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ABSTRACT

The purpose of this investigation was to determine the presence of T. cruzi antibodies and PCR in blood donors at the Central Blood Bank from Centro Médico de Occidente-IMSS Guadalajara, Jalisco, México. Samples from 166 donors (18 to 50 years of age), both sex were randomly selected from August to October, 1997. Place of residence, knowledge on triatomin bugs, and rural endemic zones visiting, were specific asked. Anti-T. cruzi IgG was determined by ELISA (Enzyme Linked Immunosorbent Assay) test. Positive blood samples were tested with PCR (Polymerase Chain Reaction), using a specific probe to T. cruzi. ELISA was positive in 9.34% donors with a IC (Interval of Confidence) of 95% of 5.32-14.72%. PCR and hybridization showed three positive cases (1.2%). No statistical difference in age and sex distribution was found. The relative risk (RR) for donor that visited rural endemic areas was 0.31. Negative answers to the questionare varied from 0.6 to 31.92%.

Key words: Trypanosoma cruzi, Blood Bank donors.

INTRODUCCION

Trypanosomiasis is a parasitic hemat and tissular disease caused by Trypanosoma cruzi; this protozoa is transmitted to humans through the feces of a triatomine in which the metacyclic trypomastigote form of the parasite is found1,2. Also, the disease can be transmitted by blood transfusion, organ transplantation, from mothers to child route, breast feeding, and rarely as a laboratory accident. The disease is occasionally found in Canada and USA3, usually related to immigrant blood donors; however in the rest of the American continent is a serious public health problem: 90 million persons live in risk zones and it has been estimated that about 20 millions persons are infected4.

The World Health Organization (WHO), and the Pan-American Health Organization (PAHO) have considered that the disease, and the risk of infection is present in Mexico5, although official data and a central program are not yet available. A nation-wide epidemiological survey performed in Mexico in 1992, revealed a 1% of seropositives6; the Centro Nacional de la

Palabras claves: Trypanosoma cruzi, banco de sangre, donadores.
Transfusion Sanguínea (National Blood Transfusion Center), has found 0.8% of seroprevalence among 24,000 blood donors. At the Instituto Nacional de Cardiología “Ignacio Chávez”, from 3,000 blood donors, a 0.3% seroprevalence was found, while in some isolated rural zones a seroprevalence of 10 to 50% have been informed.

The disease may be transmitted by blood transfusion (BT). After BT an acute phase disorder is present before the chronic stage is established. Blood transmission is recognized in the chronic phase. To date, BT is the second most important route of transmission in endemic zones where is associated to 20% of all cases, this high percentage increase the risk for the population. In countries in which the disease does not exist or is infrequent, BT is the only mode of transmission. In general, immigrants from rural to urbanized areas, looking for work opportunities are blood donors, usually are asymptomatic, apparently free of risk, but some already infected.

Trypanosomiasis emerged in 1940 in Mexico; although, is in the last few years that the interest in the study of this disease has expanded. As far as we know, only few studies have been carried out in blood banks, and only one post-transfusion case have been informed in Mexico. However, epidemiological data indicate that the problem may be increasing.

The state of Jalisco, in Mexico, is considered endemic for this parasitic disease. This state, located at 20° 41' north latitude, and 103° 21' west longitude, it's mean altitude is 1.540 m over the sea level, with an average temperature of 20.9 °C, which represent an ideal habitat for the vector. However, the frequency and mortality rate of blood transfusion associated to Chagas' disease studies in Jalisco are scarce or are subvalue.

The purpose of the present study was to determine the presence of specific IgG antibodies to Trypanosoma cruzi in blood donors from the Central Blood Bank of Centro Médico de Occidente-IMSS of Guadalajara, Jalisco, México. Positive samples were also tested using a PCR test; the risk of acquiring the infection trough blood transfusion was also determined.

Material and Methods

The study was performed in the Central Blood Bank from Centro Médico de Occidente-IMSS in Guadalajara, Jalisco, México. This is a general hospital in which a high number of patients are treated. All blood donors (BD) fulfilled the requirements of the Norma Oficial Mexicana para la Donación de Sangre Humana y sus Componentes (Mexican Official Norm on Blood Transfusion, and Blood Disposition); BD with sexual practices and glicerol 3 mL). Subsequently, the gel was treated with 0.25 M HCl solution, 0.5 M NaOH and 1.5 M NaCl each one ten minutes. Antigen was obtained from 1x108 Trypanosoma cruzi antigen, 100 µL of serum (1:250) and IgG human antibodies conjugated peroxidase diluted 1:5000. The reaction was revelated with 4-Cl and was reading in spectrophotometer SANOFI Diagnostic Pasteur PR2100 to OD 450 nm. Cutoff point (X ±2STD) was established before.

ELISA antibodies determination

Antigen preparation

Antigen was obtained from 1x10⁹ Trypanosoma cruzi epimastigotes of the Mexican Cecula strain cultured in LIT media. Flagellated parasites were harvested, washed, and lysed with an hypotonic saline solution mixed with protease inhibitors (Aprotinine 100 µg Sigma; Leupeptine 100 µg Sigma; PMSF 1 mM and PHMB-Tris 10 mM). Soluble phase was obtained after 30 minutes by centrifugation at 1,000 x g, 10 min, 4°C. Ninety six well plates (Nunc) were coated with 40 µL of soluble Trypanosoma cruzi antigen, 100 µL of serum (1:250) and IgG human antibodies conjugated peroxidase diluted 1:5000. The reaction was revealed with 4-CN and was reading in spectrophotometer SANOFI Diagnostic Pasteur PR2100 to OD 450 nm. Cutoff point (X ±2STD) was established before.

PCR Amplification

DNA was amplified using O1 5' TGGCTTGGAGGAGTTATTGT-3' and O2 5' AGGAGTGACGGTTGATCAG-3' primers. Primers were sintetized at the Instituto de Biotecnología of Cuernavaca, Morelos, and amplify a Trypanosoma cruzi highly preserved, and repetitive DNA genomic region. Amplification was performed in 50 µL of a regulatory solution 10 x (1000 mM Tris HCl pH 8.3, 500 mM KCl), 1.5 mM MgCl₂, 200 µM of each of the triphosphated deoxynucleotides (dTTP, dGTP, dTTP, Perkin-Elmer CETUS Gene Amp dNTPs Part. No. N808-0007), 50 ng of each primer, 2.5 U/100 µL of *Thermus Aquaticus* DNA polymerase (Perkin-Elmer-CETUS Gene Amp PCR Reagent Kit Part. No. N801-0060) and 500 ng of problem DNA. The reaction mixture was placed in a Perkin-Elmer CETUS N801-0150 thermal cycler with the following program: five minutes at 94°C, 45 seconds at 58°C, two minutes at 72°C, and 40 minutes at 4°C.

Fluorescein-labeled probe hybridization

After PCR amplification, products were placed in an electrophoresis agarose gel for 40 minutes at 80 volts, and stained (with a mixture 6x of xilencianol 25 mg, bromophenol blue 25 mg, and glicerol 3 mL). Subsequently, the gel was treated with 0.25 M HCl solution, 0.5 M NaOH and 1.5 M NaCl each one ten minutes after BT an acute phase disorder is present before the chronic stage is established. Blood transmission is recognized in the chronic phase. To date, BT is the second most important route of transmission in endemic zones where is associated to 20% of all cases, this high percentage increase the risk for the population. In countries in which the disease does not exist or is infrequent, BT is the only mode of transmission. In general, immigrants from rural to urbanized areas, looking for work opportunities are blood donors, usually are asymptomatic, apparently free of risk, but some already infected. In Trypanosomiasis emerged in 1940 in Mexico; although, is in the last few years that the interest in the study of this disease has expanded. As far as we know, only few studies have been carried out in blood banks, and only one post-transfusion case have been informed in Mexico. However, epidemiological data indicate that the problem may be increasing.

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minutes, and finally with 1.5 M Tris-HCl pH 8-NaCl to neutralization. DNA was transferred to a nylon membrane (Hybon-N) through saline shafted with a SSC 20X solution, overnight. After transference was completed, DNA was exposed to UV for one to two minutes to fix it to the membrane. An specific anti- 
*T. cruzi* fluorescein-labeled-probe was denatured through boiling and freezing. Membrane prehybridization was performed with a prehybridization solution (SSC 20X, SDS 10%, 0.5% blocking reagent, and 1 mg/mL salmon sperm DNA) for one hour at 65°C. Prehybridization was discharged, and the hybridization solution (prehybridization solution plus denatured 
*T. cruzi* probe) was added for an overnight incubation period, at 65°C. Then, astringent washing were done with SSC 2X-SDS 0.1% and SSC 0.2X-SDS 0.1% for 15 minutes at 65°C each one. After buffer-1 washing (Tris- HCl 0.1M pH 7.5 and NaCl 0.15M) for 10 minutes, at room temperature, the membrane was incubated with an antifluorescein antibody (Renaissance NEN™ life Science Products). Antibody was diluted in 1:100 buffer-2 (buffer-1 plus blocking reagent 0.5%) and incubated for one hour at room temperature. Finally, the membrane was developed with Luminol antifluorescein (Renaissance NEN™ Life Science Products) and exposed to a X-ray film.

**Results**

Blood donors studied were 166; 111 (66.9%) were males; age ranged from 18 to 50 years; 157 (94.6%) lived in an urban area while 9 (5.4%) in rural areas as can be observed in table 1. All patients were negative for the infectious agents investigated. Fifteen sera (9.03%) were positive for 
*T. cruzi* by ELISA with a 5.2-14.72% IC at 95%. Three of the 15 positive sera 1.2% a 320 bp amplification was obtained with PCR. This finding was confirmed with the specific 
*T. cruzi* fluorescein-labeled-probe hybridization as shown in table 1and figure 1.

The majority of the seropositive BD (n=12) lived in an urban area (Guadalajara metropolitan zone) and three in rural areas (Sta. Cruz Astillero, Ameca, and Atononlco). Eight of them were females, and their age ranged was from 20 to 45 years, as can be seen in table 2.

The questionnaire showed that 148 (89%) of BD known the triatome, 46 (27.7%) have observed the triatome in their homes, and 20 (12%) visited frequently endemic rural zones. From the 15 positive BD, all knew the triatome, therefore it was not possible to calculate the RR. When we compared this group with those who manifested that the triatome was observed in their homes, the RR was 0.39 times greater than to those who visited the endemic rural zones (0.31 times). The questionnaire non-response rate was 0.6 to 30% as shown in table 3.

**Discussion**

In this study, we found a high prevalence of 
*T. cruzi* antibodies. Similar results had been previously reported in endemic rural areas for trypanosomiasis in Mexico. The 1.2% prevalence found by PCR suggest an active asymptomatic, acute-phase disease.

In this investigation, sample were calculated by populations with less of 10,000 individuals, and the correction formula used was considering 3,450 blood donors during the time of study. When we compare the number of samples studied in this investigation and the one reported by others of 4,081, our samples represent 84% of the last number. Difference in prevalence between studies can be explain by the techinc used to determined 
*T. cruzi* antibodies or, the type of Hospital where patients were seen. In this study, patients were from a rural area where is easier to contact vectors. Also, we think that according to our results, there was a high percentage of subregister patients that were able to know the bug.

National Mexican seroepidemiological survey in 1987, using open population studies, showed that Chagas’ disease was in a heterogeneous distribution in the country, with a prevalence of 1.6, 0.5, and 0.2% variation, according to the sample dilutions used for the titer of antibodies. If we analize results published previously, we have different percentage for different states. Among them Jalisco with 1.28% to 0.1%, that’s why there are controversial to considered as high prevalence area for 
*T. cruzi*.

All patients wich ELISA-positive BD knew the triatoma, and the risk that represent the absence of knowledge of the triatoma was not calculated. In addition the RR to acquire the infection was increased when the triatoma has been observed at home and when endemic zones have been visited by the individual with positive results.

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Our findings indicate that Jalisco state has a potential risk for infection with 
*T. cruzi*, do to the anti-
*T. cruzi* antibodies levels, and the presence of the parasite, evidenced by PCR results. We must emphasize that our study was carried out in a blood bank and therefore it is possible the blood transmission through therapeutic transfusion. We think that should be advisable to include a 
*T. cruzi* test in Mexican blood banks mainly in endemic zones, to prevent iatrogenic Chagas’ disease.
### TABLE 1
Age, sex, and *T. cruzi* IgG specific antibodies in sera from blood donors.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Female -ve</th>
<th>Male -ve</th>
<th>Female +ve*</th>
<th>Male +ve*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20-25</td>
<td>22</td>
<td>32</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>25-30</td>
<td>10</td>
<td>18</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>30-35</td>
<td>5</td>
<td>17</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>35-40</td>
<td>6</td>
<td>18</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>40-45</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>45-&gt;</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>104</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

*X DO ≤ 2DST, ELISA in sera 21.

### TABLE 2
Positive IgG anti-*T. cruzi* antibodies by ELISA and PCR in blood from blood donors.

<table>
<thead>
<tr>
<th>Number of the blood donor positive*</th>
<th>PCR</th>
<th>Probe</th>
<th>Place of residence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>Sta. Cruz**</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>Ameca**</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
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</tr>
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<td>13</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>Guadalajara</td>
</tr>
</tbody>
</table>

*Cut-off DO level for ELISA X±2STD. PCR: 320 bp DNA amplification in BD blood. Probe: non-isotopic DNA hybridization PCR amplified. ** rural areas

### TABLE 3
Answers to the questionnaire related with ELISA positive antibodies

<table>
<thead>
<tr>
<th>Answer</th>
<th>Yes</th>
<th>No</th>
<th>*N/A</th>
<th>**RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Know the Triatoma n=166</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
</tr>
<tr>
<td>Triatoma has Been Seen at Home n=166</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
</tr>
<tr>
<td>Rural endemic zones visiting n=160</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
<td>Sera -ve</td>
<td>Sera +ve</td>
</tr>
</tbody>
</table>

*N/A= No answer, ** RR= Relative Risk, *** N/D= Not determined
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