Rev. Inst. Med. Trop. Sao Paulo
53(2):67-72, March-April, 2011
doi: 10.1590/S0036-46652011000200002

PROTECTIVE AND RISK FACTORS FOR TOXOCARIASIS IN CHILDREN FROM TWO DIFFERENT SOCIAL CLASSES OF BRAZIL

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SUMMARY

The aim of this study was to analyze the prevalence of *Toxocara* spp. antibodies in children from two different socioeconomic classes in the Presidente Prudente municipality, São Paulo State, Brazil, and the protective and risk factors associated with toxocariasis. One hundred and twenty-six middle-class (MC) and 126 disadvantaged children (DC) were included in this study. Anti-*Toxocara* ELISA test was performed in order to evaluate seroprevalence. A survey was applied to the children's guardians/parents in order to analyze the protective and risk factors. The overall prevalence was 11.1%, and of 9.5% (12/126) and 12.7% (16/126) for MC and DC subgroups, respectively. *Toxocara* seropositivity was inversely proportional to the family income. A high household income was considered a protective factor for toxocariasis in the total population and in both MC and DC subgroups. Being a girl was considered a protective factor for the total population and for both subgroups. Whilst being an owner of cat was a risk factor for children belonging to the total and for both MC and DC subgroups, having dog was considered as a risk factor for only the MC. Epidemiologic protective/factor risks can be distinct depending on the strata of the same population. Thus, it is relevant to evaluate these factors independently for different socioeconomic classes in order to design future investigations and programs for preventing the infection of human beings by *Toxocara* spp. and other geohelminths.

KEYWORDS: Toxocariasis; Children; Epidemiology; Seroprevalence; *Toxocara* spp.

INTRODUCTION

Toxocariasis is a zoonosis caused by nematodes of the genus *Toxocara*, most commonly *T. canis* and *T. cati*, the common roundworms of dogs and cats, respectively[1]. The disease has a worldwide distribution and it is endemic in all parts of the world, including industrialized countries where it is considered as the most prevalent helminthiasis[10,21,26,29]. In spite of being considered as is a public health hazard both in developing and developed countries, human toxocariasis has been included in the list of neglected zoonosis[19].

Humans become infected by accidental ingestion of embryonated *Toxocara* spp. eggs containing L2 larvae present in soil, especially in public areas[1]. After ingestion of eggs, the infective larvae hatch, penetrate the intestinal wall and migrate through the soft tissues of the body[10]. Most patients who are seropositive for *Toxocara* spp. show no clinical signs[21]. Toxocariasis has a wide range of clinical presentations, which are related to the degree of damage to host tissues caused by the larvae, and the number of signs and symptoms that may be present in each patient[10]. Both larval migration as well as the antigens that are secreted-excreted by the larvae can lead to an inflammatory process that may finally result in either ocular or visceral larva *migrans*[5,34].

Several studies carried out in different parts of the world were based on serological studies by using ELISA tests to detect specific antibodies to *Toxocara* spp. excretory-secretory (TES) antigens, and have focused on the factors associated with toxocariasis. Children aged from one to 15 years old are considered as the main risk group for toxocariasis as a consequence of the close contact with infected household pets or by being exposed to embryonated eggs in the environment[15]. In children, human toxocariasis is potentially associated with a variety of risk factors such as onychophagy and geophagia, pets in the household, and there is an association between high frequency of seropositivity to the parasite and socioeconomic status[10,21].

The aim of this study was to evaluate the frequency of anti-*Toxocara* antibodies in children from two different socioeconomic classes and analyze the protective and risk factors associated to toxocariasis.

MATERIAL AND METHODS

Subjects: A total of 252 children, aged from ten months old to 15 years old, whose doctors had answered a request to perform a hematological profile, were included in this study. In order to represent the different socioeconomic classes, two groups were constituted. The

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first was composed of 126 children assisted by the Brazilian public health system, representing the disadvantaged children (DC) group, whereas the other 126 who had a private health system were considered as the middle-class children (MC) group. The study was conducted in the Presidente Prudente municipality (22º07'21''S, 51º23'18''W), São Paulo State, Brazil, from August to November 2008.

The number of individuals to be enrolled in this study was established by using Epi Info 6.0 software and taking account of an estimated prevalence of 20%, an absolute error of 7% and a 95% confidence interval. A minimum of 125 individuals was estimated.

In order to avoid the participation of children belonging to the same family, individuals were included in the study by using a systematic sampling selection from the laboratorial number request.

A questionnaire was applied to parents/guardians to obtain epidemiological and socioeconomic information (sex, age, number of years of schooling of the household head, the family income, source of water used for drinking, presence of dogs and cats as pets, onychophagy or geophagia habit, presence of sand or lawn in peridomiciliar area or at school, and intake of raw or uncooked meat).

Children were excluded if their parent/guardian did not present a medical petition for blood examination or provide a signed written informed consent.

Sample collect and clinical pathology examination: For hematological and immunological tests, blood samples (5.0 mL) were collected in vacuum tubes with and without EDTA as anticoagulant from each child. Serum was separated by centrifugation and stored at -4 ºC until processing.

Blood samples were used to perform the hemogram, by using a flow cytometer flux counter (Pentra 80, HoribaDiagnostics, Montpellier, France). Differential leukocyte count was compared to direct microscopic observation of blood smears. Leukocytosis was defined as cell count more than 10,000 cells/µL and the eosinophilia was defined as absolute eosinophils count more than 500 cells/µL[32].

Antigen preparation: T. canis excretory-secretory larval antigens (TES) for ELISA was prepared as described previously[9] with some modifications[11].

Preincubation of sera with Ascaris suum Adult Worm Extract (AWE): In order to remove antibodies elicited by exposure to *Ascaris* spp., which could cross-react with *Toxocara* spp. antigens[26], test samples were preincubated with an AWE of *A. suum*[11].

Antibody detection: Serum samples were tested for anti-*Toxocara* IgG antibodies by ELISA test at a dilution of 1:320 essentially as described[11]. Polystyrene 96-well microplates (Corning, Costar, New York, NY) were coated for one hour at 37 °C followed by 18 hr at 4 °C with 1.9 µg/mL of solid-phase antigen dissolved in 0.06 M carbonate-bicarbonate buffer, pH 9.6 (100 µL/well) and subsequently blocked for two hr at 37 °C with PBS-T containing 2.5% bovine serum albumin (Sigma, St. Louis, MO). After a 40-min incubation at 37 °C, the diluted serum samples were removed and horseradish peroxidase-conjugated goat anti-human IgG (Sigma) was added at a 1:10,000 dilution (40 min at 37 °C), followed by the o-phenylenediamine substrate (0.4 mg/mL, Sigma). Absorbance readings were made at 492 nm; a cut-off absorbance value was defined as the mean absorbance reading for 96 negative control sera plus three standard deviations. A standard negative (non-reactive) serum and a positive reactive serum were used in all tests. Antibody levels were expressed as reactivity indices (RIs). RIs were calculated as the ratio between the absorbance of each test sample and the cut-off values. Samples with RIs > 1 were considered positive.

Statistical analysis: In order to compare the socioeconomic parameters (family income, mother scholarship) for the total population or MC and DC children, the data were submitted to Mann-Whitney test. The analysis of potential risk factors for toxocariasis were evaluated by Chi-square or Fisher's exact test, complemented by multivariate logistic regression analysis (variables with p-value< 0.2 in the bivariate analysis). The association between anti-*Toxocara* spp. antibodies and risk factors was expressed by odds ratio (OR) and 95% confidence interval (95% CI). All statistical analysis was made using the Statistical Package for Social Science (SPSS) version 13.0 (Chicago, IL, USA). Data presenting p-values < 0.05 were considered as significant[22].

Ethical considerations: This study was approved by the Ethical Research Committee of the University of Oeste Paulista, Unoeste, Presidente Prudente, São Paulo, Brazil (042/2007).

RESULTS

Of the total 252 children investigated, 11.1% (28/252) were positive. The frequency observed in MC and DC were, respectively, 9.5% (12/126) and 12.7% (16/126) (p = 0.5483).

Association between sex and serological test show a significantly higher proportion of positive ELISA test in male children only in the total population (Table 1). The MC was represented by 57.9% (73/126) of boys and 52.4% (66/126) girls. To be a girl was considered a protective factor for total population and for both subgroups. The ratio of protection for toxocariasis corresponded to 4.184 for female children both of total population and DC group, and to 26.316 for girls assisted by MC group.

There was no influence of age in the frequency of anti-*Toxocara* antibodies in total population and when the subgroups were compared (Table 1).

The presence of cat in the household was associated to the seropositivity for the total population and for both groups (Table 1), which was confirmed by logistic regression (Table 2). The presence of dog in the household was considered to be a risk factor by multivariate analysis for the MC subgroup (Table 1 and 2).

It was observed that educational (p < 0.0001) and economic (p = 0.0002) levels in MC were higher than DC. In MC, 15.1% (19/126) of mothers had elementary scholarship, 31.8% (40/126) had high school, and 53.2% (67/126) some college scholarship, whereas in DC, 1.6% (2/126) of mothers had frequented high school, 28.6% (36/126) had elementary scholarship and 69.8% (88/126) declared to have elementary. To have elementary scholarship was associated with an ELISA positive test in the total population (p = 0.0229) and in MC subgroup (p = 0.0003).

Table 1
Factors associated with anti-*Toxocara* IgG antibodies in a population of 252 children from different socioeconomic classes from Presidente Prudente municipality, São Paulo State, Brazil

	ELISA test		Odds Ratio (95% CI)	*p*-value*
	Positive (%)	Negative (%)		
A. Total Population				
Gender				
Male	21 (8.4)	112 (44.4)	3.027 (1.237-7.405)	0.0153
Female	7 (2.8)	112 (44.4)		
Dog in the household				
Yes	18 (7.2)	124 (49.2)	1.466 (0.648-3.318)	0.4231
No	10 (4.0)	100 (39.6)		
Cat in the household				
Yes	11 (4.4)	20 (7.9)	6.632 (2.732-16.099)	<0.0001
No	17 (6.8)	204 (80.9)		
Onychophagy				
Yes	9 (3.6)	47 (18.7)	1.784 (0.7579-4.199)	0.2260
No	19 (7.5)	177 (70.2)		
B. Disadvantaged				
Gender				
Male	11 (8.7)	49 (38.9)	2.739 (0.8916-8.413)	0.1065**
Female	5 (4.0)	61 (48.4)		
Dog in the household				
Yes	8 (6.3)	63 (50.0)	0.7460 (0.2609-2.133)	0.6008
No	8 (6.3)	47 (37.4)		
Cat in the household				
Yes	7 (5.6)	13 (10.3)	5.803 (1.866-18.240)	0.0042
No	9 (7.1)	97 (77.0)		
Onychophagy				
Yes	8 (6.3)	24 (19.1)	3.583 (1.217-10.548)	0.0277
No	8 (6.3)	86 (68.3)		
C. Middle-class				
Gender				
Male	10 (7.9)	63 (50.0)	4.048 (0.848-19.316)	0.0717**
Female	2 (1.6)	51 (40.5)		
Dog in the household				
Yes	10 (7.9)	61 (48.4)	4.344 (0.911-20.726)	0.0660**
No	2 (1.6)	53 (42.1)		
Cat in the household				
Yes	4 (3.2)	7 (5.6)	7.643 (1.841-31.729)	0.0111**
No	8 (6.3)	107 (84.9)		
Onychophagy				
Yes	1 (0.8)	23 (18.3)	0.3597 (0.044-2.932)	0.4599**
No	11 (8.7)	91 (72.2)		

IC: Confidence Interval 95% * Chi-square ** Fisher's Exact Test

Table 2
Results of the final multivariate logistic regression model including risk factors associated with anti-*Toxocara* spp. IgG antibodies in children from different socioeconomic classes from Presidente Prudente municipality, São Paulo State, Brazil

	Odds Ratio (95% CI)	*p*-value
A. Total Population		
Gender (male)	9.031 (0.094 - 0.608)	0.003
Familiar income	11.420 (0.273 - 0.708)	0.001
Cat in the household	12.449 (2.081 - 12.994)	< 0.0001
B. Disadvantaged		
Gender (male)	4.596 (0.002 - 0.755)	0.032
Familiar income	12.541 (0.010 - 0.265)	< 0.0001
Cat in the household	5.144 (1.493 - 244.092)	0.023
C. Middle-class		
Gender (male)	4.516 (0.064 - 0.895)	0.034
Familiar income	5.710 (0.224 - 0.863)	0.017
Dog in the household	4.907 (1.441 - 395.882)	0.027
Cat in the household	6.224 (1.424 - 18.988)	0.013

Most of the DC families (53.2%) received between and two times the minimum salary. In MC 41.3% mentioned having between and six and ten times the minimum salary (minimum salary reference in São Paulo, Brazil, is equivalent to approximately US$ 188.00). It was observed that the frequency of *Toxocara* spp. infection was higher in individuals whose family had the lowest monthly family income, either for total population (p = 0.0013) and DC (p = 0.0003) receiving from one up to two minimum salaries, and for MC subgroup (0.0009), in which the lowest monthly income was three to four times the minimum salary. According to Table 2, monthly family income was a socioeconomic protective factor for toxocariasis in the total population and in both subgroups.

All 252 parent/guardians interviewed reported having a drinkable water supply. Almost the total population studied claimed to have good sanitary conditions. In the MC group, 100% had good sewage services. In the DC group, 2.4% (3/126) considered the service as precarious.

Onychophagy was associated with infection in children belonging to the DC subgroup by bivariate analysis, but it was not confirmed by multivariate one.

Some variables studied were not associated to toxocariasis (Table 1). Only two parents/guardians, in each group, declared that their children received raw meat in their diet, and a dirty backyard in the house was reported by 87.70% (221/252). In seropositive children, frequency of backyard was 87.50% (14/16) in DC and 91.77% (11/12) in MC.

Eosinophilia in peripheral blood was detected in 27.78% (70/252) of population evaluated. Seven (10.0%) out of 70 children with eosinophilia were considered positive for ELISA *Toxocara* spp. test, six belonged to DC and only one to the MC group. There was no association between *Toxocara* seropositivity and eosinophilia, neither for the total population (p = 0.8257; 95% CI = 0.3450-2.103) nor for the MC (p = 0.2931; 95% CI = 0.03014-1.966)) nor for DC (p = 0.5626; 95% CI = 0.4903-4.362) children.

DISCUSSION

The main aim of our study was to compare the risk factors in different socioeconomic classes by investigation of two groups of children: one assisted by the Public Health, considered as disadvantaged children, and the other one assisted by Private Health Service, representing the middle-class.

There is a wide range in prevalence for child toxocariasis worldwide, ranging from 4.6% in the United States[28] to 57.5% among aboriginal schoolchildren of Taiwan[13], similar to that observed in the urban infant populations of Brazil, ranging from 8.7%[30] to 54.8%[16]. In our study, the prevalence in the total population was 11.1%. Surprisingly, in spite of the frequency of anti-*Toxocara* spp. antibodies in DC children being considered higher than in MC ones, there was no significant difference between the two studied groups.

Toxocariasis has been related to the hygienic conditions found among people from different socioeconomic classes, particularly in those with the lowest levels. In Northern Spain, there was a higher difference in the prevalence for toxocariasis among socioeconomically disadvantaged and middle-class children[7]. Similar results were observed in Brasilia, Brazil[6], where the frequency of anti-*Toxocara* antibodies in disadvantaged children assisted by the Public Health System (21.8%) was higher than that registered in middle-class children assisted by a Private Health System (3.0%). In both the Spanish and Brazilian studies, low income and educational level, and low standard of personal and environmental hygiene were considered as influencing the transmission of the zoonosis.

In our study, educational and economic levels in DC were significantly lower than MC, and the frequency of antibodies was inversely proportional to the monthly family income. Considering the groups, the scholarship was associated with the MC children whose mother declared to have elementary scholarship. However, logistic regression analysis indicates that a high family income turns out to be a protective factor for toxocariasis both for total population and for subgroups. The higher seroprevalence has been associated with low income and educational of households[17,20,33]. In Argentina, a prevalence of 37.9% was reported in children, and the investigators considered that the high prevalence was consistent for an infant population of low socio-economic level[3].

As reported previously[27], most of the putative risk factor associations became no longer significant after adjustment for confounding covariates by using multivariate analysis. In our study, most of the population interviewed declared to have a drinkable water supply, good sanitary conditions and to have good sewer service, making it impossible to evaluate the influence of these variables as risk factors in toxocariasis. Nevertheless, the sanitary facilities may be one of the plausible causes for the low prevalence of anti-*Toxocara* spp. antibodies in the studied population. Thus, we consider that the low frequency of antibodies in the population studied could be more related to the good sanitary facilities than to the population of the city. In Campinas, São Paulo State, Brazil, sanitary facilities was the variable correlated with positive serology for

anti-*Toxocara* antibodies. Individuals living in houses connected to the sewer network showed lower risks of infection[4].

There is a controversy with regard to the influence of the age of children in the frequency of anti-*Toxocara* antibodies. In our study, there was no association between the age of children and ELISA positive test, coinciding with those results reported previously in Brazil[2,8,16], Peru[12], Taiwan[13,14] and Sri Lanka[15].

Data in literature has described that gender was not associated with *Toxocara* spp. infection in pediatric populations[3,4,8,13,15,22,30,31]. However, to be a girl was pointed out as a protective factor for toxocariasis in our study. It is possible to consider that boys were more prone to become infected by *Toxocara* spp., corroborating the data obtained in an investigation carried out in Peru, which reported an association between toxocariasis and male children[12]. Male children in particular are more susceptible to toxocariasis due to the behavior of playing more frequently in recreational public areas whose soil is contaminated by infective eggs[1].

In the present study, onychophagy was associated, by bivariate analysis, with infection in children belonging to the disadvantaged subgroup. However, the variable was not considered as a risk factor by logistic regression. The questions concerning onychophagy/geophagia were perceived as the most difficult for interviewed individuals. It is possible that they confused the hand-to-mouth action as geophagia as well as onychophagia, which consequently led them to give us a non-confident answer. This same observation was also reported in a study carried out previously[16], in which there was an association between playing with sand and *Toxocara* spp. antibodies, but no association between toxocariasis and onychophagy/geophagia.

Another variable very controversial for the acquisition of toxocariasis by children is related to contact with dogs or cats in the household. Some authors reported a high frequency of antibodies anti-*Toxocara* spp. in subjects who raise dogs[13,16,18], cats[3,22], or both[31]. On the other hand, it has been considered that both owners and non-owners of dogs are equally at risk of being infected[8,24,31].

In our investigation, the presence of cats in the home was associated with the seropositivity for total population and for both groups studied. In addition, owning a dog was considered to be a risk factor only for middle-class children. These data corroborates the idea that either prevalence estimates or risk factors for toxocariasis may be a reflection of difference in characteristics of sampled populations. These findings suggest that it would be possible that the pets that were raised indoors allowing a close contact with the middle-class children.

Several studies involving children have been described concerning this point, showing an association[15,16,24] or not[3,6,8] between eosinophilia and toxocariasis. In our study both eosinophilia and ingestion of raw meat were not associated with pediatric toxocariasis. Due to the high number of pets in households, it may be possible to assume that the main source for *Toxocara* spp. infection for children was related to the contact with contaminated soil, while playing in the backyard of their houses.

In conclusion, this study emphasizes the importance of comparing different socioeconomic groups in the same geographic region. The data show also how parental education is important to prevent toxocariasis in the infant population. Thus, educative programs should be focused both for pediatric and adult individuals. Furthermore, discussion among experts in toxocariasis should be considered to promote standardization of epidemiological studies, in order to improve the comparison of results of surveys from different regions and to ensure the best measures to prevent the disease in the population.

RESUMO

Fatores de risco e protetores para toxocaríase em crianças de duas diferentes classes socioeconômicas do Brasil

A finalidade do presente estudo foi avaliar a prevalência de anticorpos anti-*Toxocara* spp. em crianças de duas diferentes classes sociais do município de Presidente Prudente, São Paulo, Brasil, e os fatores protetores e de risco associados à toxocaríase. Foram incluídas no estudo 126 crianças de classe média (CM) e 126 de baixa renda (BR). O teste ELISA foi realizado para avaliar a seroprevalência. Um questionário foi aplicado aos pais ou responsáveis pelas crianças para análise dos fatores protetores e de risco. A prevalência na população foi de 11,1%, sendo de 9,5% (12/126) e 12,7% (16/127) para os subgrupos CM e BR. A seropositividade foi inversamente proporcional à renda familiar. Observou-se que uma alta renda familiar foi considerado um fator de proteção tanto para a população total como para ambos os subgrupos CM e BR. Da mesma forma, ser criança do sexo feminino foi outro fator de proteção para a população total e para os dois subgrupos. Possuir gato foi um fator de risco para a população total e para os dois subgrupos estudados, enquanto que possuir cão foi considerado como fator de risco apenas para as crianças de classe média. Os fatores protetores e de risco podem ser diferentes em uma mesma população a depender do estrato social. Dessa forma, é relevante avaliar esses fatores independentemente para diferentes classes sócio-econômicas para elaboração de futuros estudos e programas de prevenção à infecção humana por *Toxocara* spp. e outros geohelmintos.

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Received: 21 September 2010
Accepted: 7 December 2010