

VISCERAL LARVA MIGRANS: A SEROEPIDEMIOLOGICAL SURVEY IN FIVE MUNICIPALITIES OF SÃO PAULO STATE, BRAZIL

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SUMMARY

An enzyme-linked immunosorbent assay (ELISA), employing antigens from *Toxocara canis* larvae and the absorption of suspected sera with *Ascaris lumbricoides* extracts was used in a seroepidemiological study performed in five municipalities of São Paulo State, Brazil (São Paulo, Campinas, Santos, Marília and Presidente Prudente) in order to determine the frequency of antibodies to *Toxocara*. In 2,025 blood samples collected, 806 proceeded from male subjects and 1,219 from females; 483 samples were collected from subjects under 15 years of age and the remaining 1,542 from subjects aged 15 years or over. Among the 2,025 sera investigated, 3.60% had antibodies to *Toxocara* at significant levels. A moderate predominance of infection with *Toxocara* among male subjects (3.72%) was observed, although the difference was not statistically significant when this rate was compared with that for female (3.28%). Related to age, a higher frequency of positive results was detected among subjects under 15 years (6.41%) against the older group (2.53%). A trend of more elevated rates of infection was observed in municipalities with high demographic densities (São Paulo, Campinas and Santos). Nevertheless, such findings only appeared to be statistically significant in subjects younger than 15 years.

KEY WORDS: Visceral Larva Migrans; *Toxocara* sp.; *Toxocara canis*; Seroepidemiological survey.

INTRODUCTION

The visceral larva migrans syndrome (VLM) was defined by BEAVER et al³ in 1952, as the outcome of prolonged migration of helminth larvae within the bodies of unusual hosts, especially man.

The helminth commonly involved in the etiology of VLM are worms of the family Ascari-

dae, parasites of dogs and cats and pertaining to the genus *Toxocara*, where *T. canis* is, doubtless, the most important species on account of its patterns of tissue migration and ability of surviving in unusual hosts^{2, 8}. Moreover, eggs of *T. canis* have been more frequently found contaminating the soil than those of the other animal ascarids^{5, 6, 24}, and the great majority of larvae,

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already identified by biopsy in human cases of VLM, has been found to belong to this species.

The essential factors for the occurrence of VLM are present in Brazil: a large number of dogs infected with *T. canis*^{5, 12} and the frequent contamination of the soil by eggs of this helminth^{5, 6, 13}. However, neither the rates of infection in the population, nor the ways of transmission are well known in this country.

This work aims to determine the frequency of antibodies to *Toxocara* in sera of people living in five municipalities of São Paulo State, evaluating for the first time in Brazil the dissemination of this nematode, in ample contingents of the population.

MATERIALS AND METHODS

Serum samples from 2,025 subjects, resident in five municipalities of São Paulo State, selected as to represent areas of the plateau and the coastline of the state, were examined. The plateau areas included four municipalities: two of them

situated in metropolitan regions with elevated demographic density (São Paulo and Campinas), the other two, with lower population density (Marília and Presidente Prudente). The littoral area was represented by a municipality of elevated population density (Santos).

The main physical and demographic characteristics of each municipality are shown in Table 1.

The distribution of blood samples, collected during 1983, according to municipality, age and sex, is displayed in Table 2.

The enzyme-linked immunosorbent assay (ELISA) was applied to these serum samples in order to detect antibodies to toxocaral infection, according to a method described by ENGVALL & PERLMANN¹¹ and modified by CYPRESS et al⁸.

The antigen used was obtained from second and third stage larvae of *T. canis*, maintained

TABLE 1
Main physical and demographic characteristics of five municipalities of São Paulo State examined for anti-*Toxocara* antibodies, during 1983.

Municipalities	Area (km ²)	Altitude (m)	Human Population*			Canine Population**	Demographic density (Inhab/km ²)
			Urban	Rural	Total		
São Paulo	1,509	760	8,337,241	155,985	8,493,226	900,833	5,688.7
Campinas	890	706	591,557	73,002	664,559	72,901	850.9
Santos	753	3	414,710	1,971	416,681	42,702	574.7
Marília	1,154	674	107,299	14,475	121,774	12,544	102.0
Pres. Prudente	555	475	129,646	7,200	136,846	14,146	247.0

* According to Population Census performed in 1980.

** According to Permanent Committee of Rabies Control of Health Secretary of São Paulo State, in 1982.

TABLE 2
Distribution of 2,025 blood samples, collected during 1983, in five municipalities of São Paulo State, examined for anti-*Toxocara* antibodies.

Municipality	São Paulo		Campinas		Santos		Marília		Pres. Prudente	
	< 15	≥ 15	< 15	≥ 15	< 15	≥ 15	< 15	≥ 15	< 15	≥ 15
Sex										
Male	71	139	59	45	54	99	26	146	34	133
Female	70	313	37	134	53	103	39	142	40	288
Total	141	452	96	179	107	202	65	288	74	421

in 0.1 M borate buffer and hatched with a tissue homogenizer Potter (Scientific Glass Apparatus, Co, Inc.) in ice bath. After complete homogenization, the solution has been centrifuged at 2,000 rotations per minute, during 20 minutes, and the supernatant, carefully decanted, has formed the antigenic fraction.

The ELISA values for reaction titer were determined through absorbance rates, read with a filter of 410 nm, using a spectrophotometer "Microelisa reader", model MR 580 (Dynatech Laboratories Inc.).

After testing sera of subjects without epidemiological antecedents for toxocaral infections and a sample, provided by the Center for Diseases Control (Atlanta, USA), with confirmed positive results for antibodies do *Toxocara*, the dilutions considered significant were those with absorbance rate equal to or above 0.30. In this way, sera that presented reaction titers equal to or above 1:160 have been considered as having antibodies to *Toxocara* at significant levels.

Due to the high prevalence of infection with *Ascaris lumbricoides* in the municipalities where the survey was carried out, only the results with titers equal or above 1:160 after absorption with *Ascaris lumbricoides* extract, obtained from cuticles of specimens of this helminth, were accepted as valid.

The found frequencies of *Toxocara* antibodies were adjusted to conform to the age and

sex distribution of São Paulo State population (Census performed in 1980). The test of Chi-square, with or without correction for continuity, depending of the number of elements of the samples was applied, for the statistical analysis of the results. In the comparison of more than two proportions, partition of the Chi-square was used for providing more detailed analysis after a significant overall test.

RESULTS

Among the 2,025 sera examined, 70 (3.46%) showed the presence of antibodies to *Toxocara* at significant levels. After adjusting for sex and age the rate of subjects with indications of previous infection with *Toxocara* larvae rose to 3.60%.

The frequencies of antibodies to *Toxocara* observed in each municipality, arranged according to sex and age group, are displayed in Table 3. The overall rates have also been adjusted according to sex and age distribution of the population in each municipality (Population Census of 1980).

No significant difference has been found between the positive rates of antibodies to *Toxocara* when the sexes are compared. In relation to age, however, the rate was significantly more elevated in subjects aged less than 15 years.

TABLE 3
Frequency (%) of anti-*Toxocara* antibodies in 2,025 subjects examined in five municipalities of São Paulo State, in 1983, according to age and sex.

Municipality	Age		< 15			≥ 15			Total		
	Sex		Male	Female	Total (*)	Male	Female	Total (*)	Male	Female	Total (**)
São Paulo			9.86	2.86	6.40	2.16	2.56	2.36	4.73	2.66	3.69
Campinas			3.39	5.41	4.39	2.22	5.22	3.73	2.61	5.28	3.95
Santos			14.81	11.32	13.09	1.01	1.94	1.48	5.62	5.00	5.31
Marília			3.85	2.56	3.21	1.37	2.11	1.74	2.20	2.26	2.22
Pres. Prudente			2.94	2.50	2.73	3.01	2.78	2.89	2.99	2.69	2.84

(*) Adjusted to age distribution of population, according to Census performed in 1980.

(**) Adjusted to age and sex distribution of population, according to Census performed in 1980.

The positive rates of *Toxocara* infection in each municipality did not have a significant difference in the overall analysis by the Chi-square test, but when the cities are grouped according to the location and density of population, forming three distinct groups as indicated in "Materials and Methods" and, at the same time, considered two groups of age (lesser than 15 years and equal to or over 15 years) a significant difference appeared (Tables 4 and 5). Then, a more elevated rate has been found in the municipalities with higher densities of population, for the group of subjects younger than 15 years.

TABLE 4

Frequency (%) of anti-*Toxocara* antibodies in subjects aged less than 15 years, in municipalities grouped according to location and density of population.

Municipalities	Examined	Positive rate	Corrected rate
São Paulo - Campinas	139	2.88	2.96
Marília - Pres. Prudente	237	5.49	5.58
Santos	107	13.08	13.09

$\chi^2 = 10.8680$ ($p < 0.05$)

TABLE 5

Frequency (%) of anti-*Toxocara* antibodies in subjects aged equal to or above 15 years, in municipalities grouped according to location and density of population.

Municipalities	Examined	Positive rate	Corrected rate
São Paulo - Campinas	709	2.40	2.42
Marília - Pres. Prudente	631	2.69	2.75
Santos	202	1.49	1.48

$\chi^2 = 1.0395$

DISCUSSION

The finding that 3.60% of the samples examined had antibodies to *Toxocara* at significant levels suggest that, in the municipalities studied, a considerable part of the population is probably infected with larvae of *Toxocara* sp.. Similar surveys, carried out in other countries, revealed patterns of infection similar to ours^{9, 15, 19, 21, 23, 26}. In this way, these results indicate that it is possible to carry out seroepidemiolo-

gical surveys, on a large scale, in order to ascertain the rates of human infection with *Toxocara* larvae, even in regions where the prevalence of infection with *A. lumbricoides* is elevated, as it occurs in the five municipalities studied⁷, since the sera are previously submitted to absorption with extracts of *A. lumbricoides*.

The analysis of the results from each of the municipalities surveyed showed some aspects deserving especial attention.

The selection of the municipalities has followed a predefined criterion in order to constitute three subgroups. In the first one, the municipality studied (Santos) was located in the coastline and presented a relatively elevated density of population (574.7 inhabitants per square kilometer). The other two subgroups included municipalities located in the plateau, away from the coastline, with different values of demographic densities: high in one group (São Paulo and Campinas) and low in the other (Marília and Presidente Prudente).

In an overall comparison of the results from the five municipalities (Table 3) the differences were not statistically significant, but a trend toward greater frequencies of subjects with antibodies to *Toxocara* in the municipalities with more elevated population densities was noted. On the other hand, the analysis of the results from the three groups of municipalities (Tables 4 and 5) revealed highly significant differences in the positive rates for anti-*Toxocara* antibodies, with more elevated values for subjects younger than 15 years, living in São Paulo, Campinas and Santos, municipalities with higher densities of population. This finding might be consequence of a closer contact between men and dogs in these municipalities (Table 1), providing a greater exposure, especially of children, to an environment contaminated by dog faeces.

Regional differences in the rates of human infection with *Toxocara* have already been observed in other countries. In the USA, the greatest serological prevalence of toxocaral infection was found in the South and Northeast but these differences tend to disappear when the rates are adjusted according to sex, age and race⁹. In France and also in the USA, rates of serological

prevalence and number of cases of VLM were found to be larger in the rural areas^{9, 10}. In Japan, MATSUMARA & ENDO²¹ obtained positive reaction for *Toxocara* antibodies more frequently among inhabitants of urban areas, compared with people living in rural areas or fishing villages. Therefore even in the Japanese case, higher rates of urbanization, linked with greater population density, showed a relation with more elevated frequencies of men infected with *Toxocara* larvae.

The discrepancy between the frequency of antibodies to *Toxocara* found in the littoral areas of Japan²¹ and that found in this paper, probably is due to the fact that the Japanese littoral regions surveyed were fishing villages, with low density of population, in contrast to the municipality of Santos, in Brazil. As a matter of fact, this comes to reinforce the role of demographic concentration as a factor of risk in the transmission of toxocariasis to man.

Among these findings, one deserves especial attention: the higher frequency of antibodies to *Toxocara* found in subjects lesser than 15 years, living in Santos, which was significantly greater than the levels found in the subgroups of subjects younger than 15 years living in São Paulo-Campinas and Marília-Presidente Prudente.

An explanation for this finding would be the influence of the environment characteristics of the littoral area providing the contamination of the beaches with dog faeces and the close, long and regular contact of children with the sand contaminated with *Toxocara* eggs. In support of this hypothesis comes the fact that, in the municipality of Santos, it was found a prevalence of infection with *A. lumbricoides* — nematode which epidemiology is, in many aspects, similar to that of *T. canis* — more elevated than in other municipalities of the State⁷. However, it must be noted that, despite its great resistance to environmental factors, *Toxocara* eggs are submitted, in the sand of beaches, to extremely adverse conditions to its survival^{1, 17}, as the excessive exposure to sunlight and the high salinity of the soil. Certainly there are other variables, not explored in this paper, related to the greater frequency of the infection with *Toxocara* among the young inhabitants of Santos, which deserve a more detailed investigation.

In general, the results obtained revealed among male subjects a moderate predominance of *Toxocara* infection, with no statistical significance, that can be explained by the greater frequency of antibodies to *Toxocara* in the subgroup formed by male subjects younger than 15 years (Table 3). For subjects with age equal to or above 15 years, an inverse trend was observed: rates slightly more elevated in women. Data from literature indicate that, in general, these rates are greater in men^{10, 15, 18, 21, 25}.

For a long time, human infection with *Toxocara* larvae was considered as a characteristic of children^{3, 15, 16, 22}, but recently there have been evidences of the occurrence of this infection in adults^{4, 10, 20, 21}. EHRHARD & KERNBAUM¹⁰ found that, in 350 patients with VLM, 17.7% were adults. MATSUMARA & ENDO²¹ found evidences of infection with *Toxocara* larvae in adult women, in Japan. More recently, MAGNAVAL et al²⁰ and GLICKMAN et al¹⁴ reported a great number of cases of VLM in adults, in a region of France. Our findings indicate that, in our country, the human infection with *Toxocara* is more frequent in the group younger than 15 years. Nevertheless, the hypothesis that the infection occurs also in older groups is not discarded, because of the finding of a considerable number of subjects older than 15 years with positive rates for *Toxocara* antibodies, in the five municipalities studied.

The results of this work, while they show how frequent is the occurrence of the infection in the five municipalities, strengthen the importance of VLM as a health complaint for the population of the State of São Paulo and probably of Brazil. They also call the attention to the possibility that densely populated areas, where a simultaneous increase of dog population occurs, turn out to be areas of greater risk of VLM for man.

RESUMO

Síndrome de Larva Migrans Visceral: inquérito soroepidemiológico em cinco municípios do Estado de São Paulo, Brasil

Realizou-se inquérito soroepidemiológico, para detecção de anticorpos anti-*Toxocara*, em

2.025 soros de indivíduos residentes em cinco municípios do Estado de São Paulo (São Paulo, Campinas, Santos, Marília e Presidente Prudente), através de técnica imunoenzimática (ELISA) e emprego de antígenos obtidos a partir de larvas de 2º — 3º estágio de *Toxocara canis*. Após absorção com extratos de *Ascaris lumbricoides*, revelou-se a presença de anticorpos anti-*Toxocara*, em níveis significativos, em 3,60% dos soros examinados, com predominância de indivíduos com idade inferior a 15 anos (6,41%). Observou-se, ainda, tendência a ocorrerem índices mais elevados de infecção por larvas de *Toxocara* entre indivíduos residentes em municípios com maior densidade demográfica (São Paulo, Campinas e Santos), especialmente no grupo com menos de 15 anos de idade.

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