Seroepidemiology of *Trypanosoma cruzi*, Etiologic Agent of Chagas’ Disease, in US Blood Donors

David A. Leiby, Elizabeth J. Read,* Bruce A. Lenes, A. Jeffrey Yund,* Robert J. Stumpf, Louis V. Kirchhoff, and Roger Y. Dodd

A comprehensive seroepidemiologic study was conducted in two Red Cross regions (Los Angeles and Miami) to determine the prevalence of *Trypanosoma cruzi* antibodies in at-risk blood donors, to identify additional risk factors, and to assess the likelihood of transmitting *T. cruzi* by transfusion. At-risk and control donors were stratified by a broad risk question, tested for *T. cruzi* antibodies, and if confirmed as seropositive, enrolled in case-control and lookback investigations. A total of 299,398 donors were queried; 23,978 at-risk and 25,587 control donations were tested, and *T. cruzi* antibodies were confirmed in 34 donors (33 and 1, respectively). Seropositive donors shared one risk factor; birth/extensive time in a *T. cruzi*-endemic area. Lookback studies identified 11 recipients, all negative for *T. cruzi* antibodies. Screening strategies that use a question are unlikely to identify all seropositive donors. The lack of definitive data on the risk of transmission by transfusion indicates additional studies of donors and recipients are needed.

The protozoan parasite *Trypanosoma cruzi*, the etiologic agent of Chagas’ disease, is endemic in portions of Mexico, Central America, and South America, where it is estimated to infect 16–18 million people [1, 2]. Humans usually acquire the infection when feces from a hematophagous triatomine insect (reduvius bug) infected with *T. cruzi* contaminate the conjunctivas, the oral or nasal mucosae, or the bite wound produced by the feeding insect. After an initial, generally mild acute phase, most infected persons enter a lifelong, asymptomatic indeterminate phase characterized by low-grade parasitemias and easily detectible antibodies to *T. cruzi*. Years or decades later, however, up to 30% of chronically infected persons develop symptomatic Chagas’ disease, manifested by cardiac and gastrointestinal dysfunction that results in ~50,000 deaths per year [3].

During the past several decades, several million persons have emigrated to the United States from countries in which Chagas’ disease is endemic [4], and 50,000–100,000 of these immigrants may harbor chronic *T. cruzi* infections [5]. These infected persons represent a largely “silent” reservoir of *T. cruzi*, posing a risk of infecting recipients of blood donated by them. Indeed, 4 acute cases of *T. cruzi* infection acquired in the United States and Canada through blood transfusion have been described, 3 of which involved *T. cruzi*-infected donors from *T. cruzi*-endemic areas in Latin America [6–9]. Additionally, these 4 cases occurred in immunocompromised patients, and thus it is likely that additional cases have occurred in immunocompetent persons but were not recognized as Chagas’ disease [3]. The extent to which public health, and in particular blood component safety, is threatened in the United States by *T. cruzi*-infected blood donors remains unclear. Estimates of the seroprevalence of *T. cruzi* in the United States vary widely (0.06%–4.9%) [5, 10–12]. A related study estimated that 1 in 340 blood donors in California has at least one reported risk factor for *T. cruzi* infection, but this study did not include antibody testing [13]. All these studies were designed primarily to determine seroprevalence or risk of *T. cruzi* infection in selected populations of blood donors, but they did not address directly issues relevant to transfusion medicine, such as the likelihood of *T. cruzi* transmission by blood transfusion.

The present study was designed to determine the prevalence of antibodies to *T. cruzi* in blood donors with and without risk for prior exposure to the parasite and to define risk factors useful for developing new strategies for identifying infected donors. An additional goal was to determine the rate of *T. cruzi* transmission attributable to transfusion.

**Methods**

**Study population and risk question.** From May 1994 through June 1995, all community whole blood donors at the American
Red Cross (ARC) Southern California (Los Angeles and Orange Counties) and South Florida (metropolitan Miami) regions were stratified on the basis of their response to a single broad risk question regarding possible T. cruzi exposure. In the Southern California Region, the study population also included all platelet apheresis and directed whole blood donors. Initially, donors were asked the following question: ‘‘Have you ever been in Mexico, Central America, or South America for >4 weeks?’’ We found, however, that some persons born in a T. cruzi–endemic country did not consider that they had spent >4 weeks in the designated T. cruzi–endemic area. Therefore, during the 10th month of the study, we changed our question to include birth as a risk factor: ‘‘Were you born in Mexico, Central America, or South America, or Have you ever spent >4 weeks in any of those places?’’ This latter question was used during the remainder of the study.

The broad risk question was administered by donor health historians as part of the usual interviews before donation. Spanish language versions of the risk question and all donor forms were available if requested by donors. Each donor who responded ‘‘yes’’ to the question submitted a serum or plasma sample for T. cruzi antibody testing. In Southern California, a comparable number of ‘‘no’’ donors was randomly selected as controls, while in South Florida approximately two ‘‘no’’ donations were chosen for each ‘‘yes’’ response.

**Laboratory testing.** Serum or plasma samples were tested for antibodies to T. cruzi (Chagas Antibody Enzyme Immunoassay Generation 2.0; Abbott Laboratories, Abbott Park, IL) as described in the manufacturer’s product insert. If a sample was reactive initially, it was retested in duplicate and considered repeat-reactive if one or both of the two repeat tests were reactive. Samples that were initially nonreactive or those for which both of the repeat tests were nonreactive were considered nonreactive. All EIA testing was done at the Southern California Region’s research laboratories in Los Angeles.

All samples identified as repeat-reactive by EIA underwent confirmatory testing at the ARC’s Holland Laboratory (Rockville, MD) using a radioimmunoprecipitation assay (RIPA) [14]. These samples were assayed in parallel with 3 negative and 3 positive control sera, the latter obtained from parasitologically confirmed cases of Chagas’ disease. Diagnostic confirmation of seropositivity by RIPA was defined as the presence of bands in autoradiographs indicative of antibodies specific for the 72- and 90-kDa glycoproteins of T. cruzi. Any specimen that was EIA repeat-reactive and RIPA-positive was considered a confirmed seropositive, and its donor was included in case-control and lookback studies.

**Donor and blood product management.** All units of blood donated by persons at risk for T. cruzi infection were quarantined until EIA testing was complete. Units with nonreactive EIA results were released from quarantine, but those with repeat-reactive EIA test results were destroyed. All donors of repeat-reactive units were indefinitely deferred from subsequent donations, regardless of RIPA test results. Repeat-reactive donors were provided information about their test results, donor deferral status, T. cruzi infection, and Chagas’ disease and were referred to community physicians for further care.

**Case-control analysis.** All confirmed positive donors were invited to participate in the case-control study. A questionnaire designed to identify factors associated with T. cruzi infection was administered to each participant by a trained nurse-epidemiologist. Questions addressed the donor’s medical history, perinatal risk factors, occupational history, parenteral exposure, environmental exposure, and for female participants, pregnancy history. For each index case enrolled, at least 3 EIA-seronegative control cases selected randomly from donors who had answered ‘‘yes’’ to the broad risk question were interviewed.

**Lookback investigations.** Lookback investigations were initiated for previous donations of all donors who were confirmed seropositive for T. cruzi. For all blood components from previous donations, records were tracked to determine the final disposition of each component. Recipients of transfused components were identified by the transfusing facility and contacted by their primary physician or by letter to obtain blood samples for T. cruzi antibody testing and information about their clinical status. Serum or plasma samples from recipients were tested by EIA and RIPA or alternative tests when access to samples was not permitted.

**Statistical analyses.** Case-control data were analyzed by univariate analysis (Epi Info, version 6.03; CDC, Atlanta). For each risk factor univariate crude odds ratios (ORs) and 95% confidence intervals (CIs) were determined. Additional statistical analyses were performed when appropriate using analysis of variance and χ² analysis. \( P < .05 \) was considered significant in all cases.

**Results**

**Prevalence of antibodies to T. cruzi.** During the 14-month study period, 299,397 blood donors were queried...
about broad risk for exposure to T. cruzi. Overall, 8.24% of donors responded affirmatively, with a significantly \( \chi^2 = 759.2, P < .001 \) larger percentage of Miami donors (12.04%) responding “yes” than Los Angeles donors (7.73%). Results of EIA testing are summarized in table 1. Of the 49,465 donations tested by EIA, 34 were confirmed as seropositive by RIPA, 33 (0.14%) from 23,978 “yes” respondents and 1 (0.004%) from 25,478 “no” respondents (table 1). It was subsequently learned, through a case-control interview, that this latter donor had answered the risk question in the negative, despite the fact that she was born and had lived in El Salvador. Her comprehension of English was poor, but she had not requested a Spanish donor form, and thus she may have misunderstood the question.

The rate of false-positive EIAs, defined as an EIA repeat-reactive test that was negative on RIPA testing, was comparable for “yes” and “no” respondents (0.16% and 0.13%, respectively; \( \chi^2 = 0.9, P \) not significant [NS]). The EIA showed a specificity of 99.9% with a positive predictive value of 32.4%. This specificity calculation was based on the assumption that all donations negative by EIA would have been negative by RIPA. Among samples from donors with a “yes” response, the rates of EIA repeat reactivity and RIPA-confirmed seropositivity were not significantly different (\( \chi^2 = 1.7, P = NS \) and \( \chi^2 = 0.4, P = NS \), respectively) in Los Angeles (0.32% and 0.15%, respectively) compared with rates in Miami (0.19% and 0.09%, respectively). On the basis of the assumption that there were no seropositive persons among the untested donors, the estimated rates of confirmed seropositivity for all donations were comparable for the two regions: 1 in 8809 (30/264,279) for Los Angeles and 1 in 8780 (4/35,118) for Miami because of a lower proportion of “yes” respondents in Los Angeles.

**Case-control studies.** Of the 34 donors confirmed as seropositive for T. cruzi, 26 participated in the case-control study. The

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases no. (%)</th>
<th>Controls no. (%)</th>
<th>Crude OR (95% CI) or statistic*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17–69</td>
<td>18–72</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>38.9</td>
<td>40.5</td>
<td>( P = .62^1 )</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (48)</td>
<td>51 (57)</td>
<td>0.72 (0.26–2.0)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (52)</td>
<td>38 (43)</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;8th grade</td>
<td>7 (28)</td>
<td>1 (1)</td>
<td>34 (3.9–1559.0)</td>
</tr>
<tr>
<td>Less than high school diploma</td>
<td>11 (44)</td>
<td>2 (2)</td>
<td>34 (6.2–332.0)</td>
</tr>
<tr>
<td>High school diploma only</td>
<td>13 (52)</td>
<td>11 (12)</td>
<td>7.7 (2.5–24.0)</td>
</tr>
<tr>
<td><strong>Family income (controls, n = 82)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;$10,000)</td>
<td>3 (12)</td>
<td>0</td>
<td>Undefined</td>
</tr>
<tr>
<td>(&lt;$20,000)</td>
<td>11 (44)</td>
<td>11 (13)</td>
<td>5.1 (1.7–16.0)</td>
</tr>
<tr>
<td>(&lt;$30,000)</td>
<td>17 (68)</td>
<td>21 (26)</td>
<td>6.2 (2.1–18.0)</td>
</tr>
<tr>
<td><strong>Of Spanish-speaking origin</strong></td>
<td>25 (100)</td>
<td>50 (56)</td>
<td>Undefined</td>
</tr>
<tr>
<td><strong>Hispanic ethnicity (cases, n = 23; controls, n = 84)</strong></td>
<td>21 (91)</td>
<td>13 (15)</td>
<td>57 (11–530)</td>
</tr>
<tr>
<td>Hispanic or other clearly linked to T. cruzi–endemic area (cases, n = 23; controls, n = 84)</td>
<td>22 (96)</td>
<td>18 (21)</td>
<td>81 (11–3377)</td>
</tr>
<tr>
<td><strong>Birth in Mexico, Central America, or South America</strong></td>
<td>24 (96)</td>
<td>35 (39)</td>
<td>37 (5.4–1550.0)</td>
</tr>
<tr>
<td><strong>Health (1 = excellent to 5 = poor)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worse than very good (2)</td>
<td>13 (52)</td>
<td>13 (15)</td>
<td>6.3 (2.1–19.0)</td>
</tr>
<tr>
<td>Worse than good (3)</td>
<td>5 (20)</td>
<td>1 (1)</td>
<td>22 (2.2–1052.0)</td>
</tr>
<tr>
<td><strong>Recognize vector</strong></td>
<td>6 (24)</td>
<td>6 (7)</td>
<td>4.4 (1.1–18.0)</td>
</tr>
<tr>
<td><strong>Ever live in a simple house</strong></td>
<td>18 (72)</td>
<td>20 (22)</td>
<td>8.9 (3.0–28.0)</td>
</tr>
<tr>
<td><strong>Time spent in T. cruzi–endemic region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cases, n = 24; controls, n = 86)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (years)</td>
<td>20.6</td>
<td>7.4</td>
<td>( P &lt; .001^1 )</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>22 (92)</td>
<td>27 (31)</td>
<td>24 (5.2–219.0)</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>21 (88)</td>
<td>22 (26)</td>
<td>20 (5.2–113.0)</td>
</tr>
</tbody>
</table>

**NOTE.** OR is calculated as odds that confirmed seropositive donor would have risk factor divided by odds that control group would have risk factor. CI, confidence interval.

* Values represent tests for differences in continuous variables.

† By analysis of variance.
remaining 8 chose not to participate or were lost to follow-up. For statistical analyses, the case-control questionnaire from the seropositive donor who responded ‘no’ to the broad risk question was excluded. When cases and controls were compared, no differences were identified in medical history (not shown), transfusion history (not shown), or visual recognition of the vector, all of which represent risk factors for exposure to T. cruzi (table 2). In contrast, index and control cases showed significant differences for mean time spent in an endemic region, 20.6 years and 7.4 years, respectively. Furthermore, with one exception, all index cases identified were born in countries where T. cruzi is endemic (tables 2, 3). The exception was a 17-year-old woman born in Los Angeles to parents who had been born and had resided in El Salvador for many years (mother) and in T. cruzi–endemic areas of Mexico (father). This donor had traveled to each of these areas, and thus she may have acquired T. cruzi from an infected insect or, less likely, via congenital transmission. On the basis of birth and time spent in T. cruzi–endemic areas, the observation that our index cases identified themselves as Hispanic or of Spanish-speaking origin was expected. Last, the index cases showed a strong tendency toward lower socioeconomic levels than controls, specifically for measures of education level, family income, and having lived in simple housing (e.g., mud walls, unmilled logs and sticks, thatched roof) (table 2). This final characteristic is generally thought to be a risk factor for T. cruzi infection [4], and 72% of our seropositive donors had lived in such houses.

Lookback investigations. Lookback investigations were carried out for all 9 confirmed seropositive donors who had previously donated blood. The dispositions of 69 components prepared for transfusion are presented in figure 1. Of the 14 recipients who could be contacted, 3 declined to be tested, 1 was reportedly tested by an immunofluorescence assay at the discretion of the recipient’s physician, and the remaining 10 recipients were tested using EIAs (8 by ARC with Abbott EIA and 2 by others with Gull EIA [Gull Laboratories, Salt Lake City]). All 11 recipients tested for T. cruzi antibodies were negative.

Discussion

Chagas’ disease has long been recognized as a serious and widespread public health problem in Latin America, with frequent transmission of T. cruzi by blood transfusion known to be a significant part of the overall problem [4]. In contrast, this illness has only become a focus of attention in the United States in recent years. Reservoirs of T. cruzi–infected wild animals and insect vectors exist throughout portions of the southern and southwestern United States. Moreover, 4 cases of autochthonous Chagas’ disease have been reported, the most recent of which occurred in 1983 [15–18].

More recent events, however, highlight the increased potential for transmission of T. cruzi by blood transfusion here. Several million persons have emigrated from endemic countries of Latin America to the United States and Canada, thereby creating a risk for transmission of T. cruzi in our hospitals. Four such cases of transfusion-associated transmission have been reported [6–9]. Information regarding this immigrant population, especially the subpopulation that donates blood, is limited. Previous blood bank studies have identified confirmed seropositive donors in several California and Texas cities [10–12]. Additionally, parasite-confirmed cases of T. cruzi infection have been reported among Nicaraguan and Salvadoran immigrants living in Washington, DC [5]. A survey in California reported that 2.4% of blood donors had lived in T. cruzi–endemic areas for more than a year [13]. Along the same lines, we conducted a survey of 3000 randomly selected blood donors in six ARC US collection regions, using our stratification question, and found that 42 (2.5%) of the 1688 donors who participated responded “yes” to the broad risk question (unpublished data). On the basis of this survey and the present seroprevalence study, we estimate that at least 2.5% of blood donors nationwide have geographic risk of exposure to T. cruzi, with higher rates in certain areas, such as Miami and Los Angeles. Thus, it may be inferred that blood donors seropositive for T. cruzi are not restricted to a limited number of US metropolitan areas but can be found throughout the country.

A key issue is the extent to which the existence of T. cruzi infection among US blood donors impacts the health of the transfused population. This study showed no evidence of transmission to 11 recipients of blood from seropositive donors. However, the upper 95% CI of this observation does not exclude the possibility of a 28% infectivity rate, a figure that is compatible with the 13%–49% rates reported from South America [19–23]. Still, the absence of observed transmission is intriguing because the 4 documented transfusion cases in the United States and Canada established that this route of transmission does occur in areas that are not T. cruzi–endemic and includes cases involving donors who left T. cruzi–endemic areas 15–20 years earlier, as is well known in T. cruzi–endemic countries. Parasites were present in the blood of seropositive persons, including 5 of 10 seropositive
Figure 1. Disposition of blood donated previously by donors identified as seropositive for antibodies to T. cruzi. Flow diagram indicates disposition of blood components derived from previous donations made by T. cruzi-seropositive blood donors and test results for recipients of transfused components. Components transfused to recipients and tested for antibodies to T. cruzi included packed red cells (PRC), platelet concentrate (PLC), fresh frozen plasma (FFP), and cryoprecipitate (Cryo).

Central American immigrants identified by Kirchhoff et al. [5] in Washington, DC.

Because transmission by transfusion is likely to occur in the United States, other factors may have contributed to our failure to observe transmission by transfusion. First, anticipated rates of transmission by transfusion are based on published studies from South America that used different test procedures and algorithms to define persons with T. cruzi infection. It is possible that we did not observe positive cases that would have been identified by alternative testing strategies (correctly or incorrectly), causing our rate to be comparatively low. Second, in the 4 North American transfusion cases mentioned above, all recipients were transfused with at least one unit of platelets, while only 1 of our 11 lookback recipients received platelets (figure 1). Platelets are prepared under centrifugation conditions that concentrate T. cruzi and are subsequently maintained under conditions that favor parasite survival (22–24°C, shelf life of 5 days, enriched plasma environment). Recipients of platelets, moreover, are often immunocompromised [24, 25] and therefore may be more likely to have easily recognizable, fulminant courses of acute T. cruzi infection. The immune status of the tested recipients in our study was not determined. As a caveat, platelet transfusion occurs less frequently in South America, but transmission by transfusion is common nonetheless, perhaps due in part to the transfusion of whole blood in some regions [26].

Our lookback studies are continuing, and we hope to develop more precise estimates of transmission risk. In the meantime, our observations have important consequences for the development of intervention strategies. The concept of screening donors by questionnaire and deferring those at risk appears inherently attractive,
although it may well be regarded as discriminatory and may also have the undesirable effect of eliciting untruthful responses [27].

Deferral of all donors with geographic risk for *T. cruzi* infection would likely result in the loss of as much as 2.5% of the US donor population, with some regions such as Miami deferring up to 12% of eligible donors. Of greater importance, even a broad risk question such as the one we used failed to identify all seropositive donors. One seropositive donor gave an incorrect negative answer and, of interest, 1 seropositive person provided a positive answer although she had been born in the United States and had visited a *T. cruzi* endemic area for <4 weeks. Furthermore, our data show that attempts to increase the specificity of such questioning would materially reduce its sensitivity; commonly recognized risk factors for *T. cruzi* infection, such as substandard housing or an ability to recognize the insect vector, failed to identify a meaningful proportion of seropositive donors (table 2).

On the basis of these findings, it does not appear likely that a strategy of testing only those donors judged to be at risk by questionnaire would be effective. Thus, the most effective approach would likely be testing of all donors, despite the fact that the vast majority would not have any risk of *T. cruzi* infection. However, no tests for screening of blood for antibodies to *T. cruzi* have been licensed in the United States. This situation places a premium on understanding the true frequency and public health risk of transfusion-transmitted *T. cruzi* in the United States. In particular, additional comprehensive studies are needed to examine levels of parasitemia in donors, as are lookback evaluations that incorporate characteristics of blood products transfused (e.g., component type, age, number of units transfused) and the health status of the recipients.

Acknowledgments

We thank the following persons: Krista L. Kerr, Holland Laboratory, for sample and RIPA management; Dezy MacDowell and Martina Lutcher for testing, ARC Southern California Region, Los Angeles; Susan R. Nystrom and Patricia P. Tallent for donor issues, disease and blood transfusion (in Spanish [English abstract]). Bol Of Sanit Panam 1989;11:849–51.


24. Martina Lutcher for testing, ARC Southern California Region, Los Angeles, for serologic testing of 2 lookback specimens with Gull EIA.


References


