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Prevalence of anti-*T. canis* antibodies in stray dogs in Mexico City

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Abstract

Toxocara canis is a common intestinal helminth found in dogs. In humans, it is a cause of *Visceral Larva Migrans* (VLM), a zoonosis rarely studied in Mexico. The aim of this study is to examine, by means of the indirect haemaglutination test (IHAT), the prevalence of antibodies of *T. canis* in the serum of stray dogs in Mexico City.

Methods and materials: 141 stray dog serum samples from three different districts of the city were analyzed: Iztacalco (49), Iztapalapa (49) and Coyoacan (43). In each location three study groups were formed. Group I with 35 dogs (less than a year old), Group II with 91 dogs (ages $1 \leq n < 6$) and Group III with 15 dogs (ages 6 and over). An extract of raw adult *T. canis* worms was used as an antigen. Finally, a modified version of Boyden's IHA serological test was carried out.

Results: Out of the 141 sera, 94 (40 males and 54 females) proved positive (dilution titres of from 1:32 to 1:4096) with a global infection prevalence of 66.7%. The frequency of infected dogs in Iztacalco was 61.2%, 51% in Iztapalapa and 90.7% in Coyoacan. The largest seroreactivity was found in Group II (ages 1–6) with 61 positive tests and a total frequency of 43.3%.

Conclusions: The high seroprevalence of anti-*T. canis* antibodies found in the dogs of the study population is an indicator of the contact which exists between these animals and the parasite. This is the result of the high degree of contamination of the soil of Mexico City with the parasite's eggs. Paradoxically, Coyoacan, with more green areas, is also the most polluted municipality. Statistical analysis confirms this. Dogs seek green areas to defecate. There exists a serious risk for the population of being infected with *Visceral larva migrans*.

Keywords: *T. canis*; Toxocariasis; Serology; Visceral larva migrans

1. Introduction

Toxocara canis is the etiological agent for Toxocariasis, an intestinal parasitosis that affects dogs and other canines (Beaver, 1956; Schantz, 1989; Glickman et al., 1978; Fisher, 2003).

Antibodies produced in dogs as a response to infection by *T. canis* can be detected from 1 to 2 months after birth (Matsumura et al., 1984). This is due to the fact that the puppies are born with parasites because the foetus is infected in the uterus. What happens is that the larvae, previously encapsulated in the mother's tissues,

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are reactivated and, during their migration, cross through the placenta, thus infecting the foetus. Infection subsequent to birth may occur in one of several ways: through the mother's milk, by the ingestion of larvae from eggs left by infected dogs in their faeces or, more rarely, by the ingestion of paratenic hosts which contain the larvae of *T. canis* in their tissues (Sprent, 1958; Overgaauw et al., 1998; Webster, 1958; Glickman and Schantz, 1981).

During its migration through the host's tissues, the larva induces a humoral immune response characterized by high levels of immunoglobulin of types IgG, IgM, IgA and IgE (Matsumura et al., 1984; Elefant et al., 2006). Human Toxocariasis was first described under the name of Visceral larva migrans (VLM) (Beaver, 1956). The larva's migrations produce granulomas in the human liver, lungs, brain and eyes. Sight loss and, in some cases, death results (Good et al., 2004); Teyssot et al., 2005; Eberhardt et al., 2005). VLM is highly related to lack of hygiene and geophagy so that the disease presents itself with higher frequency in infants and small children (Beaver, 1956; Schantz, 1989; Glickman et al., 1978; Sprent, 1958; Matsumura et al., 1984; Elefant et al., 2006).

There are approximately 1,394,000 stray dogs in Mexico City. That is, there is one dog for every seven inhabitants. 90% are in the age of reproduction. Thus, the population increases by 128,000 animals each year. The dogs in this study were chosen because it is government policy to eliminate animals which no longer have owners. This is both because they are carriers of diseases (especially rabies) and because of an increase in attacks on the inhabitants, especially by packs of dogs. The animals in this study were euthanized in accordance with the protocol NOM-033-200-1995 of the Ministry of Health. It should be noted that the 3 canine control centres of the city are situated close to the University. Given the close relationships humans have with dogs and the non-existent information provided to public health officials about the dog's humoral immunity to *T. canis*, it seemed important to measure the prevalence of specific antibodies against the *T. canis* antigen in stray dogs in Mexico City. The indirect haemaglutination test was used for this purpose. The determination of *T. canis* by parasitological methods has already been widely used. There are few studies using the serological approach.

2. Methods and materials

From February 3 to March 11 of 2005 a descriptive seroepidemiological study of the prevalence of the specific antibodies against the *T. canis* antigen was carried out on the blood serum of 141 stray dogs in Mexico City. The city is located at 2400 meters above sea level, it has a mild humid climate with heavy rains during summer and the beginning of autumn. The average temperature is 16.7 °C (INEGI, 2005)

2.1. The collection of the biological material

The dogs used in the study were collected and euthanized in their corresponding Canine Control Centres (CCC) found in three districts of Mexico City: Coyoacan, Iztacalco and Iztapalapa. Socioeconomically, Coyoacan – a middle-class residential area – is superior to the other districts which border extreme poverty. The number of dogs present is: Coyoacan (64,853), Iztacalco (59,965), Iztapalapa (254,387) (INEGI, 2005). Only those in Coyoacan have access to veterinary treatment. Each animal was identified with a tag that showed its gender, breed, approximate age and district of origin. Three age groups were established. Group I with 35 dogs (less than a year old), Group II with 91 dogs (ages $1 \leq n < 6$) and Group III with 15 dogs (ages 6 and over). Their age was established by examining the development of their teeth.

2.2. The collection of the blood samples

A volume of 5 mL of blood was extracted from each dog with a cardiac puncture using vacutainer equipment. (This method was used because the dogs had already been euthanized.) It was left to coagulate at room temperature for an hour to allow retraction before transferring it to the Immunoparasitology Laboratory in the Faculty of Medicine of the UNAM (the National University of Mexico). Once in the laboratory, it was centrifuged at $500 \times g$ for 5 min to obtain serum. Each sample of serum was separated into 0.5 mL aliquots and was stored in freezers until being processed.

2.3. The collection of the adult parasites

These had been obtained previously in the course of carrying out other studies. (Martínez et al., 1997). The process used was as follows. The small intestine of each dog was separated, when eviscerating the euthanized dogs in their corresponding Canine Control Centre, by means of an incision on the right flank, having tied their pyloric and cecal extremities. Subsequently, these organs were placed in wide mouthed glass jars with 600 mL of isotonic saline solution at 0.9%, before being taken to the laboratory.

Each intestine was cut longitudinally and the mucosa was carefully revised for adult *T. canis* worms. These were washed in sterile isotonic saline solution (SISS) at 0.9% and incubated at room temperature for 48 h at 25 C in SISS with penicillin (1000 U.I./mL) and streptomycin (1% mg/mL). Immediately afterwards, the parasites were washed with SISS to eliminate any excess of antibiotics and kept at −20 °C for further use.

2.4. The preparation of the antigens

The antigen extract was obtained by applying the sucrose-acetone method to adult *T. canis* (Beltrán et al., 1974). The protein concentration was determined using Lowry's method (Lowry et al., 1951) and standardized at 3 mg/mL. Finally, the antigens were stored at −20 °C for further use.

2.5. The production of the rabbit control serum

The specific antiserum for *T. canis* was obtained from 3 albino male New Zealand rabbits raised in the animal colony of the Medical Faculty of the UNAM in accordance with the protocol NOM-062-200-1996 of the Ministry of Health. These were immunized with the adult antigen using the following method: on the first day, the right thigh was inoculated with 0.5 mL of an emulsion of 0.5 mL (500 μg of proteins) of the whole antigen and an equal volume of Freund's complete adjuvant. The inoculation was repeated after 2 weeks in the left thigh and again on the 42nd day using several subcutaneous incisions with the incomplete Freunds adjuvant. Five days later, 15 mL blood was collected and the serum separated using a centrifuge at 500 × *g* for 5 min.

The negative control serum was obtained from rabbits raised under similar conditions by the Faculty of Medicine.

2.6. Haemaglutination test

The IHA technique (Boyden, 1951) was used as an immune diagnostic test and the same protocol as the Center of Disease Control (CDC) based in Atlanta, Georgia was followed (Csizmas, 1960;Avrameas et al., 1969; Becht, 1968). In the IHAT, sheep erythrocytes, washed in a buffer of phosphate (pH 7.2), sensitized with tannic acid, were distributed in 96-well micro-titration plates .0.05 μL were placed in each well. Each serum was diluted at different concentrations to determine the quantity of *T. canis* antibodies in each sample. The largest titer was given by the last dilution which showed less than 50% agglutination. In order to be considered positive, the cut off point was taken to be 1:32 or greater. This cut-off point was determined from previous experience, accumulated in the laboratory, in the analysis of human populations. This test, apart from being reasonably economic, has the advantage of detecting a smaller number of false positive cases in open populations (Parija and Sahu, 2003) Elisa for example would have exceeded our budget.

2.7. Statistical analysis

The differences in the distributions, by the municipality of origin, gender, breed and age, of the dogs which showed serological reactions, were obtained using intervals, degrees of association (X^2) and frequency distributions from the data base and the SPSS and JMP 5.0 statistical packages.

3. Results

Table 1 shows the results obtained by IHAT from 141 dog sera studied by means of an antigen of *T .canis*. These are shown by municipality of origin, age group and gender. In Fig. 1 it can be observed that 136 sera showed dilution titers from 1:2 to 1:4096. There were 94 positive sera (66.7%) and the distribution of the titers is given in the figure. Positive dilutions were considered to be those for values of 1:32 or more. 141 stray dogs with an average age of 45 months (S.D. 28.48) made up the sample. Their age was from 45 to 120 months. 76 (53.9%) were male and 65 (46.1%) female. The most

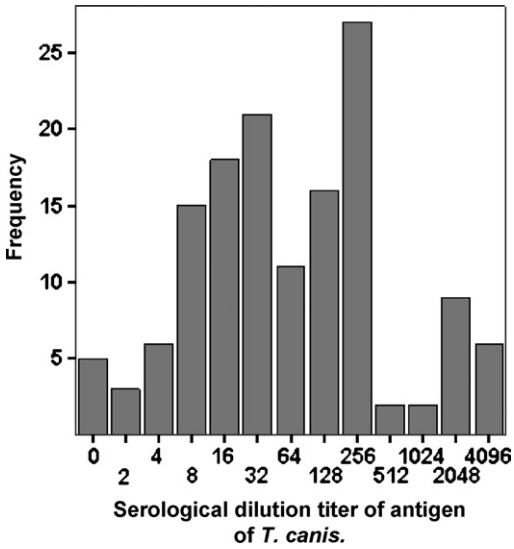

Fig. 1. Frecuency distribution of antigen delution of *T. canis*.

Table 1
Frequency distribution of serologic reactivity by municipality, breed, age and sex of 141 dog sera analyzed by IHAT

Municipality	Breed	Age	Gender	Serological reactivity to *T. canis*		Total
				Negative	Positive	
Coyoacán	Pure	I	F	0	1	1
			M	0	2	2
		II	F	0	1	1
			M	0	3	3
		III	F	0	0	0
			M	0	0	0
	Mixed	I	F	0	6	6
			M	0	1	1
		II	F	2	7	9
			M	2	16	18
		III	F	0	1	1
			M	0	1	1
Iztacalco	Pure	I	F	0	0	0
			M	0	0	0
		II	F	2	1	3
			M	4	7	11
		III	F	0	0	0
			M	1	0	1
	Mixed	I	F	0	5	5
			M	2	3	5
		II	F	7	7	14
			M	2	7	9
		III	F	0	0	0
			M	1	0	1
Iztapalapa	Pure	I	F	0	1	1
			M	0	0	0
		II	F	0	1	1
			M	1	0	1
		III	F	0	0	0
			M	0	0	0
	Mixed	I	F	6	3	9
			M	3	2	5
		II	F	6	2	8
			M	4	9	13
		III	F	2	4	6
			M	2	3	5

N.B. (1) Group I, less than a year old, Group II ages $1 \leq n < 6$, Group III ages 6 and over. (2) The cut-off point to be considered positive was 1:32 or greater.

frequent age, in the three municipalities, was 1 year 6 months (Group II). These are animals which are at the age of reproduction (Fig. 2).

The breeds of the animals studied were as follows: 116 were mixed breeds (82.3%) and 25 were pure breeds (17.7%). The latter included: Bull terriers, Boxers, Alaskan malamutes, Dalmatians, Dachshunds, Poodles, a Rottweiler, a Napolitan Mastiff, a SharPei, a Chihuahua and a Maltese. No significant difference was found between the breed of the animal and positive seroreactions, $p > 0.876$ using X^2 (Table 2).

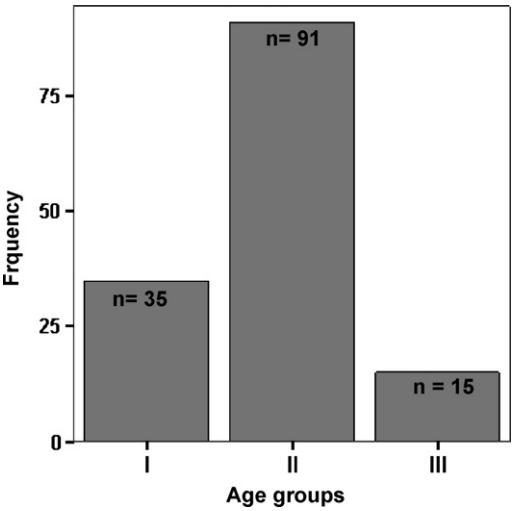

Fig. 2. Frecuency distribution of age groups.

When a comparison was made between the gender of the animals and positive serological reaction, no significant difference emerged for $p > 0.232$ (Table 3). In the distribution of frequencies by municipality, however, a significant difference did appear with a $p < 0.0001$ (Table 3).

Fig. 3 shows serological reaction by municipality. Coyoacan can be seen to have the largest number of infected animals. The confidence interval of this

Table 2
Serological reactivity by breed

Breed	Serological reactivity		Total
	Negative	Positive	
Pure	8	17	25
Mixed	39	77	116
Total	47	94	141

Table 3
Frequency distribution of serological reaction by municipality and gender

Municipality	Gender	Serological reactivity to *T. canis*	
		Negative	Positive
Coyoacan	Female	2	16
	Male	2	23
Iztacalco	Female	9	13
	Male	10	17
Iztapalapa	Female	14	11
	Male	10	14

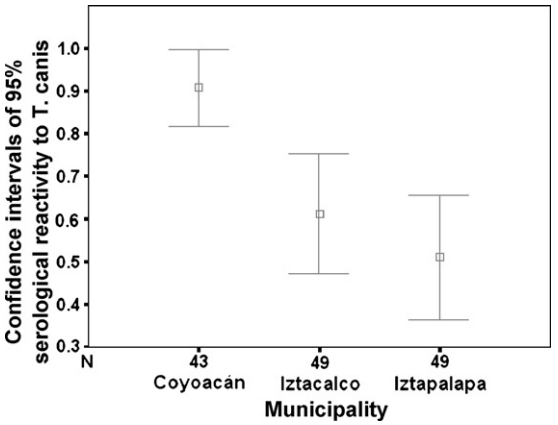

Fig. 3. Confidence intervals of 95% in the serological reactivity to an antigen of *T. canis* by municipality.

municipality does not overlap with those of the other municipalities. There is no significant difference between Iztacalco and Iztapalapa. Thus, Coyoacan shows the highest seroreactivity to the antigen of *T. canis*, having 39 results with positive dilution titers (90.7%) from the 43 dogs analyzed. Iztacalco presented 61.2% and Iztapalapa 51%.

Taken as a whole, Coyoacan showed a higher frequency of positive cases in both males and females (Table 3).

The distribution by gender and municipality showed no significant differences in positive seroreactivity, tested by confidence intervals of 95% (Fig. 4).

4. Discussion

The detection of specific antibodies has been used as an instrument of immunodiagnosis in the treatment of human Toxocariasis and *T. canis* (Glickman, 1978; Glickman et al., 1978; De Savigny, 1975). The present investigation is perhaps the first study to carry out the detection of anti-*Toxocara* antibodies in a dog population in Mexico. Although it is true to say that serological analysis and analysis by the faecal flotation method are not directly comparable, nevertheless, the titers with a value higher than 1:32 suggest the presence of infection in these animals. Moreover, serological analysis registers the previous immunological response of the infected animals. Matsumura et al. (1984) state that, "Our recent observations indicated that the IgM antibody activities were found even in chronically infected adult dogs." They also suggest that, "the materials continuously excreted or secreted from the larvae may be related to the IgM antibody activities produced throughout life."

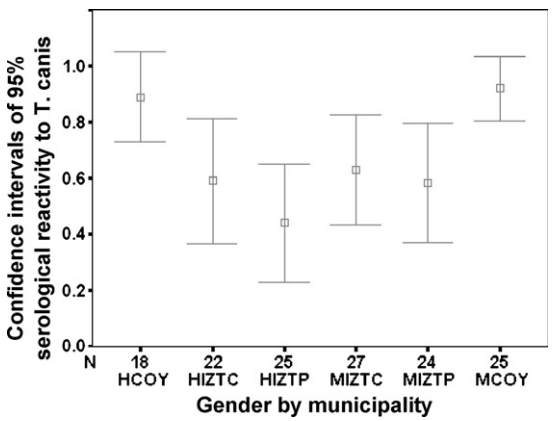

Fig. 4. Confidence intervals of serological reactivity to an antigen of *T. canis* in dog sera by gender and municipality of Mexico City analyzed by IHAT.

Because of all this, an important epidemiological factor is the presence of LMV in the child population. The habit of providing children with puppies as pets increases the risk of parasite transmission. The population at large is unaware that young dogs are exactly those most likely to cause infection. It has recently been reported that even contact with hair carrying eggs of *T. canis* from infected animals may result in infection (Wolfe and Wrigt, 2003).

In addition to the above-mentioned problems, there also exists a high degree of contamination of gardens, public parks, playing fields, sidewalks and traffic refuges in Mexico City (Vázquez et al., 1996; Martínez et al., 1998). These researchers showed that the rate of contamination of *T. canis* eggs in the soils of the municipalities of Iztacalco, Iztapalapa and Coyoacan (the very areas where the present investigation was carried out) to be 25, 14.5 and 10.9%, respectively. Martínez et al. (1998) found 16% of pet dogs in Coyoacan to be carrying *T. canis* eggs. Ponce-Macotela et al. (2005) reported a rate of 18% and Eguia-Aguilar et al. (2005) reported 13.3% in stray dogs. Such a degree of contamination constitutes a health risk for dogs, children and adults alike. This situation is also reflected in the degree of contamination reported in the child population of the municipality of Coyoacan of Mexico City (Martínez et al., 1997). As can be seen from the statistical evidence, this municipality is the most contaminated and also has the most extensive green areas. The former is a result of the latter. More parks and gardens mean more space for dogs to contaminate and be contaminated. Coyoacan is what, in Mexico, is called a "colonial" area. An essentially pre-twentieth century zone has been preserved by a middle class invasion which has left a patchwork of rich and poor. This might

also be a factor in the apparent paradox. Resistance to the infection in adult dogs is related to age, sex, breed and exposition to the infection (Greve, 1971). The prevalence of *T. canis* infection in the dogs in this study turned out to be relatively high if compared to that found in places like Germany (22.4%) (Barutzki and Schaper, 2003), Australia (38%) (Blake and Overend, 1982), Italy (33.6%) (Habluetzel et al., 2003), Japan (4.3%) (Itoh et al., 2004), United States (3.1%) (Hackett and Lappin, 2003) and the rest of Latin America (53%) (Schantz, 1989).

The high titers of anti-*T. canis* antibodies reported in adult dogs in this study differ from the observations made by the authors who think this age group develops higher immunity (Borchert, 1981). Nevertheless, cross reaction with other nematodes (Ancylostomatidae, for example) or the fact that the infection of *T. canis* might be at the stage of tissue migration, cannot be entirely ruled out as possible causes of this high seroreactivity. In our case, we believe that the high seroreactivity found in animals of Group II and the higher antibody titers (of 1:4096) found in the animals of Group III may be related to the frequent re-infections that the animals suffer by walking freely in the highly contaminated spaces of Mexico City.

It must be noted that in our investigation we found the presence of the *T. canis* antigen in dogs less than a year old (24 of the 35 samples collected).

Dog susceptibility to *T. canis* infection is an important epidemiological factor when calculating and establishing appropriate means for zoonosis control. It also gives us an estimate of the *T. canis* larva contamination in the city as well as showing us the risk humans are exposed to.

The measures we think are feasible for preventing Toxocariasis are: improvement of sanitary conditions in the city; surprise round-ups of stray dogs; promotion of ''owner-responsibility'' (i.e. collecting their dog's faeces and putting them into the organic rubbish or main sewerage).

Prevention: taking into account that the life cycle of the parasite is 2 months, it is suggested that pets should ideally be treated with antiheminth medicine every month. However, for reasons of economy and education, it seems more likely that such measures would be taken only every 3 months. Owners should make sure puppies are not infected before being given to children, washing the animals' hair, and giving the animal veterinary attention at least every 6 months.

Dog birth control with responsible sterilization.

5. Conclusions

The high seroprevalence of anti-*T. canis* antibodies found in the dogs of the studied population is an indicator of the contact which exists between these animals and the parasite. This is the result of the high degree of contamination of the soil of Mexico City with the parasite's eggs. There exists a serious risk for the population of being infected with Visceral larva migrans.

The seropositive dilutions to the antigens of *T. canis* with a value higher than 1:256 show processes of reinfection which are almost certainly associated with the interaction of these animals with other dogs in parks and gardens.

The results show that a higher level of urbanization brings with it a greater number of green areas, parks and gardens. These, in turn, have become reservoirs of canine faecal material which are a source of infection for both canine and human populations

References

Avrameas, S., Taudou, B., Chuilon, S., 1969. Glutaraldehyde, cyanuric chlorhide and tetraazotized *o*-dianisidine as coupling reagents in the passive heamagglutination test. Immunol. Chem. 6, 67–76.

Barutzki, D., Schaper, R., 2003. Endoparasitres in dogs and cats in Germany 1999–2002. Parasitol. Res. Suppl 3:S148–150.

Beaver, P.C., 1956. Parasitological reviews: larva migrans. Exp. Parasitol. 5, 587–621.

Becht, H., 1968. Properties of erythrocytes stabilized with use in and indirect haemagglutination test with influenza virus rnp-antigen. J. Immunol. 101, 18–22.

Beltrán, H.F., Gómez, P.A., Figueroa, V.V., 1974. Immunological characterization of antigenic fractions of *Trichinella spiralis larvae*. In: Charles, W.K. (Ed.), Trichinellosis. Intext Educational Publisher, N.Y., pp. 175–186.

Blake, R.T., Overend, J., 1982. The prevalence of *Dirofilaria inmitis* and other parasites in urban dogs in north-eastern Victoria. Aus. Vet. J. 58, 111–114.

Borchert, A., 1981, Parasitología Veterinaria 3ª Ed. Editorial Aribia, España, p. 228.

Boyden, S.V., 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J. Exp. Med. 93, 107–120.

Csizmas, L., 1960. Prepartion of formalinized erythrocytes. Proc. Soc. Exp. Biol. Med. 103, 157–160.

De Savigny, D.H., 1975. *In vitro* maintenance of *T.canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic test for visceral larva migrans. J. Parasitol. 61, 781–782.

Eberhardt, O., Bialek, R., Nagele, T., Dichgans, J., 2005. Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature. Clin. Neurol. Neurosurg. 107, 432–438.

Eguia-Aguilar, P., Cruz-Reyes, A., Martinez-Maya, J.J., 2005. Ecological Análisis and Description of the Intestinal Helminths Present in Dogs in México City, vol. 127. pp. 139–146.

Elefant, G.R., Shimizu, S.H., Sanchez, M.C., Jacob, C.M., Ferreira, A.W., 2006. A serological follow-up of toxocariasis patients after chemotherapy based on the detection of IgG, IgA, and IgE antibodies by enzyme-linked immunosorbent assay. J. Clin. Lab. Anal. 20164–20172.

Fisher, M., 2003. Toxocara cati: an underestimated zoonotic agent. Trends. Parasitol. 19, 167–170.

Glickman, L., Schantz, P.M., 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol. Rev. 3, 230–250.

Glickman, L., Schantz, P., Dombroske, R., Cypess, R., 1978. Evaluation of serodiagnostic test for visceral larva migrans. Am. J. Trop. Med. Hyg. 27, 492–498.

Good, B., Holland, C.V., Taylor, M.R.H., Larragy, J., Moriarty, P., ÓRegan, M., 2004. Ocular toxocariasis in schoolchildren. Clin. Infect. Dis. 15, 173–178.

Greve, J.H., 1971. Age resistance to *T.canis* in ascarid-free dogs. Am. J. Vet. Res. 32, 1185–1192.

Habluetzel, A., Traldi, G., Ruggieri, S., Attili, A.R., Scuppa, P., Marchetti, R., Menghini, G., 2003. An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. Vet. Parasitol 113, 243–252.

Hackett, T., Lappin, M.R., 2003. Prevalence of enteric pathogens in dogs of north-central Colorado. J. Am. Anim. Hosp. Assoc. 39, 52–56.

Instituto Nacional de Geografía, Estadística e Informática (INEGI). Distrito Federal, 2005. Anuario Estadístico. INEGI, Aguascalientes, México.

Itoh, N., Muraoka, N., Aoki, M., Hagaki, T., 2004. Prevalence of *Toxocara canis* infection in household dogs. Kansenshogaku Zasshi 78, 114–119.

Lowry, O.H., Rossenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein in measurement with folin phenol reagent. J. Biol. Chem. 193, 265–275.

Martínez, B.I., Gutiérrez, Q.M., Fernández, P.A., Pérez, L.M., Vázquez, T.O., García, Y.Y., 1997. Reactividad serológica a antígeno de *T. canis* en una población escolar. Rev. Mex. Patol. Clin. 44, 86–89.

Martínez, B.I., Fernández, P.A., Vázquez, T.O., Ruiz, H.A., 1998. Frecuencia de *T. canis* en perros y áreas verdes del sur de la ciudad de México. Distrito Federal. Vet. Méx. 29, 239–244.

Matsumura, K., Kazuta, Y., Endo, R., Tanaka, T., 1984. Detection of circulating toxocaral antigens in dogs by sandwich enzyme-immunoassay. Immunology 51, 609–612.

NOM-033-ZOO-1995. Sacrificio humanitario de los animales domésticos y silvestres.

NOM-062-ZOO-1996. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio.

Overgaauw, P.A., Okkens, A.C., Bevers, M.M., Kortbeek, L.M., 1998. Incidence of patent *T. canis* infection in bitches during the oestrous cycle. Vet. Quart. 20, 104–107.

Parija, S.C., Sahu, P.S., 2003. A serological study of human cysticercosis in Pondicherry, South India. J. Commun. Dis. 35, 283–289.

Ponce-Macotela, M., Peralta-Abarca, G.E., Martinez-Gordillo, M.N., 2005. Giardia intestinalis and other parasites: prevalence in adults dogs from the southern part of Mexico City. Vet. Parasitol. 15, 1–4.

Schantz, P.M., 1989. Toxocara larva migrans now. Am. J. Trop. Med. Hyg 41 (Suppl.), 21–34.

Sprent, J.F., 1958. Observations on the development of *T. canis* (Werner, 1782) in the dog. Parasitology 47, 184–209.

Teyssot, N., Cassoux, N., Lehoang, P., Bogadi, B., 2005. Fuchs heterochromic cyclitis and ocular toxocariasis. Am. J. Ophthalmol. 139, 915–916.

Vázquez, T.O., Ruiz, H.A., Martínez, B.I., Merlín, P.P., Tay, Z.J., Pérez, T.A., 1996. Contaminación de suelos por huevos de *Toxocara* sp. en parques públicos y jardines de casa-habitación de la ciudad de México. Bol. Chil. Parasitol. 51, 54–58.

Webster, G.A., 1958. On prenatal infection and the migration of *T.canis* Werner 1782 in dogs. Can. J. Zool. 36, 435–440.

Wolfe, A., Wrigt, I.P., 2003. Human toxocariasis and direct contact with dogs. Vet. Rec. 152, 419–422.