

HUMAN TOXOCARIASIS: A SEROEPIDEMIOLOGICAL SURVEY IN THE AMAZONIAN CITY OF YURIMAGUAS, PERU

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SUMMARY

The aim of this study was to determine the seroprevalence of the infection by *Toxocara* in the general population of the Amazonian city of Yurimaguas, Peru. From March to August 2008, a total of 300 subjects were sampled and tested by means of a *Toxocara* ELISA-IgG test. A clinical and epidemiological questionnaire was used to assess the symptomatology and risk factors associated with human toxocariasis. The overall rate of seropositivity was 35.66%, with a significant high proportion in children ($p < 0.001$). The clinical evaluation revealed that 95.33% of the seropositive group had some type of symptomatology: headache (66.36%), respiratory compromise (63.55%), abdominal pain (54.21%), cutaneous signs (40.19%) and ocular manifestations (36.45%), and almost all of them were statistically significant ($p < 0.001$). Furthermore, 56.07% of the seropositive subjects presented at least one intestinal pathogen parasite with predominance of helminthes, but without significant association ($p = 0.334$). The analysis of risk factors showed only that the use of public places and geophagia exhibited a significant association with the seropositivity ($p < 0.001$). Clinical, serological and epidemiological findings associated to infection with *Toxocara* were observed in the present study and future studies should be done to assess this serious health problem.

KEYWORDS: Toxocariasis; Epidemiology; Survey; ELISA; Peru.

INTRODUCTION

Human toxocariasis is an accidental parasitic zoonosis caused by larval stages of *Toxocara canis* and *T. cati*, the roundworms commonly found in the intestine of dogs and cats, respectively. Adult females of *Toxocara* can produce up to 200,000 eggs per day, which are excreted into the environment with the feces of their definitive hosts; these eggs become infective within three to five weeks, depending upon certain environment conditions such as the temperature and humidity. When embryonated, *Toxocara* eggs are accidentally ingested by humans from soil or contaminated food, infective larvae hatch in the small intestine and migrate through the body, but fail to develop to adult stages within this aberrant host^{5,22,24}.

The clinical spectrum of human toxocariasis, which varies from asymptomatic infections to severe organ injury, is determined mainly by the parasite load, the sites of larval migration, the inflammatory response and eosinophilia²⁴.

Visceral larva migrans (VLM) and ocular larva migrans (OLM) are the two main clinical forms recognized in the literature. VLM or visceral toxocariasis is characterized mainly by fever, hepatomegaly, splenomegaly, respiratory disorders, hypergammaglobulinemia and eosinophilia; OLM

is the result after a larval invasion to the eye and their pathological effects including leucocoria, chorioretinitis, optic papillitis, endophthalmitis, can lead to a partial or complete loss of vision. Nevertheless, other syndromes such as covert toxocariasis (CT), neurotoxocariasis (NLM) and other forms have also been described^{12,22,24,33,34}.

Because of the difficulty of detecting *Toxocara* larvae in biopsy tissues, the diagnosis of human toxocariasis is essentially based on immunological assays such as the ELISA test using the excretory-secretory antigens from infective *Toxocara* larvae (TES) to detect anti-*Toxocara* antibodies, which have good sensitivity and specificity^{14,18,29}. With the development of this test, several seroepidemiological studies also have been possible^{5,22,33}.

The frequency of human infections by larval stages of *Toxocara* is high in tropical and developing countries, and is generally associated with low socioeconomic groups¹⁵, with rates ranging from 1% in Spain²⁵ to 86% in Santa Lucia³⁶; from 3.6% to 24.7% in Brazil^{2,4}; 47.5% in Colombia¹; from 34.9% to 66.6% in Venezuela^{10,21}, and from 37.9% to 39% in Argentina^{3,27}. In Peru, a few reports have informed of frequencies from 7.8% to 44.92% in rural populations from different places^{7-9,19,30,31}. However, seroepidemiological studies about human toxocariasis in Peruvian Amazonian populations have not been reported.

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The aim of this study was to determine the seroprevalence of infection with *Toxocara* in Amazonian inhabitants from the district of Yurimaguas, Department of Loreto, Peru, and its association with clinical and epidemiological data.

MATERIAL AND METHODS

This study was carried out from March to December 2008, in the Amazonian district of Yurimaguas (Latitude 05° 55' S and Longitude 76° 06' W). The city of Yurimaguas is located at the northwest corner of the Peruvian Amazon at the confluence of the rivers Huallaga and Paranapura, and has a mean altitude of 180 m above sea level and it is the capital of the province of Alto Amazonas, Department of Loreto, Peru. Yurimaguas has a tropical climate with a mean temperature of 28 °C (between 21.5 and 35 °C) and an average relative humidity of 90%.

This district has an approximate population of 61,123 inhabitants. People from rural areas have poor socioeconomic conditions, usually live in neighborhoods and work mainly in trades, agriculture and fishing. From this background, a total of 300 subjects from eight months to 82 years old accepted to participate in this research after their agreement with this study.

An individual questionnaire (including an informed consent) was made and the following clinical data were registered: anamnesis data, clinical signs and symptoms (dry cough, bronchospasms, bronchitis, asthma, hepatosplenomegaly, abdominal pain, leucocoria, strabismus, retinitis, visual loss, headache, convulsions, sleep disturbance and other signs of central nervous system involvement, pruritus, skin rash, chronic weakness, and fatigue); the epidemiological data and risk factors included age, sex, presence of dogs and/or cats at home, the use of public places (i.e. central squares and public parks), history of pica and/or geophagia. This research had the approval of the Ethical Research Committee of the Institute of Tropical Medicine, UNMSM.

For immunological evaluation, blood samples were collected in vacutainer tubes without anticoagulant to obtain serum, which was separated and stored at -20 °C until use. For parasitological examination, stool samples also were collected fresh or preserved in 10% saline-formalin solution at room temperature.

Anti-*Toxocara* IgG antibodies were detected in serum samples by ELISA-IgG test using TES antigens and each serum sample was previously absorbed with total *Ascaris suum* adult extract in order to avoid the cross-

reactions in the immunoassay²⁹. All sera were tested at 1:200 dilutions and all stages of the 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 above the cut-off value were considered as positive. Additionally, all positive sera were then serially two-fold diluted to determine the relative antibody concentration by titration (the inverse of each dilution).

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 test²⁰.

All data were introduced on an Excel spreadsheet and a statistical analysis was carried out using the program Epi-Info version 6.0. Chi-square test or Fisher's exact test and Odds Ratio (OR) were performed in order to confirm the mean differences between groups. The level of significance selected was $p < 0.05$ and with a significance level of 95%.

RESULTS

From a total population of 300 subjects (112 male and 188 female), 107 subjects were positive to the ELISA-IgG test, giving an overall frequency of 35.66%. From these 107 subjects with positive serology, 4.66% had titers of 200, 8% had titers of 400, 6.33% had titers of 800 and 16.66% had titers of 1600 (Table 1).

The overall frequency of serum anti-*Toxocara* IgG antibodies in male subjects was 42.86% (48/112), whereas the overall frequency in female subjects was 31.38% (59/188). The association between gender and positive serology in the total population showed differences apparently significant in male subjects (Chi-square = 4.03; OR = 1.64; $p = 0.045$). On the other hand, an analysis between frequencies of seropositivity and each age group showed a higher significant association in the group of children from eight months to 10 years old (Chi-square = 11.04; OR = 2.62; $p = 0.0009$) (Table 1).

The analysis of the signs/symptoms suggestive of toxocariasis among the studied population showed that 95.33% of the seropositive group had at least one type of sign or symptom in comparison with a 78.75% found in the seronegative group. The most frequent clinical findings present in seropositive subjects were headache (66.36%), respiratory signs or symptoms (63.55%), abdominal pain (54.21%), cutaneous signs (40.19%), ocular signs or symptoms (36.45%) and hepatic compromise (7.47%), and almost all of them were statistically associated to positive serology (Chi-square = 14.56; $p = 0.0001$; OR = 5.5) (Table 2).

Table 1
Frequency of anti-*Toxocara* antibodies according to the age groups in people from the Amazonian city of Yurimaguas, Peru, 2008

Sex	Age groups										Total
	0 to 10		11 to 20		21 to 40		40 to 60		61 to 82		
	Pos*	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	
Male	17	11	6	11	10	20	12	17	3	5	112
Female	15	16	12	24	19	49	11	34	2	6	188
Total	32	27	18	35	29	69	23	51	5	11	300

* Pos = seropositive; Neg = seronegative

Table 2
Distribution of signs and symptoms as related to serology for toxocaríasis in people from Yurimaguas city, Peru, 2008

Clinical findings*	Anti- <i>Toxocara</i> IgG antibodies				Bivariate analysis		Multivariate analysis***	
	Seropositive (n = 107)	%	Seronegative (n = 193)	%	p value**	OR (95% CI)	p value	OR (95% CI)
Symptomatic (any)	102	95.33	152	14.56	< 0.001	5.50 (2.10-14.40)		
Pulmonary manifestations	68	63.55	46	23.83	< 0.001	5.57 (3.33-9.32)	0.008	2.921 (1.33-6.40)
Hepatic manifestations	8	7.47	18	9.33	< 0.001	0.79 (0.33-1.87)		
Ocular manifestations	39	36.45	57	29.53	0.5854	1.37 (0.83-2.26)		
Headache	71	66.36	11	5.69	< 0.001	32.63 (15.74-67.63)	< 0.001	14.502 (4.21-49.92)
Abdominal pain	58	54.21	92	47.67	0.278	1.30 (0.81-2.09)		
Weakness and/or fatigue	30	28.03	11	5.69	< 0.001	6.45 (3.07-13.52)	< 0.001	35.52 (14.38-87.75)

*Some subjects had more than one sign/symptom; **p < 0.05 = significant value (Chi-square); *** variables in the model: sex, age, pulmonary and hepatic manifestations, headache, and weakness and/or fatigue.

The parasitological analysis was carried out only in 223 subjects because some people would not accept collecting their stool samples. Even so, the study revealed that 70.40% (157/223) of the population were infected. The protozoan parasites found were as follows: *Blastocystis hominis* in 22.43% , *Giardia lamblia* in 5.61% , *Entamoeba histolytica/E. dispar* in 0.93% , and *Cyclospora cayetanensis* 0.93%. From subjects with positive serology, 56.07% (60/107) had at least one intestinal parasite. The most prevalent intestinal nematodes found in the study population were: *Ascaris lumbricoides* (12.15%) , *Hymenolepis nana* (5.61%) , and *Trichuris trichiura* (1.87%). However, their association with a positive serology revealed not to be significant (Chi-square = 0.93; p = 0.334; OR = 1.26).

The analysis of risk factors evidenced that the presence of dogs and/or cats within the houses showed no significant association with seropositivity (Chi-square = 2.6; p = 0.107; OR = 1.64). Nevertheless, the use of parks or other public places showed a significant association with the seropositivity as well as a previous history of pica or geophagia (p < 0.001) (Table 3).

DISCUSSION

Toxocaríasis is a helminthic zoonosis with a worldwide distribution and, until now, it continues to be an almost unknown disease among the health professionals or general population.

In the present study, we have observed an overall anti-*Toxocara* antibodies frequency of 35.66% in the general population from the Amazonian city of Yurimaguas, with a higher significant proportion in children from eight months to 10 years old, suggesting that they were the main group in contact with the parasite and the main group at risk of toxocaríasis, as reported by other authors^{22,24,33}. This value is among the values of frequency previously found in other places from our country^{7-9,30,31} and relatively similar with other studies from other Latin American countries^{2,3,21,27}.

From the seropositive subjects, 16.66% (50/107) had titers of 1600 for anti-*Toxocara* IgG antibodies, mainly in children from one to 10 years old, suggesting a strong immunological response due to the larval infection by *Toxocara*.

Table 3
Distribution of risk factors as related to serology for toxocaríasis in people from Yurimaguas city, Peru, 2008

Risk factors	Anti- <i>Toxocara</i> IgG antibodies				Bivariate analysis		Multivariate analysis**	
	Seropositive (n = 107)	%	Seronegative (n = 193)	%	p value*	OR (95% CI)	p value	OR (95% CI)
Ownership of dogs and/or cats at home	89	83.18	145	75.13	0.107	1.64 (0.90-2.99)		
Use of parks and/or public places	93	86.92	127	65.80	< 0.001	3.45 (1.83-6.52)	< 0.001	0.14 (0.021-0.394)
History of pica or geophagia	28	26.17	12	6.22	< 0.001	5.35 (2.59-11.05)	< 0.001	0.223 (0.105-0.477)

*p < 0.05 = significant value; **variables in the model: sex, age, use of parks and/or public places, and history of pica or geophagia.

In agreement with other authors, we have also found statistically significant differences between gender and positive serology indicating that male subjects are more associated at risk of toxocariasis than female subjects, probably because of their close contact with soil, as is the case with this population, who are farmers^{4,5,21,22,26}.

The clinical assess of the symptomatology suggestive of toxocariasis indicates to us a highly significant association with positive serology and more evidence of toxocariasis cases in this population ($p < 0.001$). Clinical findings such as respiratory compromise (asthmatic syndrome, bronchial spasm, bronchitis or dry cough) were highly significant ($p < 0.001$) as well as headache ($p < 0.001$) and weakness or fatigue ($p < 0.001$). On the other hand, clinical findings such as hepatic compromise and abdominal pain, ocular and cutaneous manifestations were also frequent, but less associated to the positive serology.

It is interesting to mention that the clinical findings associated to the positive serology were more frequently found in children between one and 10 years old (data not shown). On this point, many authors are in agreement that toxocariasis cases are more frequently associated and diagnosed in pediatric patients. However, VLM cases are less frequent in comparison with other forms of toxocariasis, such as incomplete VLM or covert toxocariasis^{22,24,34}.

Taking into consideration that the clinical manifestations found in these children are associated with middle or high titers of anti-*Toxocara* antibodies, we think that they could be presenting some clinical picture compatible with covert toxocariasis. Unfortunately, we could not take additional blood samples for hematological examination in order to assess their levels of leukocytes and eosinophils. Therefore, this is a limitation of our study.

Other clinical findings frequently associated to toxocariasis are hepatic compromise or abdominal pain. Nevertheless, the result of the multivariate analysis reveals a poor association with the positive serology, suggesting other causes for their presence in the studied population. It is possible that intestinal parasites (found in a high frequency in this population) could be the main cause for the presence of these symptoms.

On the other hand, visual loss and/or ocular pain, which are associated to ocular toxocariasis, have a poor association with the positive serology, suggesting other causes for their presence in the studied population. Similarly, cutaneous signs also were present in seropositive subjects, but they were insufficient to be associated to the serology, contradicting the findings of other authors^{14,17}.

Ownership of pets such as dogs or cats and their presence at home is frequently associated with toxocariasis, as mentioned by many authors^{5,22,24,28,34-37}. However, this fact was not associated with the seropositivity in the studied population ($p = 0.107$). On the other hand, the use of public parks or places by those people were highly associated with the positive serology ($p < 0.001$), suggesting that *Toxocara* eggs might be present in those places and to be a main source for infection in this population. Canine feces are frequent in public places and playgrounds in other countries, as reported by other authors around the world^{1,5,15,22,24,33}. Although limited studies about soil contamination with *Toxocara* eggs in Peru have revealed a frequency ranging from 30% to 80%¹⁹. It is possible that the infection occurring in this population might have also happened

by an accidental ingestion of infective *Toxocara* eggs from the soil of those public places.

Geophagia (eating soil) is a specific type of pica that increases the risk of toxocariasis, especially in children living in homes with infected puppies and kittens or as atypical behavior of playing on the soil^{5,22,33}. In this study, we have found that geophagia was significantly associated to positive serology ($p < 0.001$). Moreover, association of geophagia with toxocariasis were reported in several studies around the world, since human toxocariasis has been described as a soil transmitted zoonosis^{2,5,17,22,24}.

A high frequency of intestinal parasites might occasionally be found together with *Toxocara* infection and the statistical association between them could be close related. Nevertheless, this kind of association was not significant with regards to the positive serology ($p = 0.334$). However, their presence shows that this population has poor hygiene habits and sanitary behavior.

We also note that the presence of mixed infections between *Toxocara* and other helminthes might increase the frequency of seropositivity using conventional serological tests, but with the previous serum absorption with *Ascaris suum* antigens, we might diminish or avoid the possible cross-reactions, as recommended by other authors^{6,23}.

The high rate for toxocariasis frequency found in this study could be the result of several factors, but doubtless the most important is the social characteristics of the rural neighborhoods from Yurimaguas city, a tropical town with many areas of unpaved streets, deficient in the daily cleaning of them and dogs living on the streets. On this point, many authors report that similar conditions found in people living in tropical places from other countries are more associated to contracting toxocariasis^{5,11,15,32,33}.

Clinical, serological and epidemiological findings associated to *Toxocara* infection were observed in the present study. Future studies about soil contamination with *Toxocara* eggs should be performed to better assess and control the magnitude of this serious health problem, and community education programs should be developed to inform people about zoonotic parasitosis and promote the social concept of responsible pet ownership.

RESUMO

Toxocariase humana: inquérito soropidemiológico na cidade amazônica de Yurimaguas, Peru

O objetivo do presente estudo foi estimar a soroprevalência da infecção por *Toxocara* em população geral da cidade de Yurimaguas, Peru. Entre os meses de Março e Agosto de 2008, foi estudado um total de 300 pessoas por exame imunológico mediante *Toxocara* ELISA-IgG teste. Uma ficha clínico-epidemiológica foi utilizada para avaliar a sintomatologia e os fatores de risco associados à toxocaríase. A frequência geral de anticorpos na população foi de 35,66% com proporção significativamente maior de positividade em meninos de um a 10 anos ($p < 0.001$). A avaliação clínica revelou que 95,33% do grupo de soropositivos apresentava algum tipo de sintomatologia associada à toxocaríase: 66,36% cefaléia, 63,55% sintomas respiratórios, 54,21% dor abdominal, 40,19% sinais cutâneos e 36,45% manifestações oculares e quase todos estes sinais foram estatisticamente associados ao resultado

da sorologia ($p < 0.001$). Além disso, 56,07% das pessoas com sorologia positiva tinham pelo menos algum parasito intestinal patogênico e com predominância de helmintos, mas sem nenhuma associação significativa ($p = 0.334$). A análise dos fatores de risco mostrou que o uso de lugares públicos e história de geofagia tiveram significativa associação com a sorologia positiva ($p < 0,001$). Conclui-se que existem evidências clínicas, sorológicas e epidemiológicas de infecção por *Toxocara* na população estudada e futuros estudos são necessários para avaliar este sério problema de saúde pública.

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