HUMAN INFECTION AND RISK OF TRANSMISSION OF CHAGAS DISEASE IN HIDALGO STATE, MEXICO

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Abstract. New zones with risk of infection for Chagas disease were reported in San Antonio Tezoquipan, Caltimacan, and El Ahorcado in the Hidalgo State of Mexico. Antibodies to Trypanosoma cruzi were detected by enzyme-linked immunosorbent assay and indirect hemagglutination assay in human serum samples. Study subjects were also given an electrocardiogram. Trypanosoma cruzi was isolated from triatomines collected and its virulence was determined in BALB/c mice. Seropositive persons were found in the three regions studied and seroprevalence of T. cruzi ranged between 3.25% and 5.13%. Six of eight seropositive persons had cardiac alterations. The species of triatomines detected were Triatoma barberi, Triatoma mexicana, and Triatoma dimidiata, and at least one of each species was infected with T. cruzi. Entomologic indexes from the zones were determined, and El Ahorcado showed the greatest risk of infection. In this region, we found more virulent isolates of T. cruzi in Triatoma barberi, and the highest human seroprevalence for T. cruzi.

INTRODUCTION

Chagas disease is caused by the flagellate protozoan Trypanosoma cruzi, which is transmitted primarily by hematophagous insects from the subfamily Triatominae. This disease represents a major public health problem in Mexico and other countries in Latin America. A total of 8–9 million people in Mexico, the Andes region of South America, and Central America are infected with T. cruzi and 25 million are at risk for infection.1–3 Public health authorities in Mexico have established epidemiologic surveillance and vector control programs, and several seroepidemiologic surveys have been reported.4–5 Data on human infection, infected vectors, or behavior of T. cruzi strains have also been reported.6–8 However, few studies have reported these aspects of Chagas disease in one specific region. To establish control programs, identification of regions at risk for transmission and infection, humans infected with T. cruzi, triatomines, and virulence of T. cruzi isolates is needed.

Although data on transmission and control of Chagas disease have been reported for some states in Mexico (Puebla, Colima, Jalisco, Chiapas, Veracruz, Yucatán, Guerrero, and Oaxaca),9–16 there is little information available on other states in Mexico, including Hidalgo. In 1992, the National Serologic Survey reported that the seroprevalence of Chagas disease in Hidalgo was 1.5%.6 However, this study provided data only for Mezquital and Apan in Hildago, which represent only 2 of the 84 municipalities in this state. Triatoma barberi and Triatoma mexicana have been detected in Metztitlán and Santiago de Ayala.17 Triatoma mexicana has been identified in the northern region of Hidalgo, and Triatoma dimidiata and Triatoma gerstaeckeri have been identified in the northern and central regions of the state.18 This study also showed that Triatoma dimidiata and Triatoma mexicana were infected with T. cruzi.19 The purpose of this study was to identify new endemic zones of Chagas disease in Hidalgo.

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MATERIALS AND METHODS

Study area. The study was conducted in three southwestern zones in Hidalgo State: 1) El Ahorcado (municipality of Teozoautla, 20°29’N, 99°28’W, altitude = 2,070 meters above sea level), 2) San Antonio Tezoquipan (municipality of Alfa-jayucan, 20°28’N, 99°28’W, altitude = 2,020 meters above sea level), and 3) Caltimacan (municipality of Tasquillo, 20°32’N, 99°22’W, altitude = 1,720 meters above sea level). El Ahorcado and San Antonio Tezoquipan have an average annual temperature of 25°C and an average relative humidity of 15%, and Caltimacan has an average annual temperature of 30°C and an average relative humidity of 70%.19

Sampling design. The total number of houses in each locality was 21 in El Ahorcado, 163 in San Antonio Tezoquipan, and 359 in Caltimacan.19 The number of dwellings studied in each region was calculated by including 10% of infested houses in accordance with reports of the Secretary of Health in Hidalgo (absolute precision of 5% and a 95% confidence level). The number of dwellings selected was 20, 78, and 105 in these three regions, respectively. In each locality, the number of people living in selected houses (17 in El Ahorcado, 22 in San Antonio Tezoquipan, and 23 in Caltimacán) was proportional to the total number of inhabitants in each region (63, 628, and 1,567, respectively).6

Ethical considerations. The study protocol was reviewed and approved by the ethics committee of the Institute of Health Sciences of Universidad Autónoma del Estado de Hidalgo. This work was performed according to Reglamento de la ley General de Salud en Materia de Investigación, which reported forearm venipuncture as a minimal risk procedure and requires oral consent when illiterate participants are invited to participate in a clinical or epidemiologic study. When feasible, a written informed consent letter was used in which each individual agreed to participate.

Vector sampling. After informed consent was obtained, volunteers searched for triatomines for 30–60 minutes inside and outside their dwellings. Triatomines were collected into plastic vials. Specimens were classified according to the keys of Lent and Wygodzinsky.20

Determination of metacyclogenia in triatomines. Feces of triatomines was obtained by applying abdominal pressure to triatomines, mixed with phosphate-buffered saline, and exam-
ined for flagellates by direct microscopy at a magnification of 400×. Fecal samples containing flagellates were dried, fixed with methanol, stained with Giemsa for 10 minutes, and washed with water for three seconds. Samples were examined by direct microscopy at a magnification of 1,000× for *T. cruzi*, and parasites were identified by morphologic criteria and stages. The number of each stage was calculated as a percentage of the total number of parasites observed.

**Isolation of *T. cruzi* and determination of virulence in mice.** To isolate *T. cruzi* found in triatomines, two male BALB/c NIH mice (6–8 weeks old; weight = 20–30 grams) were inoculated intraperitoneally with flagellate parasites found in triatomine feces. Blood was obtained once a week from the tails of the mice and examined by microscopy. Specimens containing *T. cruzi* were stained with Giemsa and identified by morphologic features. If *T. cruzi* was observed, the virulence of these parasites was determined. Parasites were collected, counted in a Neubauer chamber, and diluted with 0.85% NaCl to obtain a concentration of 5 × 10⁵ trypanosomes/200 μL. To determine the virulence of *T. cruzi* isolates, 10 female mice (6–8 weeks old; weight = 25–30 grams) were inoculated intraperitoneally with 200 μL of each isolate. Parasitemia was determined by obtaining blood from the tails of inoculated mice. Ten microliters of blood was mixed with 90 μL of 0.87% NH₄Cl to lyse the red blood cells. After five minutes, parasites were collected and counted in a Neubauer chamber by microscopy at a magnification of 400×. Parasitemia was determined every third or fourth day over a 60-day period. Mortality in mice inoculated with each *T. cruzi* isolate was also recorded.

**Histopathologic analysis.** Histopathologic analysis of mice was conducted according to the procedure of Melo and Brenner. Briefly, brain, lung, heart, esophagus, stomach, small intestine, large intestine, kidney, spleen, liver, and skeletal muscle were removed, and tissues were fixed in 10% formaldehyde and embedded in paraffin. Five 7-μm-thick sections were separated by 50 intervals and stained with hematoxylin and eosin. Slides were evaluated for amastigote forms of *T. cruzi* by microscopic observations at a magnification of 1,000× in 50 random fields.

**Human blood samples.** Blood samples (5 mL) were obtained from 21 persons in San Antonio Tezoquipan, 39 in El Ahorcado, and 154 in Caltimacan. All donors ranged in age from 7 to 64 years (mean ages: 40, 47, and 24 years, respectively, and 46%, 50%, and 62%, respectively, were female). An electrocardiogram (ECG) was performed for all donors in their homes. Serum samples were tested for antibodies against *T. cruzi* by an indirect enzyme-linked immunosorbent assay (ELISA) and an indirect hemagglutination test (IHA).

**Enzyme-linked immunosorbent assay.** The ELISA (Enzyme Chagas Interbiol kit; Interbiol S.A. de C.V., Mexico, City, Mexico) was carried out according to the manufacturer’s instructions. Absorbance was measured spectrophotometrically at 492 nm. An absorbance (optical density) greater than 0.2 was considered a positive result. Each test was done in duplicate.

**Indirect hemagglutination test.** The IHA (Chagas/IHA Interbiol Kit; Interbiol S.A. de C.V.) was carried out according to the manufacturer’s instructions. A well with a red area greater than 50% of total diameter of the well was considered a positive result.

**Electrocardiographic study.** The ECG was conducted using a 12-derivation electrocardiograph (six channels, Kenz-ECG; Suzuken Co. Ltd., Nagoya, Japan) at a paper speed of 25 mm/second. Analysis and interpretation were conducted blindly, and classification of the ECG results as normal or abnormal was made according to the deductive method of ECG interpretation and the criteria of our medical personnel.

**Data analysis.** To estimate the risk of infection, the following entomologic indexes were determined: infestation (percentage of triatomine-positive houses), density (rate of triatominae found in dwellings), natural infection (percentage of *T. cruzi*-infected triatomines), and colonization (percentage of triatomine-positive houses with instar stages) (Table 1). Descriptive methods were used to determine seroprevalence of *T. cruzi*. The chi-square test was used to determine associations between serologic and ECG results, and between triatomines infected with *T. cruzi* and different regions.

## RESULTS

**Risk of infection.** Table 1 shows the entomologic indexes of the three study areas. El Ahorcado had the highest infestation index and highest density index, a higher proportion of dwellings infested with triatomines, and a higher proportion of triatomines infected with *T. cruzi*. The index of infected triatomines in San Antonio was lower and similar to that in Caltimacan.

**Identification of triatomines and isolation of *T. cruzi*.** Triatomines were collected in intradomestic and peridomestic locations (Table 2). *Triatoma barberi* was the only species

<table>
<thead>
<tr>
<th>No. of dwellings with triatomines</th>
<th>El Ahorcado</th>
<th>San Antonio Tezoquipan</th>
<th>Caltimacan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infestation index</td>
<td>30% (6/20)</td>
<td>10.25% (8/78)</td>
<td>21.9% (23/105)</td>
</tr>
<tr>
<td>No. of triatomines infected with</td>
<td>29</td>
<td>15</td>
<td>65 (36 T. dimidiat 29 T. mexicana)</td>
</tr>
<tr>
<td>Overcrowded index</td>
<td>4.83 (29/6)</td>
<td>1.87 (15/8)</td>
<td>2.82 (65/23) 2.4 to T. dimidiat 2.4 to T. mexicana</td>
</tr>
<tr>
<td>Colonization index</td>
<td>33.3% (2/6)</td>
<td>25% (2/8)</td>
<td>34.8% (8/23)</td>
</tr>
<tr>
<td>Natural infection index</td>
<td>0.15% (4/65)</td>
<td>5.55% to T. dimidiat 6.89% to T. mexicana</td>
<td></td>
</tr>
</tbody>
</table>

* Infestation index = no. infested houses/no. studied houses × 100; density index = total number of captured triatomines/total number of studied houses; overcrowded index = number of houses with nymphal phases/no. of infested dwellings with *T. cruzi* × 100; natural infection index = no. of triatomines infected by *Trypanosoma cruzi*/no. of captured triatomines × 100.
present in San Antonio Tezoquipan and El Ahorcado (third stage in San Antonio Tezoquipan and fifth stage in El Ahorcado, males and females in both communities). In Caltimacán, *Triatoma mexicana* (fifth stage, males and females) and *Triatoma dimidiata* (third, fourth, and fifth stages, males and females) were collected. All three species of triatomines collected were infected with *T. cruzi*. Epimastigotes predominated in *Triatoma barberi* collected in San Antonio Tezoquipan, and in *Triatoma dimidiata* and *Triatoma mexicana*, while trypomastigotes predominated in *Triatoma barberi* in El Ahorcado. The *T. cruzi* infection rate triatomines ranged between 6.66% and 10.35%. The highest number of infected triatomines were in *Triatoma barberi* collected in El Ahorcado. There were no significant differences among the three species in the three zones studied in the rate of triatomines infected with *T. cruzi* ($\chi^2 = 0.53, P > 0.05$).

**Virulence of *T. cruzi* isolated from triatomines.** Table 3 shows the virulence of *T. cruzi* isolates. We obtain three isolates of *T. cruzi* in El Ahorcado, one in San Antonio Tezoquipan, and three in Caltimacán. Parasites isolated from *Triatoma barberi* resulted in higher mortality in mice than those from *Triatoma mexicana* or *Triatoma dimidiata*. All but two isolates of *T. cruzi* were infective to mice and had tropisms for heart and skeletal muscle. One isolate obtained from *Triatoma barberi* (Aho-1) and one from *Triatoma dimidiata* (Caldim) produced inflammatory tissue reactions in the spleen. Parasitemia was detected 7–61 days after infection.

**Serologic and electrocardiographic studies in humans.** Humans were considered positive for infection with *T. cruzi* when their serum samples were positive by IHA and ELISA (Table 4). The highest seroprevalence of *T. cruzi* infection was detected in people living in El Ahorcado (5.13%), and the lowest seroprevalence was detected in Caltimacán (3.25%). Seropositive individuals ranged in age from 8 to 63 years; the younger seropositive people lived in Caltimacán. No predominate ECG alteration was detected in seropositive donors. In San Antonio Tezoquipan, the only seropositive donor detected did not show any ECG alterations. No relationship was found between antibody detection with any type of ECG alteration. Each of three regions studied had seronegative individuals with ECG alterations.

Serologic results were associated with ECG results in El Ahorcado ($\chi^2 = 14.33, P < 0.001$) and Caltimacán ($\chi^2 = 51.64, P < 0.001$), but not in San Antonio Tezoquipan ($\chi^2 = 0.11, P > 0.05$). In El Ahorcado, 34 seronegative persons had normal ECGs results, three seronegative persons had abnormal ECG results, and two seropositive persons had abnormal ECG results. In Caltimacán, 144 seronegative persons normal ECG results, 5 seronegative persons had abnormal ECG results, 4 seropositive persons had abnormal ECG results, and one seropositive persons had normal ECG results. In San Antonio Tezoquipan, one seropositive person had normal ECG results, and 18 seronegative persons had normal ECG results, and 2 seronegative persons had abnormal ECG results. Seropositive people who ranged in age from 8 and 23 years old (4 of 5) were found only in Caltimacán. Three of the five seronegative individuals with ECG alterations in Caltimacán ranged in age from 71 to 75 years.

**DISCUSSION**

We report the risk of infection with *T. cruzi* in three regions of Hidalgo State, Mexico by entomologic indexes, identify areas endemic for Chagas disease on the basis of persons seropositive for *T. cruzi* by two serologic tests with abnormal ECG results, and the presence of triatomines infected with *T. cruzi*. In El Ahorcado, we found the highest entomologic indexes (infestation, density, and natural infection) among
the regions studied, i.e., one of three dwellings had approximately 1.5 triatomines per house, and 1 of 10 triatomines was infected with \textit{T. cruzi}. In El Ahorcado, we found the highest human seroprevalence for \textit{T. cruzi} and isolates of \textit{T. cruzi} with the highest virulence for mice.

We found \textit{Triatoma barberi} in two of the three regions studied. This species of triatome is important in Mexico because of its transmission capacity.\cite{3,26} \textit{Triatoma barberi} showed the highest rate of infection with \textit{T. cruzi} (10.35\% in El Ahorcado and 6.66\% in San Antonio); the infection rate was 5.56\% for \textit{Triatoma dimidiata} and 6.9\% for \textit{Triatoma mexicana}, although this difference was not statistically significant ($\chi^2 = 0.53$, $P > 0.05$). \textit{Triatoma barberi} had the highest frequency of trypomastigotes, which indicates that it can transmit the infective phase of \textit{T. cruzi}. Isolates obtained from \textit{Triatoma barberi} showed the highest virulence, while isolates from \textit{Triatoma mexicana} and \textit{Triatoma dimidiata} were avirulent. The risk of transmission and infection may be related to the species of triatome. Another study showed that Zacoalco and Tuxueca had the largest number of acute cases of Chagas disease and \textit{T. barberi} was found in these regions.\cite{27} Cardenas and others showed that strains of \textit{T. cruzi} that produce more histopathologic lesions (El Capulin and La Mesa) were isolated from \textit{Triatoma barberi}.\cite{28} Magallón-Gastelum and others found that \textit{Triatoma barberi} was the species with highest natural infection index (35\%), and Tuxueca was the zone with the highest number of human infections.\cite{11}

Conversely, we showed that \textit{Triatoma mexicana} had the lower rate of infection with \textit{T. cruzi}, which is similar to the results reported by Vidal-Acosta and others.\cite{18} Thus, the risk for Chagas' disease depends on the triatamine species and the strain of \textit{T. cruzi}. Some strains and vectors are found in specific areas. In our study \textit{Triatoma barberi} was found in San Antonio Tezoquipan and El Ahorcado, which are near each other, and \textit{T. cruzi} isolated from these zones showed similar virulence. However, triatomines and isolates from Caltimacan showed different results; this zone has different climatologic conditions and is located a greater distance from the other two zones. Although El Ahorcado showed the highest risk of infection and transmission of Chagas disease, the other two zones studied also had seropositive individuals and triatomines infected with \textit{T. cruzi}.

Studies conducted in Mexico on Chagas disease seroepidemiology have shown a heterogeneous distribution of human infections.\cite{12,14,15,25,26} Results of this work show that seroprevalence in the regions studied was higher than the figure reported in the national seroepidemiologic survey conducted between 1987 and 1989 (1.5\% for Hidalgo State and a national seroprevalence of 1.6\% versus 1.94–5.13\% in this study).\cite{3,4,6,12} However, we cannot compare both studies because different zones were analyzed. Another reason for the discrepancy in the values could be differences in the diagnostic sensitivity of the techniques, as demonstrated elsewhere.\cite{29} It is not known what factors determine high or low endemicity in various zones. Nevertheless, the presence of vectors, lack of knowledge of triatamine-borne infectious, absence of vector control, low socioeconomic status in populations, regions with poor housing, and reinfestation with \textit{T. cruzi} enhance the possibility of infection with \textit{T. cruzi} because of parasite transmission.\cite{3,4,6,12}

We found an association between seropositive results and

### Table 3
Serologic results and electrocardiographic (ECG) alterations in infected persons*

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of studied individuals</th>
<th>No. of individuals (prevalence)</th>
<th>Sex/Age, years</th>
<th>ECG alteration</th>
<th>No. of individuals</th>
<th>Sex/Age, years</th>
<th>ECG alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Ahorcado</td>
<td>39</td>
<td>2 (5.13%)</td>
<td>M/63</td>
<td>AFB</td>
<td>3</td>
<td>F/73</td>
<td>RBBB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F/30</td>
<td>SB</td>
<td></td>
<td>F/19</td>
<td>ST</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M/39</td>
<td>RBBB</td>
</tr>
<tr>
<td>San Antonio Tezoquipan</td>
<td>21</td>
<td>1 (4.76%)</td>
<td>F/60</td>
<td>Normal</td>
<td>2</td>
<td>F/27</td>
<td>LBBHB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F/31</td>
<td>LBBB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F/43</td>
<td>LBBB</td>
</tr>
<tr>
<td>Caltimacan</td>
<td>154</td>
<td>5 (1.94%)</td>
<td>M/8</td>
<td>ST</td>
<td>5</td>
<td>F/71</td>
<td>LBBB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F/15</td>
<td>Normal</td>
<td></td>
<td>F/75</td>
<td>AFHB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F/8</td>
<td>ST</td>
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</tbody>
</table>

* IHA = indirect hemagglutination assay; ELISA = enzyme-linked immunosorbent assay; AFB = anterior fascicular block; RBBB = right bundle branch block; SB = sinus bradycardia; ST = sinus tachycardia; RBBHB = right bundle branch hemiblock; LBBHB = left bundle branch hemiblock; LBBB = left bundle branch block; AFHB = anterior fascicular hemiblock; VE = ventricular extrasystole.

### Table 4
Behavior of \textit{Trypanosoma cruzi} isolates in mice*

<table>
<thead>
<tr>
<th>Region</th>
<th>Isolates of \textit{T. cruzi}</th>
<th>Mean parasitemia peaks (day)</th>
<th>Mean ± SD first day of parasitemia</th>
<th>Mortality rate</th>
<th>Mean ± SD duration of parasitemia ± SD (day)</th>
<th>No. of infected organs</th>
<th>Inflammatory reaction (tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Ahorcado</td>
<td>Aho-1</td>
<td>21</td>
<td>7</td>
<td>60%</td>
<td>48</td>
<td>4 (heart, muscle, spleen, brain)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Aho-2</td>
<td>23</td>
<td>11</td>
<td>80%</td>
<td>37</td>
<td>3 (heart, muscle, brain)</td>
<td>+ (spleen)</td>
</tr>
<tr>
<td></td>
<td>Aho-3</td>
<td>27</td>
<td>15</td>
<td>60%</td>
<td>61</td>
<td>2 (heart, muscle)</td>
<td>–</td>
</tr>
<tr>
<td>San Antonio Tezoquipan</td>
<td>SA</td>
<td>26</td>
<td>12</td>
<td>40%</td>
<td>37</td>
<td>2 (heart, skeletal muscle)</td>
<td>–</td>
</tr>
<tr>
<td>Caltimacan</td>
<td>Tas-Mex</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Tas-dim</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Tas-dim</td>
<td>ND</td>
<td>18</td>
<td>25%</td>
<td>36</td>
<td>3 (heart, skeletal muscle, spleen)</td>
<td>+ (spleen)</td>
</tr>
</tbody>
</table>

* ND = no data.
abnormal ECGs results. Although two seropositive individuals had normal ECG results, these individuals may have been infected by avirulent T. cruzi or may have had been more resistant to infection. Infection with T. cruzi in humans can cause cardiac problems because some isolates of T. cruzi had tropisms to heart and skeletal muscle. However, we do not know if cardiac abnormalities caused by T. cruzi in humans are similar to those caused by T. cruzi in mice.

We did not find any specific ECG alterations in seropositive individuals. However, some persons had blockages in various cardiac locations, which is a characteristic clinical finding in Chagas disease.

We found no abnormal ECG results that predominated in seropositive persons. This result suggests that localization of T. cruzi in a human host is a random event. We also found seronegative individuals with abnormal ECG results due to other causes, a result that has been previously reported.

We found infected children in Caltimitcan, which indicates that this is a region with active infection, as previously reported. Among seropositive individuals in region, there was no association between ECG results and age or sex. This result was different from that in other studies, which showed correlations between ECG results and these characteristics. Other studies in Brazil and Ecuador reported that the ECG abnormalities were more prevalent in older subjects, especially males. However, our results are similar to those of a seroepidemiologic survey in Mexico in which 74.5% of the seropositive persons were less than 39 years of age.

We have identified zones endemic for Chagas disease in the Hidalgo State of Mexico on the basis of the presence of triatomines, characteristics of isolates of T. cruzi, and seropositive individuals with cardiac abnormalities. Entomologic indexes showed that three zones are at risk for this disease. These findings will enhance development of Chagas disease control programs in these regions. In addition, more epidemiologic studies are needed to identify seropositive individuals and triatomines infected with T. cruzi.

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