Update on Seroprevalence of Anti-\textit{Trypanosoma cruzi} Antibodies among Blood Donors in Northeast Mexico

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Abstract. Chagas disease has become frequent in non-endemic areas, where it can be transmitted by blood transmission. Therefore, we explored seroprevalence of anti-\textit{Trypanosoma cruzi} antibodies among blood donors at the Cardiology Hospital, Mexican Institute of Social Security at Monterrey, Nuevo León, by both an enzyme-linked immunosorbent assay and indirect hemagglutination. Blood samples from 1,000 healthy blood donors were selected. A seropositivity of 2.8% was shown among the studied population, of which 2.59% (21/809) were inhabitants of Nuevo León, whereas 3.07% (2/65) and 3.96% (5/126) were from Coahuila and Tamaulipas, respectively. Our result is higher than that of a previous study from 1998, where a prevalence of 0.5% was reported. This once again corroborates the importance of installing a surveillance program to detect and prevent the transfusion of \textit{T. cruzi} from asymptomatic blood donors in blood banks located in urban cities recognized as non-endemic.

INTRODUCTION

Chagas disease is an endemic infection caused by the protozoan parasite \textit{Trypanosoma cruzi}. It is a major health problem in rural and, more recently, urban areas, where 15 million people are infected and > 28 million are at risk of being infected.\footnote{Address correspondence to Lucio Galavíz-Silva, Laboratorio de Parasitología, Facultad de Ciencias Biológicas, Universidad B. Avenue, Universidad, S/N, Ciudad Universitaria, San Nicolás de los Garza, CP 66451 Nuevo León, Mexico. E-mail: lgs12167@yahoo.com} Unexpectedly, 60% of them live in urban areas, and ~50% are in a latent period.\footnote{A sample size of 1,000 blood donors (8.3%) was calculated from a total of 12,000 donors per year, with an absolute precision of 1.96, confidence level of 97.5%, and \( E = 1.36 \), which exceed the calculated sample size. Nine hundred twenty-six men and seventy-four women were randomly selected, with a female: male ratio of 1:12.5, as representative of the sex composition of the larger donor population (constituted mainly by relatives or friends’ patients) at the Cardiology Hospital. According to the protocol described in the Technical Norm for Blood Banks in Mexico, the criteria included age between 18 and 50 years, weight > 50 kg (Table 1), clinically healthy, and seronegative for hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis B virus surface antigen (HBsAg), \textit{Brucella abortus} (BrA), venereal disease (VDRL), and human immunodeficiency virus (HIV).} Ten years ago, positive blood donors were reported with a prevalence of 0.5% (of 431) in Nuevo León state.\footnote{The study population was determined with an estimated prevalence for \textit{T. cruzi} antibodies of 0.5%, reported previously. A sample size of 1,000 blood donors (8.3%) was calculated from a total of 12,000 donors per year, with an absolute precision of 1.96, confidence level of 97.5%, and \( E = 1.36 \), which exceed the calculated sample size. Nineteen hundred} Therefore, our objective was to update the prevalence of Chagas disease among donors in northeastern Mexico, a non-endemic area, with two different serologic tests, as suggested by the World Health Organization.\footnote{Data analysis. The \( \chi^2 \) test (\( P < 0.05 \)) was used to determine significant dependence between serologic test (positive/ negative), age range (years), and birthplace (Tamaulipas, Nuevo León, Coahuila), using SPSS software v. 15 (Chicago, IL).}

MATERIALS AND METHODS

Study area. Nuevo León is located in northeast Mexico and has a border with Texas (27°49’N, 23°11’S and 98°26’E, 101°14’W; Figure 1). Monterrey is the capital of the state and is an urban city, with a population of ~4,199,292 inhabitants, of which 96,326 were born in different states of Mexico.\footnote{The indirect hemagglutination assay. The indirect hemagglutination assay (IHA; SERODIA-Chagas; Fujirebio, Tokyo, Japan) was carried out according to the manufacturer’s instructions. Reactive samples at dilution \( \geq 1:32 \) were considered a positive test. All samples were analyzed in duplicate, including the control positive and negative sera described above.} The Cardiology Hospital belongs to the Mexican Institute of Social Security (IMSS), a principal center of medical care for 1,035,330 workers\footnote{Eleven serological positive samples at dilution \( 1:32 \) were considered a positive test.} of Nuevo León and neighbor states (mainly Coahuila and Tamaulipas).\footnote{The samples from blood donors were collected from a peripheral vein using a Vacutainer system. The serum was separated by centrifugation (1,200g for 10 minutes), aliquoted in 1.5-mL tubes, and stored at \(-20^\circ\text{C}\) until use.}

Study population. The sample was determined with an estimated prevalence for \textit{T. cruzi} antibodies of 0.5%, reported previously. A sample size of 1,000 blood donors (8.3%) was calculated from a total of 12,000 donors per year, with an absolute precision of 1.96, confidence level of 97.5%, and \( E = 1.36 \), which exceed the calculated sample size. Twenty-six men and seventy-four women were randomly selected, with a female: male ratio of 1:12.5, as representative of the sex composition of the larger donor population (constituted mainly by relatives or friends’ patients) at the Cardiology Hospital. According to the protocol described in the Technical Norm for Blood Banks in Mexico, the criteria included age between 18 and 50 years, weight > 50 kg (Table 1), clinically healthy, and seronegative for hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis B virus surface antigen (HBsAg), \textit{Brucella abortus} (BrA), venereal disease (VDRL), and human immunodeficiency virus (HIV). Ethical consideration. The study protocol was reviewed and approved by the ethics committee of the Universidad Autónoma de Nuevo León and was performed according to protocol described in the Technical Norm for Blood Banks in Mexico (IMSS).

Serologic test for Chagas disease. The samples from blood donors were collected from a peripheral vein using a Vacutainer system. The serum was separated by centrifugation (1,200g for 10 minutes), aliquoted in 1.5-mL tubes, and stored at \(-20^\circ\text{C}\) until use.\footnote{The enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA; Chagatest ELISA recombinant v. 3.0; Wiener Laboratory, Rosario, Argentina) was carried out according to the manufacturer’s protocol. Absorbance was measured spectrophotometrically at 450/620 nm (Multiscan MS; Thermo Labsystem, Waltham, MA). Each test was carried out in duplicate. Positive (sera from chronic chagasic patients from Brazil and Mexico were obtained from the Instituto Nacional de Cardiología Ignacio Chavez and CINVESTAV-IPN, D.F., Mexico) and negative controls (sera from healthy individuals) were included in each test.} Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA; Chagatest ELISA recombinant v. 3.0; Wiener Laboratory, Rosario, Argentina) was carried out according to the manufacturer’s protocol. Absorbance was measured spectrophotometrically at 450/620 nm (Multiscan MS; Thermo Labsystem, Waltham, MA). Each test was carried out in duplicate. Positive (sera from chronic chagasic patients from Brazil and Mexico were obtained from the Instituto Nacional de Cardiología Ignacio Chavez and CINVESTAV-IPN, D.F., Mexico) and negative controls (sera from healthy individuals) were included in each test.

Indirect hemagglutination assay. The indirect hemagglutination assay (IHA; SERODIA-Chagas; Fujirebio, Tokyo, Japan) was carried out according to the manufacturer’s instructions. Reactive samples at dilution \( \geq 1:32 \) were considered a positive test. All samples were analyzed in duplicate, including the control positive and negative sera described above.

Data analysis. The \( \chi^2 \) test (\( P < 0.05 \)) was used to determine significant dependence between serologic test (positive/ negative), age range (years), and birthplace (Tamaulipas, Nuevo León, Coahuila), using SPSS software v. 15 (Chicago, IL).
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RESULTS

Seropositive blood donors were characterized by matching results of ELISA and IHA. On the basis of this sample, 28 (2.8%) showed antibodies against T. cruzi (Table 1). With regard to the birthplace of the blood donors, 80.9% (809) were born in the metropolitan area of the state of Nuevo León, and the remainder (19.1%; 191) were blood donors born from the neighbor states of Coahuila and Tamaulipas (6.5% and 12.6%, respectively); 21 seropositive donors were from Nuevo León (2.59%), 2 were from Coahuila, and 5 were from Tamaulipas. With regard to age range, this study shows that the highest frequency of seropositives was observed among blood donors 36–45 years of age (1.2%, 12/1,000). The highest frequency was also higher in women (4.05%, 3/74) than in men (2.69%, 25/926). More detailed information, with regard to seroprevalence by birthplace, sex, and age is shown in Table 1. There was no significant statistical association between serologic results (ELISA or IHA), sex composition ($\chi^2 = 5.351, P = 0.500$), and birthplace of donors ($\chi^2 = 12.011, P = 0.445$).

DISCUSSION

The results presented in this study showed evidence of infection by T. cruzi in the population of a non-endemic area of northeast Mexico, indicated by the presence of high levels of antibodies to this parasite; in this region of Mexico, the reported 0.5% prevalence of Chagas disease by the official Secretaria de Salud in 1998 is believed to be underestimated. This study showed that the seroprevalence of anti-T. cruzi antibodies in blood donors was greater than reported and may suggest the importance of blood transfusion as one of the main routes of T. cruzi transmission, which calls for urgent Chagas serologic testing of blood donors in blood banks of urban cities, where many people from endemic rural regions reside, such as in the case of Nuevo León, where in 2005 alone, 96,326 immigrants arrived, adding to the 827,453 already present, which should be further studied in future epidemiologic studies. This study is not statistically representative for inhabitants of Coahuila and Tamaulipas, because our studied population was blood donors from the Cardiology Hospital. In addition, we considered necessary a new screening of seroprevalence in an open population, because the only current information available is from a National Seroepidemiology Survey performed in 1987 and published in 1992 that showed only 0.1% of prevalence for Coahuila and Tamaulipas and 0.2% for Nuevo León; obviously, these results stress the need to update the knowledge of Chagas disease in northern geographic areas of Mexico, such as requested by some reports.

Similar results, with higher seroprevalence than reported earlier, have been shown for the cities of Tijuana, Mexicali, Ensenada, and Tecate, in which infected workers were identified as originating from the “Mixteca Oaxaqueña,” where each year > 100,000 people depart to other cities but only 25% return to their city of origin. Recent reports have shown a seroprevalence of 7.7% in blood donors from Puebla (another non-endemic area); furthermore, in the urban area of Morelos, a prevalence of seropositivity of 20% was reported. These results strongly contrast with a low prevalence published in

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**Table 1**

Seroprevalence of anti-T. cruzi antibodies (ELISA and HAI) among blood donors according to their geographical origin, sex composition, and age ranges

<table>
<thead>
<tr>
<th>Birthplace</th>
<th>Seroprevalence percentage (positives/1,000)</th>
<th>Seroprevalence by sex and birthplace percentage (positives M/N examined/positives F/N examined)</th>
<th>18–25</th>
<th>26–35</th>
<th>36–45</th>
<th>46–50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuevo León</td>
<td>2.1 (21)</td>
<td>2.51 (19/755) 0.79 (2/251) 0.2 (0/19)</td>
<td>0.79</td>
<td>0.2</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Coahuila</td>
<td>0.2 (2)</td>
<td>3.17 (2/63) 0 (0/8) 0 (0/14) 4.34 (1/23)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Tamaulipas</td>
<td>0.5 (5)</td>
<td>3.7 (4/108) 0.1 (0/21) 0.1 (0/3)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>2.8 (28)</td>
<td>2.69 (25/926) 0.3 (2.75/8/290) 0.9 (3.86/10/259)</td>
<td>0.3</td>
<td>0.9</td>
<td>1.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

M = males; F = females; BP = seroprevalence by birthplace; SP = seroprevalence by sampled population.
1992 (1.6% and 0.1–1.5% for Puebla and Morelos, respectively). In this manner, we could suggest that migration phenomenon of rural populations to industrial or tourist cities spreads Chagas disease throughout the country and increases the risk of transmission by blood transfusion in urban areas.

In the population studied, it was noted in the filled-out questionnaires that none of the individuals reported heart problems, which may indicate that seropositive individuals were infected but not sick (indeterminate cases). Similar cases for Chagas disease were reported in Guerrero, Puebla, and Morelos, but none of the seropositive individuals showed intestinal or heart problems, which could suggest that, in Mexico, the infection by \textit{T. cruzi} may be silent or non-specific. In many countries of Latin America, serologic tests are compulsory for blood donors, such as in Chile; however, in Mexico, these measures are only applied in endemic areas, according to the guidelines of the Official Mexican Standard, although endemic areas are not specified. Therefore, there is a need for health systems to develop a program for the serologic screening of blood donors for anti-\textit{T. cruzi} antibodies. This would allow the detection of persons who have been exposed to \textit{T. cruzi}, so that they can be excluded as candidates of blood donors, which would break the chain of transmission of the infection by blood transfusion. An important question in our questionnaire was the birthplace of the donor; a trend to a higher prevalence among blood donors who were born in the metropolitan area of the state of Nuevo León was observed. However, it will be interesting to analyze the donor’s parental birth place because most of the people evaluated were born or resided outside of the urban city (rural zones of Nuevo León or central and southern areas of Mexico).

We conclude that it is necessary to observe the recommendations and standards issued for the control of Chagas disease, which have been authorized and ratified in various inter-gubernatorial meetings of the region. Therefore, it is recommended that the screening of blood donors for anti-\textit{T. cruzi} antibodies be compulsory for all states of Mexico, because increased migration within the country has extended the boundaries of endemic areas, which can be further worsened by the transmission of the disease through blood transfusion. Based on our results, the transmission of \textit{T. cruzi} by blood transfusion may be an emerging risk in Nuevo León, considering that there has been an ~5-fold increase in the incidence of seropositive individuals.

Received March 2, 2009. Accepted for publication April 15, 2009.

Acknowledgments: The authors thank the personnel of the Hospital de Altas Especialidades del IMSS and Jorge Alberto Miranda Murillo and Luis Fernando Sanchez Mendoza for contacting, interviewing, and taking samples from the studied individuals. We also thank Dr. Ricardo Gómez-Flores for the English review of the manuscript.

Financial support: This investigation was supported by Programa de Mejoramiento al Profesorado, Secretaría de Educación Pública, Grant ID SEP PROMEP/103.5/07/2523 and Programa de Apoyo a la Investigación Científica y Tecnológica, PAICYT/UA.NL. CA1488-07.


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REFERENCES