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

Ignacio Martínez-Barbabosa [a,*], Manuel Gutiérrez Quiroz [b], Leticia Araceli Ruiz González [b], Elena Marcia Gutiérrez Cárdenas [a], Arturo Alpizar Sosa Edubiel [a], Jose Luis Valencia Juárez [b], Enrique Gaona [c]

[a] *Departamento de Atención a la Salud, Área de Ciencias Básicas Edif, H-102, Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100, Colonia Villa Quietud, Delegación Coyoacan, C.P. 04960 México, DF, Mexico*
[b] *Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, Mexico*
[c] *Departamento El Hombre y su Ambiente. Universidad Autónoma Metropolitana-Xochimilco, México, Mexico*

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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,

* Corresponding author.
E-mail address: imarti@correo.xoc.uam.mx (M.-B. Ignacio).

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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.