Prevalence of *Trypanosoma Cruzi* antibodies in blood donors from the Sao Paulo State, Brazil, between 2012 and 2014

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Abstract

Introduction: American tripanosomiasis (Chagas disease), the second most neglected disease in the world, is caused by the protozoan parasite *Trypanosoma cruzi*. Though natural transmission by insect vectors has been controlled, there is significant risk of *T. cruzi* transmission by blood transfusion in non-endemic regions, generally due to immigration processes from endemic areas.

Methodology: The objective of this study was to evaluate anti-*T. cruzi* seroprevalence in blood donors from the western part of São Paulo State, Brazil, by serologic and immunofluorescence confirmation tests for the period between 2012 and 2014. Currently, this region is regarded as a non-endemic area for Chagas disease.

Results: The confirmed overall *T. cruzi* seroprevalence among blood donors was 0.10%, which can be considered low compared to other Brazilian regions. Nevertheless, the distribution of the anti-*T. cruzi* antibodies within the examined region was uneven, and some areas of significantly higher prevalence were observed.

Conclusions: We could consider two tendencies in the prevalence of *T. cruzi*: (i) residual older undiagnosed cases from São Paulo State, and (ii) immigration from endemic Brazilian or South American regions. The discordance obtained for *T. cruzi* prevalence by serologic and immunofluorescence methods demonstrates that more specific routine diagnosis is needed to diminish the cost of the assays and the loss of blood supply once all seropositive blood bags are immediately discarded.

Key words: Blood donors; *Trypanosoma cruzi*; seroprevalence; blood transfusion.

Introduction

American trypanosomiasis, or Chagas disease, is the second most neglected tropical disease, is a zoonosis caused by the protozoan kinetoplastid parasite *Trypanosoma cruzi* (*T. cruzi*). Approximately 7 million people are infected worldwide, mostly in continental Latin America, with death toll rate of more than 7,000 cases per year. The infection is widely distributed within the Americas, from North America to southern Chile and Argentina. Once totally restricted to the American continent, Chagas disease has been spread to other continents due to the improvement of global travel and population movements from and to Latin America [1]. In its natural cycle, occurring exclusively in the Americas, *T. cruzi* is transmitted by large blood-sucking insects, named kissing or assassin bugs, belonging to the *Triatominae* subfamily of the *Reduviidae* family. Once the population of the *Triatominae* insects is mostly controlled [2], in its absence, *T. cruzi* can be transmitted by alternative routes which include congenital transmission, organ transplantation, oral transmission, and transmission by blood transfusion [3].

The transmission of *T. cruzi* by blood transfusion is the second most important route of parasite dissemination in Latin America and the most important in industrialized countries [4]. *T. cruzi* can withstand blood storage conditions such as low temperatures (from 4°C to -80°C), freezing, and thawing [5]; consequently, it can be transmitted virtually by all blood components (*i.e.* whole blood, packed red blood cells, granulocytes, fresh frozen plasma,

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cryoprecipitate, and platelets). The possibility of transfusion-transmitted infection depends on risk factors such as the amount of transfused blood, parasite strain, presence of parasitemia by the time of donation, immune status of the recipient, and sensibility of the used diagnostic tests [4]. The highest transfusion transmission risk has been observed for platelet concentrates in comparison to plasma and whole blood due to the higher platelet parasitic load [6]. Fortunately, only a small proportion of individuals infected with T. cruzi through blood transfusion develop clinically overt disease [7]. Additionally, the universal screening for T. cruzi in endemic countries has dramatically reduced the risk of the transfusion transmitted infection, even though the sensibility of the T. cruzi diagnostic assays can be lower compared to the viral screening tests (97%–99%). In countries where the routine screening for T. cruzi in blood banks has been fully implemented, the residual risk of transfusion-transmitted infection has been calculated to be approximately 1 in 200,000 blood donations, with clusters of geographical endemicity [4,8].

The objective of this study was to examine the prevalence of anti-T. cruzi IgM/IgG antibodies (anti-T. cruzi Ab) in blood donors for the period between 2012 and 2014 from the western part of São Paulo State, considered currently not endemic for this tropical disease.

Methodology

Geographic location

The Blood Center of Ribeirão Preto is located in the northeast part of São Paulo State (21°10′42″S 47°48′24″W), southeast Brazil, and is responsible for serological testing for bloodborne infectious diseases in volunteer blood donors recruited from Ribeirão Preto city and its branches (municipalities of Serrana, Batatais, Fernandópolis, Franca, Presidente Prudente, Araçatuba, Olimpia, and Bebedouro). The covered region corresponds to almost the entire western part of São Paulo State (Figure 1).

Volunteer blood donors

From January 2012 to December 2014, a total of 281,551 blood donations were obtained from volunteer donors from the Blood Center of Ribeirão Preto and its branches. The donations were obligatorily tested serologically for the following bloodborne infections, based on Brazilian legislation: human immunodeficiency virus-1/2 (HIV-1/2), hepatitis C virus (HCV), hepatitis B virus (HBV), human T-lymphotropic virus-1/2 (HTLV-1/2), T. cruzi, and Treponema pallidum. The inclusion criteria for donation of blood were age between 18 and 65 years, minimal body weight (~ 50 kg for men, ~55 kg for women), absence of acute infectious disease, no report of risk behavior (intravenous drug abuse, unsafe sexual practices, etc.), and no history of blood or hemoderivative application in the previous 10 years. In this study, the donors were classified based on their number of donations as first-time, lapsed (only one donation), or regular (more than two times a year).

Serological detection and confirmation of American trypanosomiasis (Chagas disease)

Serological screening for anti-T. cruzi Ab was performed obligatorily on all blood donations. For serological testing, the Gold ELISA Chagas Kit (REM, São Paulo, Brazil), detecting simultaneously IgM/IgG, was used, following the manufacturer’s instructions. For confirmation of the positive results, the indirect immunofluorescence (IF) Imunocruzi Kit (Biolab-Mérieux, Rio de Janeiro, Brazil) was applied, following the manufacturer’s instructions.

Statistics

The anti-T. cruzi Ab prevalence among volunteer blood donors was obtained dividing the number of IF-confirmed first-time T. cruzi samples by the total number of first-time blood donations collected for each year. Data regarding gender and age of the seropositive blood donors were compared using the Chi-squared ($\chi^2$) test. The statistical analysis was performed using GraphPad Prism version 5 software (GraphPad Software, San Diego, USA). A p value was considered statistically significant if it was below 0.05 (p < 0.05).
Table 1. Total number of donations obtained at the different municipalities of the São Paulo State and prevalence of anti-Trypanosoma cruzi antibodies among blood donors for the period 2012–2014.

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Total number of donations</th>
<th>First-time donations</th>
<th>Seroprevalence</th>
<th>Confirmatory test</th>
<th>Median age</th>
<th>Male / female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribeirão Preto</td>
<td>96,177</td>
<td>23,002</td>
<td>0.15% (34/23,002)</td>
<td>0.10% (24/23,002)</td>
<td>46.5 years (range 23–63)</td>
<td>75.00/25.00</td>
</tr>
<tr>
<td>Presidente Prudente</td>
<td>45,090</td>
<td>10,289</td>
<td>0.11% (11/10,289)</td>
<td>0.04% (4/10,289)</td>
<td>47 years (range 38–55)</td>
<td>25.00/75.00</td>
</tr>
<tr>
<td>Franca</td>
<td>42,984</td>
<td>9,473</td>
<td>0.08% (8/9,473)</td>
<td>0.04% (4/9,473)</td>
<td>49.8 years (range 33–63)</td>
<td>100.00/0.00</td>
</tr>
<tr>
<td>Araçatuba</td>
<td>40,286</td>
<td>9,812</td>
<td>0.12% (12/9,812)</td>
<td>0.09% (9/9,812)</td>
<td>39 years (range 23–54)</td>
<td>88.88/11.12</td>
</tr>
<tr>
<td>Fernandópolis</td>
<td>24,311</td>
<td>6,365</td>
<td>0.36% (23/6,365)</td>
<td>0.28% (18/6,365)</td>
<td>50 years (range 37–64)</td>
<td>44.44/55.56</td>
</tr>
<tr>
<td>Olimpia</td>
<td>9,594</td>
<td>2,063</td>
<td>0.19% (4/2,063)</td>
<td>0.10% (2/2,063)</td>
<td>60 years (range 59–61)</td>
<td>100.00/0.00</td>
</tr>
<tr>
<td>Bebedouro</td>
<td>9,227</td>
<td>1,743</td>
<td>0.00% (0/1,743)</td>
<td>0.00% (0/1,743)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Batatais</td>
<td>8,411</td>
<td>1,272</td>
<td>0.16% (2/1,272)</td>
<td>0.16% (2/1,272)</td>
<td>39 years (range 36–42)</td>
<td>0.00/100.00</td>
</tr>
<tr>
<td>Serrana</td>
<td>5,471</td>
<td>635</td>
<td>0.31% (2/635)</td>
<td>0.00% (0/635)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>281,551</td>
<td>64,654</td>
<td>0.15% (96/64,654)</td>
<td>0.10% (63/64,654)</td>
<td>46.8 years (range 23–64)</td>
<td>65.07/34.93</td>
</tr>
</tbody>
</table>

1 Seroprevalence determined by the Gold ELISA Chagas Kit (REM, São Paulo, Brazil); 2 Confirmation of Trypanosoma cruzi infection by utilization of Immunocruzii Kit (Biolab-Mérieux, Rio de Janeiro, Brazil); 3 Percentage of the individuals with confirmed T. cruzi infection by immunofluorescence.

Results

A total of 281,551 donations obtained from Ribeirão Preto city and its branches were screened for anti-T. cruzi Ab in the laboratory of serology, Blood Center of Ribeirão Preto, for the period between 2012 and 2014. From these donations, 64,654 (23%) were obtained from first-time blood donors. Anti-T. cruzi Ab were detected in 96 first-time blood donors (total seroprevalence, 0.15%). The indirect IF test confirmed T. cruzi infection in 0.10% of the cases with positive serology (Table 1). The seroprevalence rate for anti-T. cruzi Ab per year was 0.22% in 2012, 0.08% in 2013, and 0.14% in 2014. The IF confirmation demonstrated much lower prevalence: 0.14% in 2012, 0.06% in 2013, and 0.09% in 2014 (Table 2).

The median age of the IF positive for anti-T. cruzi Ab donors varied depending on the region. In general, the median age of infected individuals was higher: 46.8 years (47.7 years in 2012, 42.5 years in 2013, 48.8 years in 2014). However, the median age of the positive donors varied depending on region: in Ribeirão Preto, the median age was 46.5 years (range 23–63 years); in Batatais, 39 years (range 36–42 years); in Fernandópolis, 50 years (range 37–64 years); in Franca, 49.8 years (range 33–63 years); in Presidente Prudente, 47 years (range 38–55 years); in Araçatuba, 39 years (range 23–54 years); and in Olimpia, 60 years (range 59–61 years). Regarding the prevalence by gender, a higher occurrence of anti-T. cruzi Ab was observed in males than in females (p < 0.5) (Tables 1 and 2). By year, the distribution of the genders was as follows: 61% male blood donors (19/31) in 2012, 69% (9/13) in 2013, and 68% (13/19) in 2014.

The highest number of IF-confirmed T. cruzi infections (0.28% serology + IF) was observed in the municipality of Fernandópolis (population IBGE, Instituto Brasileiro de Geografia e Estatística, 2014; 67,836 inhabitants). During the study period, that location provided 6,365 first-time blood donations, from which 23 were serologically reactive to anti-T.

Table 2. Seroprevalence of anti-Trypanosoma cruzi antibodies per year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of donations</th>
<th>First-time donations</th>
<th>Seropositive donations</th>
<th>Confirmatory test</th>
<th>Median age</th>
<th>Male / female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>93,858</td>
<td>21,753</td>
<td>0.22% (48/21,753)</td>
<td>0.14% (31/21,753)</td>
<td>47.7 years (range 23–64)</td>
<td>61.30/38.70</td>
</tr>
<tr>
<td>2013</td>
<td>94,527</td>
<td>21,745</td>
<td>0.08% (18/21,745)</td>
<td>0.06% (13/21,745)</td>
<td>42.5 years (range 23–64)</td>
<td>69.23/30.77</td>
</tr>
<tr>
<td>2014</td>
<td>93,166</td>
<td>21,156</td>
<td>0.14% (30/21,156)</td>
<td>0.09% (19/21,156)</td>
<td>48.8 years (range 25–63)</td>
<td>68.42/31.58</td>
</tr>
<tr>
<td>TOTAL</td>
<td>281,551</td>
<td>64,654</td>
<td>0.15% (96/64,654)</td>
<td>0.10% (63/64,654)</td>
<td>46.8 years (range 23–64)</td>
<td>65.08/34.90</td>
</tr>
</tbody>
</table>
cruzi Ab; however, the infection was confirmed by IF in only 18 cases. The second highest prevalence (0.16%) was observed in Batatais municipality, with two confirmed by IF cases. The higher prevalence of anti-

Discussion
In Latin American countries, where American trypanosomiasis is endemic, the transmission of T. cruzi by blood transfusion is the second most important route for parasite acquisition. Hemocomponents are contaminated by apparently healthy blood donors with asymptomatic, parasitemic, or chronic Chagas disease, who are unaware of their status [8].

Our results demonstrated that the prevalence of anti-T. cruzi Ab in blood donors from the western part of São Paulo State for the period of 2012–2014 was 0.10% (IF + serology), with the highest peak in 2012 (0.14, IF + serology) (Tables 1 and 2). The obtained prevalence was similar to the seroprevalence registered in the city of Uberaba (0.2% serology without confirmation) [9]; however, it was higher compared to the cities of São Paulo (0.02% per year; study period 1996–2001) [10] and Campinas (0.05%) [11]. The last two cities, which are located within São Paulo State, represent the largest metropolitan territories not only in the State but also in the country; therefore, the anti-T. cruzi Ab seroprevalence is calculated on large sample volume with a smaller number of confirmed cases. Compared to the rest of Brazil, the obtained anti-T. cruzi Ab seroprevalence in blood donors can be regarded as low. Brazilian blood donors from various regions demonstrate anti-T. cruzi Ab prevalences of 0.47% (southern Brazil) [12], 1.4% (Londrina city, Paraná State) [13], 1.7% (Rio de Janeiro city) [14], and 1.9% (Iguatu city, Ceará State) [15]. The significant difference of the anti-T. cruzi prevalence between blood donors in the different Brazilian regions is probably due to the different sensitivity and specificity of the tests used for the blood donor screening, the year studied, the demographic characteristics of the population, the sample volume, and/or presence of vector transmission.

The obtained prevalence, however, was lower compared to the T. cruzi prevalences of blood donors from other Latin American regions (i.e., states of Veracruz, 0.5% and Puebla, 9.0%; Mexico [17], Buenos Aires [1.46%], and Argentina [18]). The South American regions with the highest T. cruzi seroprevalence such as Gran Chaco, Argentina (> 20%) [19] and Bolivia (28%) [20], cannot be compared to our results because in these locations, complete vector control has not been achieved.

Our data demonstrated uneven distribution of T. cruzi prevalence in the examined region; the highest occurrence was observed in the Fernandópolis municipality with rates of 0.28% (IF + serology). A specific characteristic of this region is its specialization in sugarcane production. All the positive individuals belonged to low socioeconomic classes and had low schooling levels. Once the economic activities of this region are related to agricultural production, one could suppose that there is a sporadic occurrence of Chagas disease transmission by Triatominae vectors. However, the median age of the infected individuals was 51.2 years. No young blood donors tested positive for Chagas disease. Though the state of São Paulo has been regarded as free of vectorial Chagas disease transmission since 1970 [21], it is highly probable that these positives represent residual undiagnosed asymptomatic cases.

On the other hand, an interesting tendency was identified in the Araçatuba region, which demonstrated lower prevalence than Fernandópolis (0.09% IF + serology, 2012–2014). Almost all the Chagas disease cases were observed in young rural workers (23–36 years) with low education originating from northeast Brazil (states of Bahia and Alagoas). In the northeast region, natural T. cruzi transmission with participation of Triatominae vectors has been observed [22]. These positive cases of Chagas disease can be regarded as introduced from endemic areas; therefore, we can consider that the T. cruzi seroprevalence in the western part of São Paulo state can result from two events: (i) entrance of immigrants from other Brazilian regions endemic for Chagas disease (or highly endemic Latin American countries such as Bolivia and Argentina), and (ii) residual undiagnosed asymptomatic cases before eradication of T. cruzi in the State.

Conclusions
The anti-T. cruzi Ab prevalence in volunteer blood donors from the western part of São Paulo State can be considered low. The current T. cruzi prevalence in this region is probably due to two events: immigrant influx...
from endemic areas with vector transmission and presence of residual undiagnosed asymptomatic cases before eradication of the vectorial transmission. In recent years, due to the increased immigration of chronically infected individuals from endemic regions, we can expect an increase of the T. cruzi seroprevalence in non-endemic areas such as São Paulo State. More reliable routine T. cruzi serology is needed to diminish the rate of false-positive results and loss of blood supply.

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