Seroprevalence of Larva Migrans of *Toxocara canis* and Evaluation of Associated Risk Factors Among Children in a Mexico-United States Border Region

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ABSTRACT

This study was performed to estimate the seroprevalence of larva migrans of *Toxocara canis* and identify associated risk factors among children in Mexicali, Mexico. Blood samples (n = 288) were collected randomly from children examined at the IMSS Clinical Laboratory and serum was tested by ELISA. The adjusted seroprevalence of toxocarosis was 10.6% (95% CI, 4.6%-12.7%). The factors evaluated were: 1) number of family members, 2) frequency of child's visits to parks, 3) administration of anthelmintics to dog(s), 4) indoor/outdoor status of dogs, 5) living conditions of dogs, and 6) dog's mobilization between house and street. None of the factors showed association with larva migrans. The study emphasizes the importance of maintaining a routine deworming schedule for dogs, limiting the access of dogs into public parks, and educating the public on the health hazards associated with failure to remove pet droppings from park grounds and other public areas.

INTRODUCTION

Toxocarosis is a zoonotic disease and a public health concern in most countries, although the prevalence of the disease is unknown in many areas.[1] *Toxocara canis* is recognized as the main causative agent of larva migrans in humans. The larvae can invade several different tissues causing a variety of clinical manifestations, including visceral larva migrans and ocular larva migrans, which are primarily seen in children.[2]

Humans (mainly children) become infected by accidental ingestion of embryonated eggs present in soil contaminated with dog feces.[3] The embryonated eggs become infective after 9-11 days at 24°C or 3.5-5 days at 30°C. In areas with dry sandy soil and temperatures higher than 37°C, the larva dies before becoming infective, while regions of similar temperatures with clay soil allow larval survival for up to 2 years.[4,5]

In humans, the seroprevalence of larva migrans due to *T canis* has been associated with the prevalence of *T canis* in dogs, the frequency and type of contact between humans and dogs, geophagy (typically in

reference to children), playing in parks, and contact with objects contaminated with infected dog feces.[3,6-8] A high seroprevalence has been found in developing countries, and a prevalence varying between 4% and 8% for visceral larva migrans in children has been reported in the United States.[3,9] In México City, a seroprevalence of 7.5% has been reported in children between 6 and 13 years of age.[10] However, there are no data that reflect the actual prevalence in children of all ages throughout Mexico.

Due to sequestration of the larvae within tissues, serology is the only method by which the prevalence of *Toxocara canis* can be determined, with enzyme-linked immunosorbent assay (ELISA) being the most frequently used method.

Preliminary studies have shown a high frequency (62.5%) of *T canis* eggs in soil sampled from parks and playgrounds in the Mexicali area[11] as well as a high seroprevalence in dogs (56.1%; 95% CI, 49.6%-62.5%).[12] These findings suggest an existing risk of infection with larva migrans due to *T canis* within the local population in Mexicali. Our goal was to assess the seroprevalence of larva migrans due to *T canis* and associated risk factors in children residing in the urban area of Mexicali, Baja California, a Mexican-American border region.

MATERIAL AND METHODS

Study Design and Characteristics of the Population

A cross-sectional study was conducted. The reference population was children between 1 and 12 years of age from the urban area of Mexicali, Baja California, Mexico. All children included in the study were evaluated at the Clinical Laboratory of the Mexican Institute of Social Security (IMSS), Gynecology-Pediatric/MF Hospital No.31, a Governmental Health Institution. Approximately 58% (437,000/760,000) of the population of Mexicali is covered by a Governmental Health Institution, and most people (~87%) are covered by the IMSS (381,501/437,000). In Mexicali, there are 5 hospitals belonging to the IMSS and 1 laboratory where all patient samples are submitted for routine clinical analysis. The IMSS laboratory serves an average of 120 patients of all ages daily.

Children between 1 and 12 years of age of both genders were included. All children had been referred to the IMSS lab by their family physician, and were included in the study regardless of original presenting complaint. Blood samples were collected randomly from 288 children seen by the Clinical Laboratory between December 2001 and March 2002. Samples with hemolysis or insufficient volume were excluded. The sample size was based upon random sampling assuming a seropositivity of 25% for *T canis* in the population,[13] at a 95% level of confidence.[14] Samples were obtained randomly from a comprehensive list of all children seen at the lab during the specified time frame. The blood samples were collected 3 days a week, and the day of sampling was randomly selected from Monday through Friday. Of the total number of children included in the study, 17.3% (50/288) were asymptomatic. Informed consent was obtained from all parents who allowed their children to participate in the study.

Collection of Information

A questionnaire was designed to collect data on each child and its association with dogs. The questions covered: 1) general information pertaining to the child: address, gender, age, number of family members, frequency of child's visits to parks, dog ownership; 2) dog handling: number of dogs in the house, anthelmintics administration, and dog mobilization between house and street; and 3) living conditions of dogs: type of surface (bare ground or grass, or concrete), indoor/outdoor status of dog. Data obtained from most questions were dichotomous. A general overview of the questionnaire is shown in Table 1. A detailed questionnaire form in Spanish is available from the corresponding author upon request.

Prior to application, the questionnaire was validated in terms of degree of difficulty. The questionnaire was completed by 30 randomly selected parents of children seen

Table 1. Overview of the epidemiological questionnaire.

Section	Main Variables
1. General information on the child	Address (excluded from final data), gender, age, number of family members, frequency of child's visits to parks, and dog ownership.
2. Dog handling	Number of dogs in the house, anthelmintics administration, mobilization of dog between house and street.
3. Living conditions of dogs	Indoor/outdoor dog; type of surface (bare ground with or without grass, or concrete).

by the Clinical Laboratory during an average 8-hour work day. The values assessing difficulty ranged from 1 through 5 (1 = easy, 5 = difficult), and 90% of the included parents rated the questions as "1." The questionnaire was then applied to the parents of all children included in the study by the first author and the lab technician. The study was performed following the guidelines of the General Health Law regulating human subject research, and following the principles of Ethics established by the Declaration of Helsinki (1964) developed by the World Medical Association, reviewed in Tokyo (1975), Venice (1983), and Hong Kong (1989).

Blood Collection

Blood samples were collected by certified personnel at the IMSS Laboratory. Three mL of blood were collected from the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in tubes containing clot activator (Vacutainer® SST). Each sample was properly labeled and centrifuged at 3,500 RPM for 10 minutes to separate the serum. The serum was transferred to 1-mL vials, labeled and stored at -20°C until further analysis.

Serology

An indirect ELISA (*Toxocara* larva Microwell ELISA), with a guaranteed 93.3% sensitivity and 87.5% specificity, was performed following the manufacturer's protocol. Sera from patients were diluted 1:64 in dilution buffer and assessed against positive and negative control sera provided by the manufacturer using an optical density (OD) at 450 nm. An absorbance of less than 0.3 OD units was considered negative, while an absorbance equal to or greater than 0.3 OD units was considered a positive reading.

Statistical Analysis

All statistical analysis was performed using the Statistical Analysis System (SAS) for Windows version 9.1.[15] Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed. The adjusted prevalence and its 95% confidence interval (CI) were obtained using the Rogan-Gladen estimator.[16] The independent variables were initially tested for univariate associations with the outcome variable using a chi-squared test.[17]

The relationship between a risk factor and the serological status (positive or negative) was evaluated using odds ratio in a univariate logistic regression analysis.[18]

The risk factors evaluated includes the child's gender, age, number of family members (>3, ≤3), frequency of visits to parks (frequent >1, rare ≤1 visit per year); number of dogs in the household (1, ≥2), anthelmintics administration (yes, no), dog mobilization between house and street (yes, no), place where dog lives (indoor, outdoor), and type of surface where dog is kept (bare ground with or without grass, concrete floor). The age of children was categorized by scholarity (not attending school = ≤3 years, kindergarten = 4-6 years, elementary school I = 7-9 years, elementary school II = ≥10 years). Any statistically significant relationship between independent and dependent variables was assessed using the Wald test.[18]

RESULTS

Of the 288 serum samples analyzed, 46.9%

(135/288) were from girls and 53.1% (153/288) from boys. The adjusted seroprevalence of *T canis* larva migrans in the study group was 10.6% (95% CI, 5.4%-13.4%) with a sera dilution factor of 1:64. The children were divided into 4 age groups according to scholarity: ≤3 years, 4-6 years, 7-9 years, and 10-12 years. The relationship between *Toxocara* seroprevalence and gender was evaluated, and no statistically significant difference ($P > 0.05$) was observed between females and males. Also, no statistically significant difference ($P > 0.05$) was found when seroprevalence values were compared among the different age groups (Table 2). Furthermore, the difference found among the age groups in males was not statistically significant.

When dog ownership was evaluated as a risk factor associated with seropositive children, 5.5% of children that owned dogs (183/288) were seropositive to larva migrans due to *Toxocara*, while 14.3% of the children without dogs in the household were seropositive (105/288). However, no significant association was observed (OR = 0.347 [0.15-0.80]) between dog ownership and seropositivity to larva migrans due to *T canis* in the present study (Table 3).

Univariate logistic regression analysis showed no association between any of the evaluated risk factors and seropositivity for *T canis* larva migrans

DISCUSSION

The adjusted seroprevalence of 10.6% (95% CI, 4.6%-12.7%) for *T canis* larva migrans in children seen at the Clinical Laboratory of the IMSS Hospital in Mexicali B.C. Mexico, is similar to that reported in children in the United States (4.6%-10.2%)[19,20] and Mexico City (7.3%),[10] but lower than values reported in children from other Latin American countries. In Bolivia, a seroprevalence of 34.6% for larva migrans due to *T canis* has been reported previously,[21] with similar numbers found in Argentinian (37.9%),[22] and Brasilian (38.8%) children.[23] Seropositivity to *T canis* was 57.5% among aboriginal schoolchildren in the mountain areas of

Table 2. Adjusted seroprevalence of larva migrans due to *Toxocara canis* in children residing in Mexicali, Baja California, Mexico, according to age and gender.*

Age (years)	Male (+)	Adjusted Prevalence %	CI 95%	Female (+)	Adjusted Prevalence (%)	CI 95%	Total	Total Prevalence (%)	CI 95%
≤3	3	11.1	4.9-13.2	0	0.0	0.0-0.0	3	5.2	1.4-7.2
4-6	5	14.9	7.5-16.8	7	17.5	9.2-19.2	12	16.3	8.4-18.1
7-9	2	5.9	1.8-7.9	5	13.6	6.6-15.6	7	15.9	8.1-17.8
≥10	2	12.2	5.7-14.2	1	5.2	1.4-7.2	3	9.9	4.2-12.0
Total	12	10.8	4.8-12.9	13	10.4	4.5-12.4	25	10.6	4.6-12.7

*Equal letters by column into age indicate no differences ($P > 0.05$).

Table 3. Association between children seropositive to *Toxocara canis* larva migrans and dog ownership.

	Dog in Household				No Dog in Household			
Gender	n	ELISA (+)	Adjusted Prevalence (%)	C.I. 95%	n	ELISA (+)	Adjusted Prevalence (%)	C.I. 95%
Male	89	5	6.8	2.3 – 8.8	46	7	18.7	10.0 – 20.3
Female	94	5	6.4	2.1 – 8.5	59	8	16.6	8.6 – 18.4
Total	183	10	6.6	2.2 – 8.7	105	15	17.5	9.2 – 19.2

OR = 0.347 (0.15-0.80).

north-eastern Taiwan according to 1 study. In Caracas, Venezuela a seroprevalence of 66.6% has been reported in children[24] and 81% seropositivity has been documented in Colombian studies.[25] In other regions, such as New Zealand, a much lower seroprevalence has been reported (0.7% ± 1.65%).[26] A possible explanation for the relatively low seroprevalence to larva migrans due to *T canis* in children in Mexicali is the hot and dry weather conditions of the region which may not allow the development of the infective stage of the *T canis* eggs.

Different studies have shown a higher frequency of *T canis* seroprevalence in male individuals,[2,10,27,28] which has been associated with behavioral differences in how boys and girls tend to play. Also, 1 study reported predominance of toxocarosis seroprevalence in adult female patients, and male children.[10,28] Previous studies have shown that when age is evaluated as a risk factor that may influence the seroprevalence of larva migrans due to *T canis*, no significant difference is found between children of varying ages.[29-31] These findings are divergent when compared to a number of other studies that have established that seropositivity increases with age.[20,32] Others studies have reported that this syndrome most frequently affects children between 2 and 5 years of age.[33] Furthermore, this zoonosis appears to predominantly affect children between a few months and 4-5 years, because children in this age group tend to practice pica and geophagy more commonly.[23,34,35] Seropositivity of larva migrans due to *T canis* can, however, be remarkably dependent on age, according to a study focused on Caribbean communities, with higher values (40%-60%) in children from 5 to 15 years of age compared to lower values in adults.[36] A definite reason for the observed difference could not be determined, but previous studies have suggested that behavioral differences between genders play an important role. Traditionally, the play behavior of boys leaves them more susceptible to infection. However, it cannot be ruled out that some unidentified aspects of how girls engage in play, through this particular region, might play a role in their increased seroprevalence.

In regard to dog ownership as a potential risk factor of larva migrans due to *T canis*, no significant association was observed between owning a dog and seropositivity in the present study—a finding which concurs with previous studies.[19] Results similar to ours have been reported from Nigeria.[31] Another study also reported that dog ownership is a significant risk factor in rural children, but not in urban children, which is likely due to the differences in living conditions and the number of dogs owned. However, dog ownership as a risk factor in toxocarosis remains a controversial topic.[30] Some researchers have reported a higher seroprevalences in individuals that have regular contact with dogs.[2,37-39] Dogs infected with *T canis* may infect people through direct contact. *Toxocara canis* eggs were found in the hair of 25% of the dogs tested in Ireland.[40] While other studies have failed to find any association between direct contact with dogs and seropositivity for *T canis*,[31,41-43] the prevalence of toxocarosis in humans has been directly related to the prevalence in dogs as well as contact with contaminated soil containing *T canis* eggs.[23,44] Some, however, do not consider direct contact with animals

a potential risk, because embryonation of excreted *T canis* ova requires a minimum of 2 weeks. This is the same reason there is no expected relationship between seropositivity and exposure to dogs and cats in the household.[45]

A relatively higher seroprevalence was expected in the current study, because results from a previous investigation had shown a high degree of soil contamination with *T canis* eggs in parks and playgrounds, indicating a high prevalence among dogs in the area.[28,33,44] The low seroprevalence value reported might be due to the fact that the study only included children from the urban region, while most other studies have reported that seroprevalence typically tend to be higher in rural areas. Differences in the presence of risk factors may be one of the explanations for rural/urban differences.[3] More importantly, the extreme environmental conditions in Baja California, including high temperatures and low humidity, may have reduced the viability of the infective larva.

This is the first structured study to report on the seroprevalence of larva migrans due to *T canis* in children residing in the urban border-region area of Mexicali, B.C. Although the detected seroprevalence is low, it still calls for enforcement of preventive and control measures to eliminate this parasite and to inform the population about the negative consequences of this disease. Furthermore, the study emphasizes the importance of maintaining a periodic deworming schedule for dogs; limiting the access of dogs into public parks, as well as educating dog owners about removal of their dog's feces from public places.

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