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Anti-Trypanosoma cruzi antibodies in dogs (Canis familiaris) from Tlalnepantla Municipality, State of Mexico


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ABSTRACT

Blood samples from 487 dogs living in irregular immigrant settlements in Tlalnepantla, México, were tested for specific anti- _T. cruzi_ antibodies with ELISA and Western-Blot techniques. Positive sera were absorbed with _Toxocara canis, Anclylostoma caninum_ and _Dipylidium caninum_ proteins to determine helminthic cross-reaction; immunodominant _T. cruzi_ proteins were used as antigen with the Western-blot technique. Pre-absorption _T. cruzi_ ELISA prevalence was 1.6% (8 cases). However, cross-reactions with _A. caninum_ and _D. caninum_ were observed, and after absorption, final prevalence (Western-blot) was only 1.2%.

Key words: _Trypanosoma cruzi_, dog, México, cross-reaction.

RESUMEN

Se colectaron los sueros de 487 perros de diferentes razas, sin distinción de sexo o edad, que habitaban en asentamientos irregulares de inmigrantes en Tlalnepantla, México. Se determinó la presencia de anticuerpos específicos anti- _T. cruzi_ usando las técnicas de ELISA y Imunooelectrotransferencia (blot). Los sueros positivos fueron absorbidos con proteínas de _Toxocara canis, Anclylostoma caninum_ y _Dipylidium caninum_ para determinar reacciones cruzadas con _T. cruzi_. La prevalencia de positivos a _T. cruzi_ pre-absorbidos fue de 1.6% (8 casos) y después de absorber fue de 1.2%. La edad y raza de los perros no se relacionaba con la presencia de anticuerpos.

Palabras claves: _Trypanosoma cruzi_, perros, México, reacción cruzada.

INTRODUCCION

_Trypanosoma cruzi_ is the causative agent of Chagas disease, and it is an important cause of morbility in several Latin American countries1-2. The main transmission mode is trough contamination of triatomine bites with their feces2.

In America, both, wild and domestic animals have been screened for this parasitic infection, and have also been identified as _T. cruzi_ reservoirs. Monkeys, opossums, armadillos, squirrels, and mice are the most common wild reservoirs while pigs, dogs, and cats are the most frequent domestic reservoirs, cows have also recently been described as domestic reservoirs in Jiutepec, in the state of Morelos, Mexico4-5. Weakness, anaemia, and splenomegaly are the main symptoms observed in infected dogs; cardiomyopathy may also be present in those animals, although not as severe as those observed in man7. Therefore, Chagas’s disease is important in dogs not only due to the seriousness of the disorder but by its role as a reservoir, and the risk that they represent when those animals are handled8. In South America, it has been informed that over 35% of dogs are infected with _T. cruzi_11-12. The domestic cycle is the most important factor in Chagas’s disease; this cycle maintains the transmission in semi-rural zones in which both reservoirs, man and animals, share the same habitat11. In addition, the World Health Organization has found nearly 150 different wild animal species are reservoirs of _T. cruzi_14.

In metropolitan Mexico City, irregular areas are settled by people usually coming from _T. cruzi_ rural endemic zones; urbanization of the disease may be induced. Immigrants bring their domestic animals; therefore, a serious public health risk may be evolving in this zone. However, the consequence of such risk has not been studied. Works on _T. cruzi_ reservoirs in this part of Mexico City are scarce.

The purpose of this study was to determine specific anti- _T. cruzi_ IgG class antibody in sera of dogs (Canis familiaris) that come from several endemic areas of the country and that...
are known to be living in the municipality of Tlalnepantla, State of Mexico which is part of Mexico City Metropolitan area.

MATERIAL AND METHODS

Serum Samples

Sera samples from 487 dogs (Canis familiaris) of different breeds living in Tlalnepantla, State of Mexico, were analysed. Dogs were provided by the Canine Central Service, and were caught without distinction of age or sex. Sera from a hybrid dog, previously inoculated three times with one million trypomastigotes of T. cruzi Coecula strain (a Mexican T. cruzi isolate) were used as positive control. Sera from 30 healthy dogs of similar age than the experimental group, living in a different zone, were used as negative control; these dogs were kept isolated, and had no risk of infection. Each serum sample was studied with triple ELISA\textsuperscript{15} and Western-Blot techniques.\textsuperscript{16,17} The antigen used consisted in 40 mg of protein obtained from the lysis of 1x10\textsuperscript{8} flagellated trypanosomas of the Coecula T. cruzi Mexican isolate get of INDRE (National Institute of Epidemiology Reference of Mexico); this isolate was cultured in LIT medium\textsuperscript{18}, re-suspended in a mixture of Tris-HCl 10 mM, NaCl 150 mM, NP-40, EDTA 2 mM, and 1 mg/mL of protease inhibitor, cocktail (P8465 Sigma Aldrich).

Cut-off point for ELISA test, was obtained from healthy dogs sera considering the average value of D.O. = ±4 STD; sera with values over this point were considered positive.

Statistical analysis

The statistical analysis included the Anova test to compare positive and negative sera according to sex, with a 95% IC and a precision of 0.05.

Helminthic cross-reaction

Total antigens derived from Toxocara canis, Dipylidium caninum, and Ancylostoma caninum, 1 mg/100 mL of each antigen was added to each dog sera which presented an anti-T. cruzi antibody (OD) higher than the OD cut-off point.

The helminth antigen was obtained from necropsy of dogs performed at the Faculty of Veterinary of UNAM; it was incubated for 1 hour at 37°C, centrifuged during five minutes at 3,800 rpm in a clean assay tube, and the supernatant was separated. The absorbed sera were used as first antibody in the Western-Blot test.

RESULTS

Cut-off point obtained by ELISA for normal dogs sera was established at D.O. = 0.9818; using this value, 8 of 487 sera were identified as positive, which represents a frequency of 1.64% (IC 95%; 0.7-3.02%). Relation of age, sex, and sero-positive dogs are shown in table 1.

DISCUSSION

Dogs living in Tlalnepantla, State of Mexico, showed a 1.64% frequency of positivity to T. cruzi antibody; this result may indicate that those animals either live in a T. cruzi endemic zone, or they were already infected with this parasite when they immigrated into the area with their owners. Also, the results may be indicative of vector translation and a possible adaptation of the insect to a new environment, or that triatoma is a natural resident of this area.

A high prevalence of this infection has been reported in dogs from Argentina, Paraguay, Chile, Panama and Brazil\textsuperscript{19-23} integral epidemiological research in which human population is included, have been developed in those countries. A prevalence of 4 to 65%, depending on the district studied, in dogs, and cats have been found. On the other hand, the probability to generate infection in the vector, from a canine population, is higher when dogs are in contact with other previously infected dogs; these dogs are a long-time source of the parasite. Therefore, a stable prevalence of the disease; with a high canine population parasitism, can exist if no environmental-control measures are taken\textsuperscript{24}.

It must be remembered that T. cruzi is a parasite originally adapted to wild ecosystems; in those wild ecosystems the parasite interacts with animals, which can be potential hosts. Such hosts usually develop a noticeable adaptation to the parasite and become reservoirs; when an ecosystem is disturbed, vertebrate animals are frequently the first animals to be displaced. Regarding vector transmission of tripanosomiasis, a vector transference to the new animals that substitute others in the disturbed ecosystem can occur; a peridomestic transmission route is first installed, and then a second domestic transmission appears\textsuperscript{25}. Subsequently, a human-domestic animal relation evolve; domestic animal my have a poor adaptation to the parasite and the disease manifest.

This type of events have been proved in endemic zones; in Mexico, a similar situation has been observed in the states of Puebla, Morelos, and Nayarit\textsuperscript{26}. These studies have demonstrated a direct relationship between domestic animals and human infection; this works have also shown a higher prevalence of T. cruzi in dogs than that found in our study.

The geographical area in which our study was performed is a relatively new urbanized zone; two types of human settlements can be observed in this area: recently built homes which are ecosystem-disturbance factors and, at the same time a disease domestication barrier; the second type is represented by the presence of irregular settlements in which poor people construct their homes in a manner that is propitious to the development of the parasitosis. In addition, people who settles these areas, come from potential endemic zones; therefore, chronically infected individuals, vector transportation, domestic animal (meanly dogs), translation as potential reservoirs, and a disturbed
environment, integrates enough elements to establish a parasite transmission cycle.

Dogs play an essential role as source of infection and in the persistence of the parasitosis; seropositive dogs are twelve more times capable of transmitting the parasite than seropositive children and 100 more times than seropositive adults. According to information from endemic zones in South America, dogs can be used as monitors of human disease.27-28

Finally, in the area studied by us, further research is needed in order to establish the prevalence of the disease in the human population, in other domestic animals, in the search of vectors, and in the frequency of infected vectors.

### Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Females*</th>
<th>Males**</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>126</td>
<td>3</td>
<td>137</td>
</tr>
<tr>
<td>4 to 8</td>
<td>46</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>8 to 12</td>
<td>18</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>12 to 16</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>3</td>
<td>288</td>
</tr>
</tbody>
</table>

**SEX**

-ve = negative  +ve = positive  

*,** p>0.05 †,†† p>0.05 ‡, §§ p>0.05

### Table 2

<table>
<thead>
<tr>
<th>Breed</th>
<th>-ve</th>
<th>+ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid</td>
<td>390</td>
<td>8</td>
<td>398</td>
</tr>
<tr>
<td>With defined breed</td>
<td>72</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>Defined breed</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>479</td>
<td>8</td>
<td>487</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Dog sera number</th>
<th>Cut-off point X±4 STD</th>
<th>Non-absorbed sera Antigens</th>
<th>Absorbed sera (DAT) Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAT T. cruzi</td>
<td>DAT T. cruzi</td>
</tr>
</tbody>
</table>
| Ser 
= Trypanosoma cruzi 1mg/mL. 
A= Ancylostoma caninum 1mg/mL. 
T= Toxocara canis 1mg/mL. |
| 84              | 0.974 ± 0.050         | + + + +                     | - - - +                     |
| 121             | 1.011 ± 0.049         | + + + +                     | - - - -                     |
| 220             | 0.976 ± 0.057         | + + + +                     | - - - +                     |
| 364             | 1.103 ± 0.026         | + - + +                     | - - - +                     |
| 391             | 1.108 ± 0.034         | + + + +                     | - - - +                     |
| 396             | 1.197 ± 0.018         | + + + +                     | - - - +                     |
| 398             | 1.033 ± 0.027         | + + + +                     | - - - -                     |
| 408             | 1.083 ± 0.032         | - - - +                     | - - - +                     |
| Positive control| 1.220 ± 0.030         | - - - +                     | - - - +                     |

**BIBLIOGRAPHY**


