toxocara* ELISA-IgG test and hematological examination. Additionally, stool samples were collected for coproparasitological examination to check cross reactions. We found frequency of positives in 32.4% (59/182) with a significant higher proportion of positivity in male children ( $p < 0.00001$ ). 71.2% of the children with positive serology (52 male and seven female), were between five and 10 years old, 77.96% had respiratory symptoms, 61.02% had ocular manifestations, 38.98% had hepatic symptoms, 38.98% had mild or moderate eosinophilia, signs statistically associated with seropositivity. 83.5% of studied population had some intestinal parasite, such as: *Blastocystis hominis* (53.3%), *Giardia lamblia* (31.3%), *Entamoeba coli* (29.1%), *Entamoeba histolytica*/E. *dispar* (1.1%), *Hymenolepis nana* (5.49%), and *Ascaris lumbricoides* (3.3%), but they had not any association with serology results. The ownership of dogs or/and cats were significantly associated with seropositivity to anti-*Toxocara* antibodies although the presence of such pets within the house was not. In conclusion, clinical and serological evidence of *Toxocara* infection exists in the studied population.

**KEYWORDS:** Toxocariasis; Frequency; ELISA test; Children.

### INTRODUCTION

The human toxocariasis, a serious worldwide health problem, is an accidental parasitic zoonosis due to human infection by larval stages of *Toxocara canis* and *T. cati*, they are common ascarids from dogs and cats, respectively, and considered the principal etiological agents of this syndrome<sup>9,21</sup>. Humans are infected after ingesting embryonated eggs in contaminated soil or tissues of infected paratenic hosts. The infective larvae hatch in the small intestine and migrate through somatic organs, preferably liver and eyes, and led to two different classical forms: visceral larva migrans (VLM) and ocular larva migrans (OLM)<sup>25</sup>.

Frequently, human infection with *Toxocara* larvae shows no clinical manifestations and evolves in an asymptomatic way. However, in some cases it can cause severe alterations such as fever, hepatomegaly, splenomegaly, respiratory symptoms, muscular pain, and anorexia, accompanied, by high eosinophilia and high titers of anti-*Toxocara* antibodies.

In the epidemiology of this parasitosis, ingestion of contaminated soil with embryonated *Toxocara* eggs is a major risk factor, though other transmission routes must be considered. A strong association

between the presence of dogs and toxocariasis has been proposed, with diverse criteria regarding the real role of the contact with the dog in the transmission of this parasitosis<sup>16,31</sup>.

The method of choice for serodiagnosis is based on ELISA technique using *Toxocara* larvae excretion-secretion (TES) antigens<sup>24</sup>, which shows 78.3% to 100% of sensitivity and 90% to 92% of specificity<sup>14,15,18,24,25</sup>. With development of this technique, frequency studies also have been possible.

Toxocariasis prevalence is higher in tropical and developing countries and is generally associated with low socioeconomic level<sup>20,28</sup>, with rates ranging from 1% in Spain<sup>22</sup> to 86% in Santa Lucia<sup>29</sup>; of 3.6% to 24.7% in Brazil<sup>2,7</sup>; 47.5% in Colombia<sup>1</sup>; of 34.9% to 66.6% in Venezuela<sup>12,20</sup>, of 37.9% to 39% in Argentina<sup>3,23</sup>. In Peru, limited reports indicate frequency of 7.8% to 20% in rural population and of 40% in subjects with ocular suspicion<sup>10,11,19</sup>.

The goal of this study was to determine frequency of *Toxocara* infection in school children from Morrope district, province of Lambayeque, Lambayeque, Peru, and its association with clinical manifestations and risk factors. At the same time, we would like to

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determine if there could be any cross reaction between serology and presence of intestinal parasites, because they are very common in this population.

## MATERIAL AND METHODS

This study was carried out from October to December 2005, in a population of school children from Morrope district, province of Lambayeque, Lambayeque, Peru. Morrope district is located at Northwest from Chiclayo City, in Lambayeque. The district has a total population of 40,356 inhabitants and a population of approximately 10,607 scholars. The central area of district (area of study) has a total population of 10,706 inhabitants with 2,827 people in school age. Kinder garden, primary and secondary schools were studied. Total population of the three schools was 259 and 182 children were selected by random sampling.

An individual record was made and the following data were registered: anamnesis data, clinical signs and symptoms (pulmonary infiltrates, bronchospasms, bronchitis, hepato and splenomegaly, abdominal pain, leucocoria, strabismus, retinitis, visual loss, convulsions and other signs of central nervous system involvement, pruritus and chronic weakness); epidemiological data and risk factors (age, sex, ownership of dogs or/and cats, their presence within home, use of public playgrounds, history of pica or geophagia) and eosinophils count: normal eosinophils (< 6%), mild (6-10%), moderate (11-15%) and severe (> 15%) eosinophilia. The protocol design, individual record, as well as use of biological fluid samples stocked at our institution had the approval of local ethical committee.

For hematological and immunological tests, blood samples were collected in Vacutainer tubes with and without EDTA as anticoagulant, respectively. Serum was separated by centrifugation and stored at -20 °C until use. For coproparasitological examination, stool samples were analyzed in fresh or preserved in 10% saline-formalin solution and stored at room temperature.

Total anti-*Toxocara* IgG antibodies were detected by ELISA-IgG test, using TES antigens<sup>24</sup>. Previously, each serum sample was absorbed with total *Ascaris suum* adult extract to diminish cross reactions<sup>24</sup>. All sera were tested at 1:200 dilutions and all stages from enzyme

immunoassay were performed at room temperature. The cut-off was set by the mean optical density (OD) of the negative reference serum, plus three times standard deviations. Serum samples with OD > 0.29 (cut-off) were considered as positive. Additionally, all positive sera were serially two-fold diluted to determine antibody concentration by titration.

Hematological examination included total erythrocyte and leukocyte count, and leukocyte differential formula. Total erythrocyte and total leukocyte count was obtained by direct microscopic observation in a Neubauer chamber; leukocyte differential formula was obtained by stained-blood smear with Wright-Giemsa stain. Eosinophilia was defined as absolute eosinophils count more than 500 cells/ $\mu$ L, according to WILLIAMS *et al.*<sup>30</sup>

Coproparasitological examination was realized by direct microscopic observation in fecal smears and by rapid sedimentation technique to detect parasites that can generate cross-reactivity in the serological test.

A database was constructed in an Excel spreadsheet to record and analyze results and Chi-square test was carried out using the software Epi-Info 6.0. Statistical significance level was 5%.

## RESULTS

From 182 children, 59 were positive (32.4%) to the ELISA-IgG test. From children with positive serology, 57.62% (34/59) had titers of 200, 22.03% (13/59) had titers of 400, 16.95% (10/59) had titers of 800, and 3.39% (2/59) had titers of 1600. Association between sex and serological test show a significantly higher proportion of positivity in male children (*Chi-square* = 43.86; *p* < 0.00001).

Ownership of dogs or/and cats showed a significant association (*Chi-square* = 16.58; *p* < 0.00001) with positive anti-*Toxocara* antibodies in the sera analyzed. However, presence of pets within home was not significantly associated with seropositivity (*Chi-square* = 0.40; *p* = 0.5261). Use of public playgrounds by children showed a significant association with seropositivity to ELISA test (*Chi-square* = 7.11; *p* = 0.0076). The presence of a history of pica or geophagia also was statistically significant (*Chi-square* = 5.17; *p* = 0.0230) (Table 1).

**Table 1**  
Distribution of risk factors as related to serology for toxocarosis in school children from Morrope district, Peru, 2005

Risk factors		Anti- <i>Toxocara</i> antibodies						<i>p</i>
		Positive		Negative		Total		
		N	%	N	%	N	%	
Ownership of dogs/cats	Yes	58	39.19	90	60.81%	148	100	0.0000*
	No	1	2.94	33	97.05	34	100	
Presence of pets within the home	Yes	19	35.85	34	64.15	53	100	0.5261
	No	40	31	89	69	129	100	
Use of public playgrounds	Yes	56	36.36	98	63.63	154	100	0.0076*
	No	3	10.71	25	89.29	28	100	
History of pica or geophagia	Yes	13	21.31	48	78.69	61	100	0.0230*
	No	46	38.01	75	61.99	121	100	

\* -Significant values; *p* ≤ 0.05

Association between presence of symptoms and positive serology was statistically significant ( $Chi\text{-square} = 32.74; p = 0.0001$ ). The signs/symptoms more predominant in children with positive serology were respiratory signs (77.96%), ocular signs (61.02%), hepatic compromise (38.98%), abdominal pain (30.51%) and cutaneous signs (27.12%) (Table 2).

**Table 2**

Distribution of signs and symptoms as related to serology for toxocariasis in school children from Morrope district, Peru, 2005

Signs and symptoms*	ELISA (+) = 59	ELISA (-) = 123
Asymptomatic	1	97
Respiratory	46	22
Hepatic	23	3
Ocular	36	10
Neurological	1	0
Cutaneous	16	14
Abdominal pain	18	13

\*Some children had more than one sign/symptom.

Association between mild or moderate eosinophilia and positive serology was statistically significant ( $Chi\text{-square} = 27.59; p < 0.00001$ ). From 59 children with positive serology, 33.9% had mild eosinophilia; 5.08% had moderate eosinophilia, and 61% had normal eosinophilia. Only 7.32% of children with negative serology had mild eosinophilia.

Copro-parasitological analysis revealed a frequency of 83.5% (152/182) in this population: *Blastocystis hominis* (53.3%), *Giardia lamblia* (31.3%), *Entamoeba coli* (29.1%), *Entamoeba histolytica/E. dispar* (1.1%), *Hymenolepis nana* (5.49%), and *Ascaris lumbricoides* (3.3%). Nevertheless, the association between parasitosis and positive serology was not statistically significant ( $Chi\text{-square} = 1.35; p = 0.2447$ ).

From 59 children with positive serology, 81.13% had at least one intestinal parasite: 52.54% had *B. hominis*, 30.5% had *G. lamblia*, 10.17% had *H. nana*, and 1.69% had *A. lumbricoides*. This case parasitized with *A. lumbricoides* had also moderated eosinophilia.

## DISCUSSION

Toxocariasis is considered a helminthic zoonosis of wide geographic distribution occurring both in developed countries and in these ones with deficient sanitary, cultural, and social structures<sup>9</sup>. This parasitosis is also a poorly diagnosed disease and it is largely unknown either to health professionals or the general population.

In the present study, we observed 32.4% of frequency for anti-*Toxocara* antibodies in this child population using ELISA-IgG test, suggesting that children in study population had contact with the parasite, and developed an immune response with production of specific antibodies. This value is similar in comparison with other studies carried out in our country<sup>10,11</sup> and in other Latin American countries<sup>1,3,7,20,23</sup>.

The association between male children and positive serology indicate they might be at greater risk for toxocariasis, because presence of pica, geophagia and poor hygiene behaviors might be more frequent

in those children. Gender does not seem to be an important factor regarding *Toxocara* infection, although a higher frequency was observed in males. Several studies have shown a higher frequency of *Toxocara* infection in male children, which may derive from differences in the play and social behaviors of boys, resulting in increased exposure to the eggs of *Toxocara* sp.<sup>2,3,25,26,29</sup>. High prevalence for *Toxocara* serology may result from various factors, the most important of which concerns living conditions associated with urban infrastructure.

The ownership of dogs or/and cats were significantly associated with seropositivity to anti-*Toxocara* antibodies. Regarding presence of animals, there is controversy as to importance of contact with dogs as a risk factor for human toxocariasis. CHIEFFI *et al.*<sup>6</sup> and SCHANTZ *et al.*<sup>26</sup>, 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<sup>13,17</sup>.

Presence of dogs or/and cats within home tended to be associated with risk of infection by *Toxocara*<sup>25,31</sup>. However, this association was not statistically significant ( $Chi\text{-square} = 0.40; p = 0.5261$ ) in the present study, contradicting findings by CHIODO *et al.*<sup>8</sup> and SCHANTZ *et al.*<sup>26</sup> showing significant association between seropositivity and dogs or/and cats within the home.

Use of public playgrounds is other important risk factor associated to toxocariasis infection, because canine stools are very frequent in many countries. In our study, this risk factor has significant association with *Toxocara* infection. It is possible that infection occurring in this population might have been brought about by inadvertent ingestion of *Toxocara* eggs contaminating soil of public places within the district. Limited studies in our country on the contaminated soil with *Toxocara* eggs revealed a frequency ranging from 30% to 80%<sup>5,19</sup>.

Highly significant association between signs/symptoms and positive serology indicates an evidence of *Toxocara* infection in this child population. However, only 39% of children with positive serology had a light or moderate eosinophilia and none had a severe eosinophilia. These data suggest that studied population could be presenting a covert toxocariasis<sup>21,28</sup>, due to unspecific clinical manifestations and the poor eosinophilia so different from classic syndromes of VLM and OLM. Children with negative serology for toxocariasis also presented symptoms, which might be due to a different etiology, i.e. intestinal parasitosis (found in 81.13% of population studied) or other unknown causes.

Respiratory symptoms was clinical feature most frequently observed in children with positive serology, and included from slight to moderated manifestations, as BUIJS *et al.*<sup>4</sup> already observed in a previous study.

GLICKMAN *et al.*<sup>15</sup> and SHARKEY *et al.*<sup>27</sup> point out that ocular toxocariasis may be negative at ELISA test, probably due to a low concentration of circulating antibodies in this form of disease. Nevertheless, 36 out of 46 children with ocular manifestations showed positive results. According to GLICKMAN *et al.*<sup>13</sup> and MAGNAVAL *et al.*<sup>21</sup> a single larva in the eye may cause serious damages, therefore, an early diagnosis is very important.

Systemic infection and high frequency of intestinal parasites may occasionally be found but significant statistical association between them is lacking. As results show, more frequent parasites are protozoa and they do not cause cross reactions with ELISA test but these results suggests that studied group was exposed to soil contamination of food; this is a principal way to get *Toxocara* infection and represent a characteristic of our group.

The high toxocaríasis frequency could be the result of various factors, but doubtless, the most important are the rural and social characteristics of Morrope district, a city with an extended area without paved streets and deficient daily cleaning, with high percentage of this population with unsatisfied basic needs and a high proportion of dogs living at home. It is well known that when dogs are present, the potential for household contamination with *Toxocara* eggs is high. Furthermore, deficient sanitary behaviors, climatic conditions and houses in poor neighborhoods, led to most of these children playing in close contact with soil since early ages.

Clinical and serological evidence of *Toxocara* infection was observed in our population. Future studies about soil contamination should be done to better assess the magnitude of this serious health problem, and community education programs should be developed to promote the social concept of a responsible pet ownership.

## RESUMO

### Evidências clínicas e sorológicas de infecção por *Toxocara* em escolares do Distrito de Morrope, Lambayeque, Peru

O propósito do presente trabalho foi estimar a frequência da toxocaríase humana em crianças do distrito de Morrope, Lambayeque, Perú. Nos meses de outubro a dezembro de 2005, 182 escolares (96 meninos e 86 meninas) foram estudados. Coletaram-se amostras de sangue para a análise hematológica e imunológica pelo teste de ELISA-IgG-*Toxocara* e amostras fecais para o exame parasitológico. A frequência geral da população foi de 32,4% (59/182) com proporção significativamente maior de positividade nos meninos. Das crianças com resultado positivo ao teste sorológico (52 meninos e sete meninas), 71,2% tinham entre cinco e 10 anos, 77,96% apresentavam sintomas respiratórios, 61,02% apresentavam manifestações oculares, 38,98% apresentavam moléstias hepáticas, 38,98% apresentavam suave ou moderada eosinofilia e todos estes sinais foram estatisticamente associados ao resultado da sorologia. A presença de algum parasito intestinal foi observada em 83,5% da população estudada: *Blastocystis hominis* (53,3%), *Giardia lamblia* (31,3%), *Entamoeba coli* (29,1%), *Entamoeba histolytica/E. dispar* (1,1%), *Hymenolepis nana* (5,49%), e *Ascaris lumbricoides* (3,3%), mas estes não tiveram nenhuma associação com a sorologia. A posse de cães e gatos revelou associação estatisticamente significativa com a positividade para toxocaríase embora a presença destes animais no domicílio não tenha revelado tal associação. Conclui-se que existem evidências clínicas e sorológicas de infecção por *Toxocara* na população estudada.

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