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Seroprevalence and Risk Factors Associated with Larva Migrans of *Toxocara canis* in Dogs from Mexicali Baja California, México

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Abstract: We conducted a cross-sectional study to estimate the seroprevalence to larva migrans of *Toxocara canis* in dogs of the urban area of Mexicali Baja California, México, for a 12 month period, from dogs attended at private veterinary clinics. Also, the risk factors associated with the seropositive values were evaluated. Sera were tested for antibodies to larva migrans of *T. canis* using a commercially available ELISA test. The dog owners were interviewed utilizing an epidemiologic questionnaire about sex, size, age, anthelmintic treatment, place where dog lives, access of dog to the street and number of dogs in the house. An adjusted seroprevalence of 56.1% (95% CI 49.6-62.5%) was obtained, while the apparent prevalence was 57.8%. Seroprevalence values were found to differ significantly ($p<0.05$) among dogs according to age and size. The risk factors associated with seroprevalence to *T. canis* were the number of dogs in the house and the access of dogs to the street, with OR of 1.90 ($p<0.01$) and 2.42 ($p<0.01$), respectively. The high seroprevalence of *T. canis* found in the present study suggests that dogs may constitute an underestimated risk of transmission for the human population and also emphasizes the importance of maintaining a periodic deworming calendar for dogs, limit the access of dogs into public parks, as well as educating dog owners about removing dog feces from public places.

Key words: *Toxocara canis*, larva migrans, toxocarosis, zoonosis, seroprevalence, epidemiologic

INTRODUCTION

Toxocarosis is a zoonotic disease caused by *Toxocara canis (T. canis)*. In dogs, the disease is characterized by general discomfort, intermittent diarrhea, anemia, respiratory and neurologic symptoms. Humans can become infected by ingesting embryonated Toxocara eggs and the symptoms can vary from intestinal discomfort, to respiratory, visceral, muscular, ocular, neurological, or cutaneous symptoms, but rarely death (Quiroz, 1999). The presence of *T. canis* in dogs has been documented in several countries, including England (Read and Thompson, 1976; Richards and Lewis, 2001), the United States (Lawrence *et al*., 1979; Acha and Szyfres, 1986; Georgi and Georgi, 1992; Schantz, 1994) and México (Luna, 1981; Cruz *et al*., 1993; Martínez *et al*., 1998), were seroprevalences between 3 and 99% have been reported. In Mexicali, the disease was documented for the first time by Luna (1981), who found by necropsy the adult parasite in the small intestine of 66% (330/500) of dogs examined at the Animal Control Facilities in the city of Mexicali Baja California. To date the actual seroprevalence of *T. canis* and the risk factors associated with this infection is unknown in Mexicali, México and the purpose of the present study was to document the status of the infection and the factors associated with its presence in dogs examined at private veterinary clinics.

MATERIALS AND METHODS

Study design and characteristics of the population: A cross-sectional study was conducted to determine the seroprevalence of *T. canis* and the risk factors associated in dogs. The reference populations were dogs that were attended at any of the 33 private veterinary clinics participating in the study from the urban area of Mexicali Baja California, Mexico. In Mexicali there are a total of 80 private veterinary clinics distributed around the city that in average examine 6 dogs daily. A total of 33 clinics accepted to participate in the study and each clinic provided a total of 10-11 blood samples. Dogs were randomly selected. The eligibility criteria were those dogs from 1 month and older of any sex and breed that attended a private veterinary clinic for any circumstance and whose

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owners agreed to participate in the study. The owners were informed of the purpose of the study and the minimal risk involved. Blood samples were collected from May 2001 to April 2002.

Epidemiologic questionnaire: A questionnaire was designed to capture data associated with dogs. The questions included. General information: address, dog gender, age and size, number of family members. Dog handling: Number of dogs in the house, anthelmintics administration and place where dog lives: Indoor or outdoor dog or both, bare ground or grass, concrete floor, access of the dog to the street (movement of dog between house and street). The outcomes of most of the questions were dichotomous. Previous to its application, the questionnaire was validated by measuring the degree of difficulty of the questions. For this, the questionnaire was answered by 30 owners of dogs and the values for the questions were from 1 to 5 (1 = easy, 5 = difficult). The resulting mean value obtained was 1.2. The questionnaire was applied by the Veterinarian from each private clinic.

Blood collection: Blood samples were collected by a certified Veterinarian at each private clinic. Briefly, 3 mL of blood was collected by punction of the cephalic vein after proper asepsis of the area with isopropyl alcohol and placed in tubes containing clot activator (Vacutainer®SST). Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred to 1 mL vials, labeled and stored at -20°C until testing. The hemolized or insufficient blood samples were excluded.

Serological analysis: A commercially available indirect Enzyme Linked Immunobsorbent Assay (Toxocara larva Microwell Serum ELISA, IVD Research Inc.) for use in humans was modified to detect antibodies against *Toxocara canis* in dog sera. Briefly, microtiter plates containing an antigen from *larva migrans of T. canis* were incubated with 100 μL of dog sera (1:20 dilution) and the positive and negative controls. All samples were tested in duplicates. After 10 min incubation, the plates were washed three times with washing solution. The immunoclomplexes formed were detected by the addition of 100 μL of rabbit anti-dog IgG peroxidase conjugate, at a 1: 1000 dilution (SIGMA Laboratories). After washing, 100 μL of tetrametilbenzidina was added to all the wells and incubated for 5 min. To stop the enzymatic reaction 100 μL of stopping solution (sulfuric acid) was added. The optical density was read at 450 nm using a spectrophotometer. For the positive control a pool of 8 serum samples was used. These sera were collected from dogs confirmed positive to *T. canis* eggs by coproparasitoscopical examination using the flotation technique described previously (Thienpont *et al*., 1985).

The rabbit anti-dog peroxidase conjugate was used instead of the Protein A peroxidase conjugated used for human sera. The specificity and sensitivity of this test was 87.5 and 93.3%, respectively. Absorbance readings <0.3 Optical Density (OD) units were considered negatives, while = 0.3 OD units were positives.

Statistical analysis: In order to estimate the seroprevalence of *T. canis*, a random sample of 344 dogs was taken, considering an estimator of p = 0.25, with a 95% confidence and a 5% precision (Scheafer *et al*., 1987). The value of P estimator was based on previous findings of 0.66 *T. canis* seroprevalence in dogs (Luna, 1981). All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1 (SAS, 2004). 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 Rogan-Gladen estimator (Greiner and Gardner, 2000). The independent variables were initially tested for univariate associations with the outcome variable using a chi-squared test (Walker, 1997).

The relationship between the risk factor and serological status (positive or negative) was evaluated using Odds Ratio (OR) in an univariate logistic regression analysis (Allison, 1999). The risk factors evaluated were size, gender, age, number of dogs in the house ($1 \geq 2$), anthelmintics administration (yes, no), place where dog lives (indoor, outdoor), ground where dog lives (bare ground or grass, concrete floor) and movement of dog between house and street (yes, no). The hypothesis to prove significance of the independent variables in the model were performed using the Wald test (Allison, 1999).

RESULTS

Seroprevalence to *T. canis*: An adjusted seroprevalence of 56.1% (95% CI 49.6-62.5%) was obtained using the Rogan-Gladen estimator (Greiner and Gardner, 2000) with a sera dilution factor of 1:20; while the apparent prevalence was 57.8%.

No significant differences ($p>0.05$) in the prevalence of *T. canis* were observed between female and male dogs (61 vs. 55.7%, respectively).

Significant differences ($p<0.01$) were observed in apparent prevalence among dogs of different age. Dogs of one year old or younger had lower seroprevalence compared to older dogs (41.4 vs. 73.7%, respectively).

Table 1: Apparent seroprevalence of larva migrans of *Toxocara canis* in dogs from Mexicali, B.C., Mexico, according to size

Size	n	Seroprevalence (%)	p	Odds ratio	CI 95% Wald
Small	123	43.9	--	1.00	Reference
Medium	156	65.3	<0.05	2.39	1.4-3.9
Big	65	66.1	<0.05	2.47	1.3-4.6
Total	344	57.8			

Adjusted prevalence: 56.1% (95% CI 49.6-62.5%)

Table 2: Beta coefficient for logistic regression model and risk factors associated with larva migrans of *T. canis* in dogs from Mexicali, B.C., Mexico

Variable	Beta coefficient	SE [a/]	P>χ^2	OR [b/]	CI 95% Wald [c/]
Intercept	-1.173	0.98	NS	1.00	0.59-1.71
Number of family members	0.006	0.27	NS	1.00	0.59-1.71
Number of dogs in the house	0.645	0.27	*	1.90	1.122-3.27
Anthelmintics administration	-0.964	0.27	**	0.38	0.219-0.652
Place where dog lives	0.184	0.44	NS	1.20	0.498-2.874
Ground where dog lives	-0.371	0.31	NS	0.69	0.367-1.272
Movement of dog	0.885	0.29	**	2.42	1.383-4.248

n = 344. NS = p>0.05, * = p<0.05, ** = p<0.01, [a/]SE = Standard error, [b/]OR = odds ratio, [c/] CI 95 % don't incluye 1, means OR was statistically different (p<0.05)

A significant association (p<0.01) was observed between dog size and seropositive to *T. canis*. So that 2.3 more *T. canis* positive cases were observed in medium size dogs and 2.4 more cases in large size dogs, with respect to small size dogs (Table 1).

Risk factors: The number of dogs in the house and the access of dogs to the street were significant risk factors (p<0.05) that influenced the prevalence to *T. canis*. Using anthelmintic treatment, number of members in the family and place where dog lives were not significant (p>0.05) risk factors (Table 2). A large number of dogs present in the house favors the presence of cases of larva migrans (adjusted OR = 1.9) when compared with those houses that only had one dog. The fact that a dog have access to the street represented 2.4 more risk of a positive result for larva migrans of *T. canis*, when compared with dogs that do not have contact with street dogs (Table 2).

DISCUSSION

An adjusted seroprevalence of 56.1% (95% CI 49.6-62.5%) to *T. canis* was found in dogs examined at private veterinary clinics in the urban area of Mexicali Baja California. This seroprevalence is higher than those reported by other researchers, were values between 13 and 45% have been observed (Kazacos, 1978; Acha and Szyfres, 1986; Richards *et al*., 1995; Overgaauw, 1997; Martínez *et al*., 1998; Overgaauw and Boersema, 1998; Eguia-Aguilar *et al*., 2005). These data reveal that the disease is endemic in this region. However, the ELISA used in this study only measured antibodies against *T. canis* and the presence of antibodies does not mean that the animal was sick at that specific time. Thus, not all dogs that had a positive result would have clinical symptoms, but yet instead it indicates that they have been exposed to *T. canis* and therefore there is a risk for humans to become infected by the ingestion of embryonated eggs that are shed in dog feces. Furthermore, the presence of toxocarosis in humans is directly proportional to the prevalence of infected dogs (Mizgajska, 2001). In 1981 a prevalence of 66% to *T. canis* was found by necropsy, in dogs confined at the Animal Control Facilities in Mexicali (Luna, 1981), similar to the seroprevalence found twenty years later in the present study, indicating that the risk for the human population is still present and that the implementation of preventive medicine programs are necessary. This study found that dogs > 1 year old showed higher seroprevalences to *T. canis* than those dogs = 1 year old. Similar results have been previously reported (Dumenigo *et al*., 1994; Laird-Perez *et al*., 2000). These results may be due to the fact that dogs are constantly getting infected with *T. canis* eggs from the contaminated environment as they grow up. However, another study has shown that seroprevelences were higher in younger dogs (Rubel *et al*., 2003). In addition, no differences in seroprevalences were observed between female and male dogs, similar to results previously reported (Chiejina and Ekwe, 1986; Dumenigo *et al*., 1994; O'Lorcain, 1994; Fontanarrosa *et al*., 2006). However, there is data that showed higher seroprevalences in male dogs (Rubel *et al*., 2003). Moreover, it is important to consider that females can pass the larva migrans via transovarial to the fetus (Quiroz, 1999). With respect to dog size, this study showed a higher seroprevalence in dogs of medium and large size and lower seroprevelence in small size dogs. A possible explanation for this result is that larger dogs ingest a bigger amount of food increasing the chances of getting infected if the food is contaminated with parasite eggs. This suggest that size can influence the presence of toxocarosis because of the larger amount of contaminated food ingested and the fact that infection can only occur by ingestion (Quiroz, 1999). Dogs that had access to the

street had a higher probability of being infected compared to those that did not go out the house premises and that are more likely to receive regular preventive treatments like anthelmintic and also are not exposed to highly parasitical environments. Since toxocarosis was found to be highly prevalent in Mexicali and that in the city exists a high population of street dogs that are infected with *T. canis*, it is necessary that physicians especially pediatricians include the toxocarosis in their differential diagnosis since children up to 5 years old are at a higher risk to become infected due to the practice of geophagy and playground habits in areas contaminated with infected dog feces (Genchi *et al*., 1990; Herry *et al*., 1997; Kazacos, 2000). The presence of a large number of dogs in the house represented a higher risk of positive cases to larva migrans maybe because is easier to maintain clean an area with one dog and the presence of more dogs represents more contamination with dog feces that contain *T. canis* eggs (Genchi *et al*., 1990).

CONCLUSION

A highly seroprevalence to *T. canis* was found in dogs attended at private veterinary clinics in Mexicali. Moreover, there are a large number of street dogs that lack a proper veterinarian care and that most likely are infected with *T. canis*, representing a continuous contamination of the environment with *T. canis* eggs by feces. Implementation of fences at public parks and home yards to prevent the access of dogs may help to decrease the contamination of soil where the *T. canis* eggs find the optimal environmental conditions to become infective for humans. This strategy might not completely eliminate the risk of infection but might decrease the possibility that dogs and children become infected in these places. Furthermore, it is necessary to promote people education, especially dog owners about collecting their dog feces from public places and the importance of using antiparasitic drugs periodically, as well as the health consequences of becoming infected. In addition, the implementation of an anthelmintic administration campaign and population control may be necessary for dogs in Mexicali.

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