

Major Article

Seroprevalence of *Trypanosoma cruzi* infection among blood donors in the state of Bahia, Brazil

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Abstract

Introduction: Chagas disease (CD) affects 5.7-7.0 million individuals worldwide, and its prevalence reached 25.1% in the state of Bahia, Brazil. There is an association between the prevalence of CD, the socioeconomic status of the population, and the risk of re-emergence due to non-vectorial transmission, such as blood transfusion. This study determined the seroprevalence of *T. cruzi* infection among blood donors in the state of Bahia, located in northeastern Brazil, and their epidemiological profile during a 10-year period. **Methods:** We performed a descriptive cross-sectional study involving a database review. Data were collected from patients with non-negative results for *T. cruzi* infection during a 10-year period. **Results:** A total of 3,084 (0.62%) samples were non-negative for *T. cruzi* infection in an initial serological screening, and 810 (0.16%) samples were non-negative in the second screening. The correlation between infection and age (30 years or older) and between infection and lower educational level (12 years or less) in the first and second screening was statistically significant. The seroprevalence of *T. cruzi* infection was higher in men in the first screening. In addition, 99.52% of the municipalities of Bahia had at least one case of CD. Livramento de Nossa Senhora and Salvador presented the highest disease prevalence and recurrence, respectively. **Conclusions:** The seroprevalence of *T. cruzi* infection in these populations was lower than that found in other studies in Brazil but was comparatively higher in densely-populated areas. The demographic characteristics of our population agreed with previous studies.

Keywords: Chagas disease. Blood donor. Seroepidemiological studies.

INTRODUCTION

Human Chagas disease (CD), also known as American trypanosomiasis, is a vector-borne tropical disease caused by the protozoan hemoflagellate *Trypanosoma cruzi*. CD was discovered and initially described by Dr. Carlos Chagas in 1909, at Lassance, Minas Gerais, Brazil¹.

In Latin American countries, CD causes 12,000 to 14,000 deaths annually² and affects 5.7 to 7.0 million people³. More affordable traveling conditions from Latin America to non-

endemic areas and increasing international migration flows have increased *T. cruzi* infection beyond the borders of Latin America, and CD reached Europe, North America, and Oceania countries⁴⁻⁶.

The seroprevalence of *T. cruzi* infection decreased in Latin America (from 6.49% to 1.28%) and Brazil (from 6.96% to 0.21%) between 1970 and 2006⁷ as a consequence of control campaigns in endemic countries, which helped reduce vectorial and transfusion transmission. Despite this decreasing trend in endemic areas, neglected regions have a higher number of cases and infected individuals.

In Brazil, the overall prevalence of *T. cruzi* infection reached 4.2% between 2000 and 2002 in the northeast, and the infection rate was higher than 5%^{8,9}. More recently, micro-outbreaks of CD occurred via oral transmission in municipalities in the interior of the states of Santa Catarina and Bahia¹⁰.

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Vectorial transmission is more common in endemic areas, in which the parasite is transmitted by contact with feces or urine of infected blood-sucking triatomine bugs (Hemiptera, Reduviidae). Other transmission routes include the consumption of contaminated foods or beverages, tissue and organ transplantation, mother-to-child transmission during pregnancy, laboratory accidents, induced or criminal infection, and blood transfusion. The transmission of *T. cruzi* via blood transfusion has increased because of urbanization and modernization of medicine¹¹ and is the predominant route of transmission in both endemic and non-endemic areas¹², especially in countries in which screening tests for CD are not mandatory in blood banks. Furthermore, *T. cruzi* tolerates extreme conditions, such as low temperatures (−80 to 4°C), and freezing and thawing¹³, indicating that the parasite can be transmitted in all blood components, including granulocytes, red blood cells, and plasma.

T. cruzi infection occurs predominantly in individuals living in rural areas⁸. However, the epidemiological status of CD has changed as a consequence of migration flows from rural areas to the periphery of large cities, where the presence of forest remnants increases the risk of disease transmission¹⁴⁻¹⁷.

Human CD is classified as a neglected transmitted disease, primarily affecting communities with higher social vulnerability, characterized by low income, poor housing and hygiene conditions, and low levels of schooling. For this reason, among parasitic diseases, CD is the most strongly associated with population vulnerability, in which the majority of seropositive individuals have these risk factors¹⁸.

Studies on CD should assess ecological, biological, social, and economic parameters to identify risk factors associated with disease development and progression. Furthermore, these studies reduce the risk of infection by producing knowledge for scientific and technological innovations.

Technologies capable of reducing the risk of transmitting infectious diseases through transfusion are critical to prevent the spread of contagious agents and ensure the quality of blood for transfusion¹⁹. Studies of discarded serological tests are capable of creating relevant technologies to improve the quality of blood transfusion. Serological screening has been used in several countries to improve public health programs. However, this type of testing is facing ethical, operational, and financial difficulties²⁰.

Training the teams responsible for collecting pre-donation information is critical, given that adequately collected epidemiological data may help avoid transmission and decrease costs in the public health system.

In addition to avoiding transmission, identifying individuals with suspected disease allows researchers to identify regions with a higher risk of transmission and the most relevant epidemiological characteristics associated with seropositivity. Moreover, identifying places of birth and residence with higher prevalence may help characterize areas with a higher risk of transmission in the state of Bahia.

Previous studies with blood donors reported that the percentage of non-negative results was higher among married

individuals, people in stable relationships⁷, and subjects with lower education levels²¹. The percentage of infection was higher in men²²⁻²⁵, although one study found that the rate was higher in women²¹. The number of cases was higher in older age groups, especially in individuals older than 30 years^{7,22,23,25,26}. Moreover, the percentage of infection between men and women differed according to age group²².

The objective of this study was to determine the seroprevalence of *T. cruzi* infection and describe the epidemiological profile of blood donors from the Hematology and Hemotherapy Foundation of Bahia (HEMOBA) with non-negative results for *T. cruzi* infection detected by screening tests during a 10-year period (February 2008 to February 2018) in the state of Bahia. The specific objectives were to determine the most relevant epidemiological characteristics of blood donors with non-negative results for CD and identify the municipalities of origin of this population.

METHODS

Study design: Descriptive cross-sectional study involving a database review.

Target population: All blood donors from HEMOBA in the state of Bahia, Brazil, examined during the study period. HEMOBA is the official blood service in Bahia, and its units cover all health macro-regions in the state;

Diagnostic criteria: Non-negative results for *T. cruzi* infection;

Selection criteria:

- **Inclusion:** Donors with non-negative results for *T. cruzi* infection detected in serological screening tests performed at HEMOBA;
- **Exclusion:** Donors with negative serology in the second screening;

Variables: Gender, age, place of residence, place of birth, education level, racial group, marital status, number of annual blood donations, and test results in the first and second screening;

Clinical selection of candidates and release of blood bags:

To minimize the risk of *T. cruzi* infection via blood transmission, the Brazilian Ministry of Health has issued several ordinances, the most recent being Ordinance 5, which was published in the Official Gazette of the Union on September 28, 2017²⁷ and lists the criteria for the clinical selection of candidates for blood donation. HEMOBA created a standard computerized questionnaire (Hemovida System) following these criteria and disclosed the data for the study. The methodology used for the release of blood bags followed the recommendation of the Ministry of Health according to Ordinance 5.

Serological screening: High sensitivity tests are mandatory. Between January 1991 and January 2015, screening tests for CD among blood donors at HEMOBA used anti-*T. cruzi* antibodies and ELISA from MUREX (FRA) and BIOMÉRIEUX (FRA). Since February 2015, HEMOBA adopted serological screening by chemiluminescence using IgG and IgM class antibodies. These antibodies were purchased from ABBOTT (IL, USA)

until February 25, 2016, and from DIASORIN (ITA) since then to date. HEMOBA uses the same serological test in donors in the second screening and sends them to a referral unit in case of positivity.

Statistical analysis: Descriptive analyses were performed using the statistical package Epi Info 2000 (CDC, Atlanta, GE, USA). The χ^2 test was used to compare proportions, and a level of significance of 5% ($p < 0.05$) and a confidence interval of 95% were adopted in all tests. Qualitative variables were expressed as absolute numbers and relative frequency.

Georeferencing: The information was georeferenced using the software Qgis version 20.0 to identify groups with high rates of non-negative results for *T. cruzi* infection according to the place of residence. The natural breaks method with five class intervals was used because the variables were discrete.

Ethics

The adopted procedures were in accordance with the ethical standards of the Research Ethics Committee of the Gonçalo Moniz Institute (FIOCRUZ, Bahia, Brazil) and with the Helsinki Declaration of 1964, revised in 1975, 1983, 1989, 1996, and 2000. The consent form was waived because the analysis was based on a database. However, no personal identification data were used to ensure the complete anonymity of blood donors. The research did not cause any physical, psychic, moral, intellectual, social, cultural, or religious risk to blood donors.

RESULTS

During the 10-year study period (February 22, 2008 to February 21, 2018), 494,010 donors and 816,346 blood samples were examined at HEMOBA. In a preliminary analysis, 3,084 (0.62%) non-negative samples were identified. All samples reactive for *T. cruzi* are considered non-negative by HEMOBA. Patients whose test results were non-negative or inconclusive were advised to return to HEMOBA to undergo a second screening. This orientation follows a protocol in which the blood center sends up to three letters to the donor's registered address, with an interval of 30 days between each of them. A total of 1,486 patients returned to the blood center for a second screening, of whom 810 (0.16%) were considered non-negative for *T. cruzi* infection.

Among the non-negative samples, 1,334 (43.26%) were analyzed by chemiluminescence using IgG and IgM antibodies and 1,750 (56.74%) by ELISA using anti-*T. cruzi* antibodies in the first screening, whereas 671 (45.15%) samples were analyzed by chemiluminescence using IgG and IgM antibodies and 815 (54.85%) by ELISA using anti-*T. cruzi* antibodies in the second screening.

The group of individuals non-negative for *T. cruzi* infection in the first and second screening (810) was analyzed by gender, age, racial group, marital status, educational level, place of residence, and place of birth.

In the first screening, the prevalence of non-negative results for *T. cruzi* infection was 0.62% (3048 samples) and was higher in men (PR, 1.0919; 95% CI, 1.02–1.17) and in blood donors aged 30 years or older (PR, 1.5863; 95% CI, 1.4676–1.7147).

The prevalence of non-negative results in donors with less than 12 years of schooling was 32% (PR, 1.3236; 95% CI, 1.2040–1.4550) when compared with those with at least 12 years of education. There was no statistically significant association between the place of birth and residence and the prevalence of non-negative serology in this population (**Table 1**).

The analysis of data from the second screening indicated that the prevalence of non-negative results for *T. cruzi* infection was 0.15% (810 samples). The prevalence was higher in blood donors aged 30 years or older (PR, 3.4497; 95% CI, 2.8547–4.1689) and donors with 12 years of schooling or less (PR, 2.6943; 95% CI, 2.1154–3.4317). There was no statistically significant association between non-negative serology, gender, and the place of birth and residence in Bahia (**Table 1**).

The data on the prevalence of non-negative results according to the marital status and racial group are shown in **Table 2**. Given the high miscegenation of the population in Bahia, it is difficult to classify the study sample into racial groups. Notwithstanding, there was no significant association between prevalence rates and racial groups.

The data on the place of residence of blood donors with non-negative results for *T. cruzi* infection were georeferenced and summarized in cartographic representations. The prevalence rates during the 10-year period in the first and second screening performed at HEMOBA is shown in **Figure 1**.

During the study period, 415 municipalities of Bahia were identified as places of residence of blood donors who presented at HEMOBA. Only the municipalities of Almadina and Jiquiriçá were not represented in the samples in this period. In addition, 608 cities outside of Bahia were identified as places of residence, with 11,656 donors (1.43%); however, these donors were not included in the geographical representations because these cities were outside the cartographic limit of the state.

According to the data from the first screening, the municipalities with the highest prevalence were Livramento de Nossa Senhora, located in the center-south of the state ($n=2$, 100%), Cotegipe ($n=14$, 6.31%), Wanderley ($n=17$, 6.69%), Sebastião Laranjeiras ($n=5$, 7.25%), Mulungu do Morro ($n=8$, 7.92%), Jaborandi ($n=4$, 8.33%), and Lajedinho ($n=1$, 10%). In the second screening, the municipalities with the highest prevalence were Livramento de Nossa Senhora ($n=2$, 100%), Boa Vista do Tupim ($n=1$, 3.85%), Wanderley ($n=11$, 4.33%), Botuporã ($n=1$, 4.7%), and Lajedinho ($n=1$, 10%).

The places of residence with at least one non-negative result per year between February 2008 and February 2018 in the second screening are shown in **Figure 2**.

The municipalities with the highest number of recurrences was Salvador (state capital) (11 out of 11 years), Feira de Santana (located in the north-central region of the state) (9 out of 11 years), Barreiras (8 out of 11 years), and Santo Antônio de Jesus (8 out of 11 years).

DISCUSSION

Human CD is endemic in Brazil, despite the success of government initiatives adopted in the past few decades.

TABLE 1: Factors associated with *T. Cruzi* infection in the first and second screening.

| Characteristics | Seroprevalence of <i>T. cruzi</i> infection (2008-2018) | | | | | | | | | |
|---|---|------|--------|-----------------|------------|------------------|------|--------|-----------------|----------|
| | First screening | | | | | Second screening | | | | |
| | Total | %* | PR | (95% CI) | p | Total | %* | PR | (95% CI) | p |
| Total | 3084 | 0.62 | | | | 810 | 0.16 | | | |
| Sex | | | | | | | | | | |
| Male | 1908 | 0.65 | 1.0919 | (1.0150-1.1733) | p < 0.05 | 470 | 0.16 | 0.9298 | (0.8088-1.0689) | ns |
| Female | 1176 | 0.59 | 1 | | | 340 | 0.17 | 1 | | |
| Age, years | | | | | | | | | | |
| ≥ 30 | 2196 | 0.44 | 1.5863 | (1.4676-1.7147) | p < 0.001† | 683 | 0.14 | 3.4497 | (2.8547-4.1689) | p<0.001† |
| < 30 | 888 | 0.28 | 1 | | | 127 | 0.40 | 1 | | |
| Place of residence is located in Bahia | | | | | | | | | | |
| Yes | 3049 | 0.38 | 1.2657 | (0.9075-1.7653) | ns | 803 | 0.10 | 1.6667 | (0.7922-3.5066) | ns |
| No | 35 | 0.30 | 1 | | | 7 | 0.06 | 1 | | |
| Place of birth is located in Bahia | | | | | | | | | | |
| Yes | 2832 | 0.38 | 1.0743 | (0.9447-1.2218) | ns | 753 | 0.10 | 1.2629 | (0.9649-1.6529) | ns |
| No | 252 | 0.35 | 1 | | | 57 | 0.08 | 1 | | |
| Years of education | | | | | | | | | | |
| ≤ 12 | 2573 | 0.66 | 1.3236 | (1.2040-1.4550) | p < 0.001† | 738 | 0.19 | 2.6943 | (2.1154-3.4317) | p<0.001† |
| > 12 | 511 | 0.50 | 1 | | | 72 | 0.07 | 1 | | |

*% of total blood donors that belong to this category between 2008 and 2018. CI, confidence interval; ns, not significant. † Significant in a multivariate logistic regression analysis. PR: Prevalence Rate.

TABLE 2: Seroprevalence distribution according to marital status and racial group.

| Characteristics | Seroprevalence of <i>T. cruzi</i> infection (2008-2018) | | | |
|-----------------------|---|------|----------|------|
| | Sample 1 | | Sample 2 | |
| | Total | %* | Total | %* |
| Total | 3084 | 0.62 | 810 | 0.16 |
| Marital status | | | | |
| Married | 1299 | 0.75 | 373 | 0.22 |
| Separated | 10 | 0.89 | 4 | 0.36 |
| Divorced | 57 | 0.62 | 20 | 0.22 |
| Judicially separated | 10 | 0.71 | 2 | 0.14 |
| Single | 1634 | 0.55 | 386 | 0.13 |
| Widow | 41 | 1.29 | 17 | 0.01 |
| Others | 33 | 0.50 | 8 | 0.12 |
| Racial group | | | | |
| Caucasian | 172 | 0.59 | 45 | 0.15 |
| Brazilian Caucasian | 1069 | 0.60 | 277 | 0.16 |
| Indian | 4 | 0.27 | 1 | 0.07 |
| Mixed | 1422 | 0.66 | 400 | 0.18 |
| Black | 417 | 0.60 | 87 | 0.13 |
| Asian | 0 | 0 | 0 | 0 |

*% of total blood donors that belong to this category between 2008 and 2018.

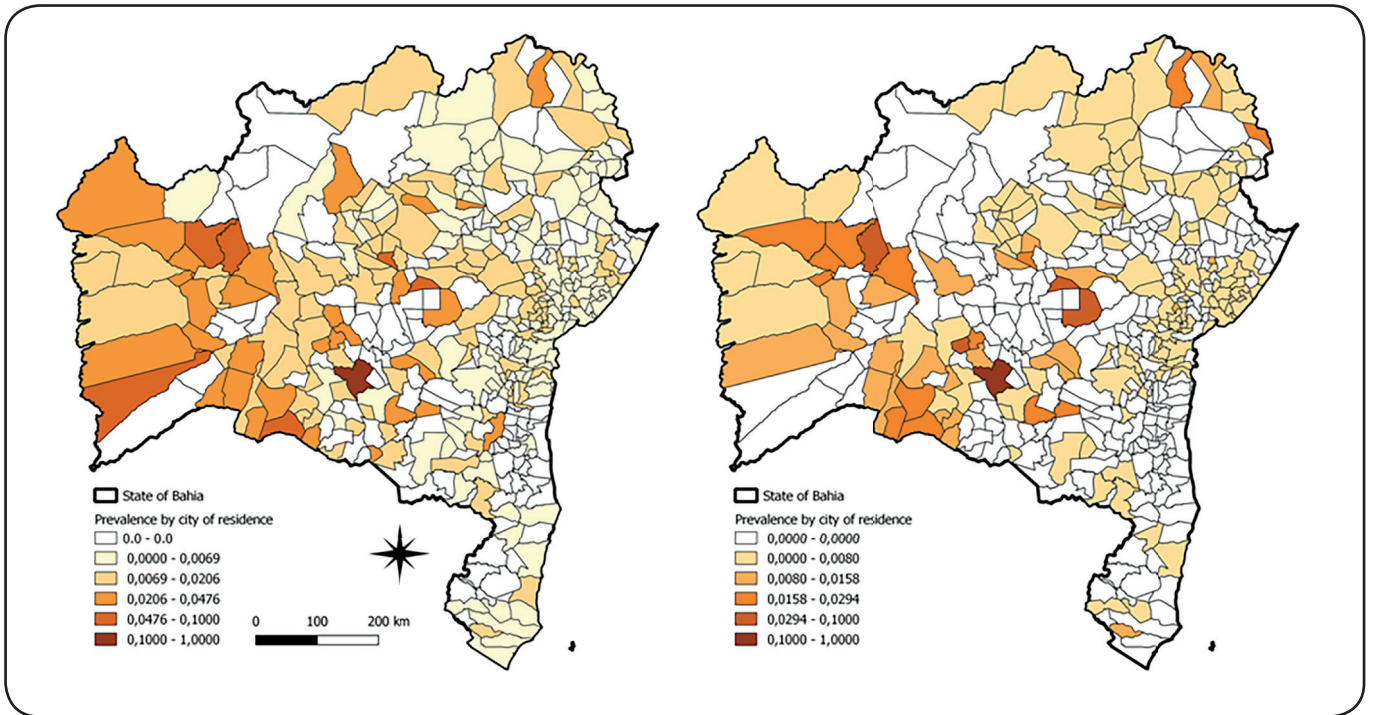


FIGURE 1: Seroprevalence of *Trypanosoma cruzi* infection in the state of Bahia in the first screening (left) and second screening (right) between 2008 and 2018.

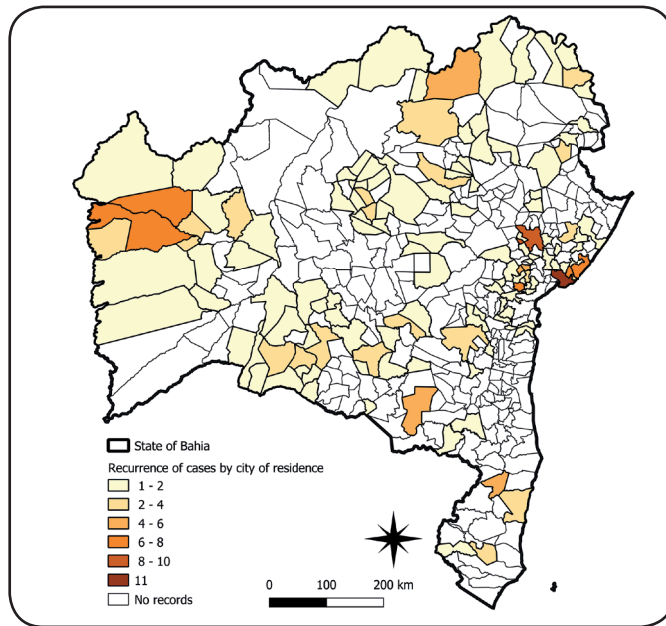


FIGURE 2: Recurrence of *Trypanosoma cruzi* infection (second sample) per year by municipality in the state of Bahia, Brazil, between 2008 and 2018.

Measures for eradicating vectorial transmission are constantly debated. However, much remains to be done because the disease is transmitted by other routes, including blood transfusion, which has gained importance in the epidemiological literature. Studies investigating the prevalence of CD in blood donors can provide quantitative epidemiological data and guide interventions in high-risk areas and population groups.

Among a population of 494,010 donors and 816,346 blood donations during the 10-year period, 3,084 (0.62%) samples were non-negative in the first screening. Among 1,486 donors, 810 (0.16%) samples were non-negative in the second screening. These rates may be underestimated because, despite strong guidance on the importance of second screening, many donors did not return to the blood units and therefore were not included in the analysis.

The prevalence rates were lower than those found in other studies conducted in Brazil but were relatively higher in densely-populated regions. Dias et al. (2000) found that the seropositivity for *T. cruzi* infection between 1987 and 1994 ranged from 0.77% to 2.22% in Bahia and from 0.69% to 0.88% in the northeast region of Brazil. Dias (1998) reported that the seropositivity between 1988 and 1990 in Brazil was 0.97% and decreased in the following years: 0.7–1.1% in 1991, 0.7% in 1993, and 0.75% in 1994; the seropositivity in the northeast of Brazil was 0.36% in 1994. In contrast, Monich (2016) found that the discard rate of donated blood in Curitiba between 2003 and 2012 varied from 0.6% to 0.9%, whereas Slavov et al. (2016) indicated that the discard rate in the state of São Paulo between 2012 and 2014 was 0.1%. It is worth mentioning that the methodology used in some studies did not involve second screening, and prevalence was calculated using donors with positive serology in the first screening. The prevalence in the present study using this approach was 0.62% (3,084 individuals).

The seroprevalence of *T. cruzi* infection was higher in individuals aged 30 years or older and individuals with 12 years or less of education in the first and second screening, and seroprevalence was significantly higher in men in the first screening. These results agree with previous studies²¹⁻²⁵. Data on seroprevalence rates according to marital status and racial group are summarized in **Table 1**.

With respect to the geographical distribution of the places of residence, 99.52% (415) of the municipalities of the state of Bahia and 608 municipalities outside the state borders had at least one diagnosed case of CD during the 10-years study period. Georeferencing was performed according to the place of residence rather than the place of birth because maternities are unavailable in many cities in the interior of the state. Moreover, the place of residence is a more reliable parameter because it represents, in most cases, the place where individuals are most exposed to infection throughout life.

Georeferencing enabled the identification of municipalities with higher seroprevalence (**Figure 1**). Increased access to blood transfusion services is a contributing factor for higher recurrence. Salvador, the state capital, is home to the HEMOBA headquarter and has the highest number of blood donors and donations, which may explain the higher rate of recurrence in this city. In addition, HEMOBA's blood collection stations were being implemented in municipalities of the state throughout the study period, which might have increased the number of blood donations.

Seroprevalence analysis of CD can accurately assess the risk of transfusion-related CD and the rate of disease transmission²⁸. This study identified high-prevalence regions in Bahia, allowing performing serological and epidemiological surveys in these areas and collecting epidemiological data to formulate health policies.

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Conflicts of Interests

The authors declare that there are no conflicts of interest associated with this study.

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