JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

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

Svetoslav Nanev Slavov[1,2], Katia Kaori Otaguiri[1,3], Mariana Tomazini Pinto[1], Vanderléia Bárbaro Valente[1], Eugênia Maria Amorim Ubiali[1], Dimas Tadeu Covas[1,2], Simone Kashima[1,3]

[1] Blood Center of Ribeirão Preto, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[2] Department of Clinical Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo; Ribeirão Preto, São Paulo, Brazil
[3] Department of Clinical, Toxicological and Bromatological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

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.

J Infect Dev Ctries 2017; 11(3):277-281. doi:10.3855/jidc.8169

(Received 27 January 2016 – Accepted 16 June 2016)

Introduction

American trypanosomiasis, or Chagas disease, 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,

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, Olímpia, 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

Figure 1. Geographical localization of São Paulo state within Brazil and overall anti-*Trypanosoma cruzi* antibody prevalence for the period between 2012 and 2014.

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 (χ^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.

Municipality	Total number of donations	First-time donations	Seroprevalence[1]	Confirmatory test[2]	Median age	Male / female (%)[3]
Ribeirão Preto	96,177	23,002	0.15% (34/23,002)	0.10% (24/23,002)	46.5 years (range 23–63)	75.00/25.00
Presidente Prudente	45,090	10,289	0.11% (11/10,289)	0.04% (4/10,289)	47 years (range 38–55)	25.00/75.00
Franca	42,984	9,473	0.08% (8/9,473)	0.04% (4/9,473)	49.8 years (range 33–63)	100.00/0.00
Araçatuba	40,286	9,812	0.12% (12/9,812)	0.09% (9/9,812)	39 years (range 23–54)	88.88/11.12
Fernandópolis	24,311	6,365	0.36% (23/6,365)	0.28% (18/6,365)	50 years (range 37–64)	44.44/55.56
Olímpia	9,594	2,063	0.19% (4/2,063)	0.10% (2/2,063)	60 years (range 59–61)	100.00/0.00
Bebedouro	9,227	1,743	0.00% (0/1,743)	0.00% (0/1,743)	-	-
Batatais	8,411	1,272	0.16% (2/1,272)	0.16% (2/1,272)	39 years (range 36–42)	0.00/100.00
Serrana	5,471	635	0.31% (2/635)	0.00% (0/635)	-	-
TOTAL	**281,551**	**64,654**	**0.15% (96/64,654)**	**0.10% (63/64,654)**	**46.8 years (range 23–64)**	**65.07/34.93**

[1] Seroprevalence determined by the Gold ELISA Chagas Kit (REM, São Paulo, Brazil); [2] Confirmation of *Trypanosoma cruzi* infection by utilization of Imunocruzi 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 Olímpia, 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 Fernandopólis (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.

Year	Total number of donations	First-time donations	Seropositive donations	Confirmatory test	Median age	Male / female (%)
2012	93,858	21,753 (23.18%)	0.22% (48/21,753)	0.14% (31/21,753)	47.7 years (range 23–64)	61.30/38.70
2013	94,527	21,745 (23.00%)	0.08% (18/21,745)	0.06%(13/21,745)	42.5 years (range 23–54)	69.23/30.77
2014	93,166	21,156 (22.70%)	0.14% (30/21,156)	0.09% (19/21,156)	48.8 years (range 25–63)	68.42/31.58
TOTAL	**281,551**	**64,654 (22.96%)**	**0.15% (96/64,654)**	**0.10% (63/64,654)**	**46.8 years (range 23–64)**	**65.08/34.90**

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-*T. cruzi* Ab observed in this municipality is probably due to the obtained small sample volume. In Bebedouro and Serrana municipalities, no cases of anti-*T. cruzi* Ab were detected in the study period. The seroprevalence of anti-*T. cruzi* Ab (serology + IF) is shown in Figure 1.

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 acquirement. 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. It is also important to note that the Chagas disease transmission risk varies significantly within the limits of countries, and therefore such an uneven distribution in a large country like Brazil could be expected [16].

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 Fernandopolis 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.

Acknowledgements

We are grateful to Sandra Navarro Bresciani for the artwork. The study was supported by the Fundação de Amparo e Pesquisa do Estado de São Paulo-FAPESP, Brazil (Grant № 2009/16623-1, CTC-1998/14.247-6 and INCTC-2008/57.877-3), and the Conselho Nacional do Desenvolvimento Científico e Tecnológico, Brazil (INCTC-573.754/2008-0).

References

1. World Health Organization (2015) Third report on neglected tropical diseases. Investing to overcome the global impact of neglected tropical diseases. Chagas disease. Available: http://apps.who.int/iris/bitstream/10665/152781/1/9789241564861_eng.pdf?ua=1. Accessed 25 April 2016.
2. Massad E (2008) The elimination of Chagas disease from Brazil. Epidemiol Infect 136: 1153-1164.
3. Bern C, Kjos S, Yabsley MJ, Montgomery SP (2011) *Trypanosoma cruzi* and Chagas' disease in the United States. Clin Microbiol Rev 24: 655-681.
4. Wendel S (2010) Transfusion-transmitted Chagas disease: is it really under control? Acta Trop 115: 28-34.
5. Martin DL, Goodhew B, Czaicki N, Foster K, Rajbhandary S, Hunter S, Brubaker AS (2014) *Trypanosoma cruzi* survival following cold storage: possible implications for tissue baniking. PLoS One 9: e95398.
6. Cancino-Faure B, Fisa R, Riera C, Bula I, Girona-Llobera E, Jimenez-Marco T (2015) Evidence of meaningful levels of *Trypanosoma cruzi* in platelet concentrates from seropositive blood donors. Transfusion 55: 1249-1255.
7. Castro E (2009) Chagas' disease: lessons from routine donation testing. Transfus Med 19: 16-23.
8. Angheben A, Boix L, Buonfrate D, Gobbi F, Bisoffi Z, Pupella S, Gandini G, Aprili G (2015) Chagas disease and transfusion medicine: a perspective from non-endemic countries. Blood Transfus 13: 540-550.
9. Lima LM, Alves NP, Barbosa V de F, Pimenta GA, Moraes-Souza H, Martins PR (2012) Prevalence of Chagas disease in blood donors from the Uberaba Regional Blood Center, Brazil from 1995 to 2009. Rev Soc Bras Med Trop 45: 723-726.
10. Sabino EC, Gonçalez TT, Salles NA, Silva GR, Chamone DF (2003) Trends in the prevalence of Chagas' disease among first-time blood donors in São Paulo, Brazil. Transfusion 43: 853-856.
11. Barjas-Castro Mde L, Guariento ME, Vincente CS, Castro V (1998) Screening blood donors for *Trypanosoma cruzi* infection in a non-endemic area of Brazil. Transfusion 38: 611-612.
12. Araújo AB, Vianna EE, Berne ME (2008) Anti-*Trypanosoma cruzi* antibody detection in blood donors in Sotuthern Brazil. Braz J Infect Dis 12: 480-482.
13. Reiche EM, Inouye MM, Pontello R, Morimoto HK, Itow Jankevicius S, Matsuo T, Jankevicius JV (1996) Seropositivity for anti-*Trypanosoma cruzi* antibodies among blood donors of the "Hospital Universitário Regional do Norte do Pará", Londrina, Brazil. Rev Inst Med Trop São Paulo 38: 233-240.
14. Silveira HJ, Mozart ON, Norberg AN, Pile EA (2003) *Trypanosoma cruzi* prevalence and clinical forms in blood donor candidates in Brazil. Rev Saude Publica 37: 807-809.
15. Sobreira AC, Gomes FV, Silva MA, Oliveira MF (2001) Chagasic infection prevalence in blood donors at the Regional Blood Donation Center of Iguatu. Rev Soc Bras Med Trop 34: 193-196. [Article in Portuguese.]
16. Stanaway JD, Roth G (2015) The burden of Chagas disease: estimates and challenges. Glob Heart 10: 139-144.
17. Sánchez-Guillén MC, Barnabé C, Guégan JF, Tibayrenc M, Velásquez-Rojas M, Martínez-Munguía J, Salgado-Rosas H, Torres-Rasgado E, Rosas-Ramírez MI, Pérez-Fuentes R (2002) High prevalence of anti-*Trypanosoma cruzi* antibodies, among blood donors in the State of Peubla, a non-endemic area of Mexico. Mem Inst Oswaldo Cruz 97: 947-952.
18. Blejer JL, Saguier MC, Salamone HJ (2001) Antibodies to *Trypanosoma cruzi* among blood donors in Buenos Aires, Argentina. Int J Infect Dis 5: 89-93.
19. Remesar M, Sabino EC, Del Pozo A, Mayer A, Busch MP, Custer B (2015) Bimodal distribution of *Trypanosoma cruzi* antibody level in blood donors from highly endemic area of Argentina: what is the significance of low reactive samples? Transfusion 55: 2499-2504.
20. Carrasco R, Miguez H, Camacho C, Echalar L, Revollo S, Ampuero T, Dedet JP (1990) Prevalence of *Trypanosoma cruzi* infection in blood banks from seven departments of Bolivia. Mem Inst Oswaldo Cruz 85: 69-73.
21. Leite OF, Alves MJ, Souza SS, Mayo RC, Andrade VR, Souza CE, Rangel O, Oliveira SS, Lima VL, Rodrigues VL, Carvalho ME, Casanova C, Wanderley DM (2001) *Triatoma infestans* under entomological surveillance for Chagas' disease is São Paulo State, Brazil. Rev Soc Bras Med Trop 34: 437-443.
22. Mendonça VJ, de Oliveira J, Rimoldi A, Filho JC, de Araújo RF, da Rosa JÁ (2015) Triatominae survey (Hemiptera: Reduviidae: Triatominae) in the south-central region of the state of Bahia, Brazil between 2008 and 2013. Am J Trop Med Hyg 92: 1076-1080.

Corresponding author

Svetoslav Nanev Slavov, PhD
Laboratory of Molecular Biology, Blood Center of Ribeirão Preto, Faculty of Medicine of Ribeirão Preto, University of São Paulo
Rua Tenente Catão Roxo 2501, CEP 14051-140
Ribeirão Preto, São Paulo, Brazil
Phone: +551621019300 ext. 9680;
Fax: +551621019309
Email: svetoslav.slavov@hemocentro.fmrp.usp.br

Conflict of interests: No conflict of interests is declared.