Review article

Chagasic infection among blood donors in Brazil: an integrative review

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ABSTRACT

Based on the literature, this work aimed to discuss infection by Chagas disease among blood donors in Brazil. Studies on the prevalence of Trypanosoma cruzi infection in donors or candidates for blood donation in Brazil are important. The prevalence of infection appears to be a sensitive indicator and can be a true marker of the risk of the transmission of Chagas disease by blood transfusion. Moreover, it serves as a marker of the level of transmission of the disease in a region, as well as a tool to characterize the epidemiological profile of individuals affected by the disease. The present study is an integrative review of the literature on chagasic infection among blood donors. An evaluation of the literature identified the epidemiological profile of blood donors infected by T. cruzi, which is characterized in general as men, over 30 years old, with a low level of schooling, low income and mainly coming from rural areas.

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Introduction

The American trypanosomiasis, also known as Chagas disease, is an important endemic parasitic disease and a medical and social problem in Brazil and in several other Latin American countries with impact both on the economy and on public health. Trypanosoma cruzi is an obligate intracellular pathogen and the etiologic agent of Chagas disease.

The infection of humans and other vertebrates, such as rodents, carnivores and primates, occurs primarily by the contact of the skin and mucosa of the vertebrate host with the stools of the triatomine contaminated by T. cruzi. However, there are other forms of transmission, such as by blood

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transfusion, congenital, laboratory accidents, organ transplantation and ingestion.  

The World Health Organization (WHO) initiatives in partnership with Latin American governments have led to the control of vectorial transmission of Chagas disease by the main vector, Triatoma infestans, in several endemic areas by preventive measures to avoid the presence of triatomines in residences. With the control of natural transmission by means of eradication of the vector in different endemic countries, blood transfusion became the main mechanism of dissemination of Chagas disease in these countries during the 1980s and 90s.

Thus, another measure to control the endemic of Chagas disease in the country was the creation of the Southern Cone Initiative in 1991. This project established that in parallel to the fight against T. infestans, another objective would be to reduce and eliminate transmission by blood transfusion by strengthening the blood bank network and consequently the effective screening of blood donors. These preventive measures have contributed positively to the increased safety of blood transfusions in Brazil.

The risk of the patient becoming infected when receiving a unit of blood from a chagasic donor is still variable. In regions with high vectorial transmission rates and a large number of infected individuals with high parasitic loads, such as Santa Cruz in Bolivia, blood transfusions account for 49% of the cases. In countries with low rates of natural transmission, such as, Argentina, Brazil, Chile and Uruguay, these rates are between 12% and 18% and the risk is even lower in non-endemic countries.

Studies on the prevalence of T. cruzi infection in donors or candidates for blood donation in Brazil are important. The prevalence of infection appears to be a sensitive indicator, and can be a true marker of the risk of Chagas disease transmission by blood transfusion. Moreover, it serves as a marker of the level of transmission of the disease in a region, as well as a tool to characterize the epidemiological profile of affected individuals. Thus, based on the literature, this study aims to discuss Chagas disease infection among blood donors in Brazil.

Methods

The present study is an integrative review of scientific publications on chagasic infection among blood donors in Brazil. The purpose of the research was to summarize the studies published in this field of interest in order to identify the topics addressed, to analyze the object of the study from the perspective of several authors and to identify their multiple determinants.

The integrative review is a type of research that presents a comprehensive methodological approach, as it allows an advanced analysis of available literature, discussions about methods and results of research, and reflections on the performance of other researchers. The main objective of this study was to obtain a broad understanding of a given phenomenon based on previously published studies.

Based on these definitions, published thematic studies about chagasic infection among blood donors in Brazil were identified. The literature search was conducted during the months of August thru December 2017 in the Virtual Health Library (VHL) database, which is an open access operational platform for technical cooperation of the Pan American Health Organization (PAHO). This platform is established as a site for the integration of health information sources to promote democratization and broader access to scientific and technical health information in Latin America and the Caribbean.

The search descriptors consisted of the following terms: “Chagas disease”, “Blood donors” and “Brazil”, using the Boolean operator “and” between each descriptor in order to find a greater number of publications on the subject. Ninety-two articles were identified by searching with the descriptor “Chagas Disease and Blood Donors and Brazil”. Inclusion and exclusion criteria were applied using the VHL platform filters.

The inclusion criteria were as follows: full articles published between 1997 and 2017, which are available electronically in the databases MEDLINE, LILACS and the State Secretariat of Health of São Paulo in Portuguese and English. The articles should have Chagas disease, blood donors, T. cruzi, blood banks, serological tests, blood transfusion, blood, prevalence and blood safety as the main subjects.

The exclusion criteria included articles prior to 1997, incomplete or unavailable articles, opinions and publications that do not refer to the main subjects. As a result 18 articles were identified, two of which were discarded because the articles did not follow the proposed methodology giving a total of 16 articles. Two more articles published in 2016 and 2017 were added to improve the discussion about this theme.

The selected articles were analyzed according to the information contained in the abstracts and subsequently each one was read in full. Thus, the main data that contained relevant information for analysis were extracted.

Results

After a careful reading, 18 articles were identified that met the inclusion and exclusion criteria. The selected articles were included in Table 1.

Discussion

Risk factors associated with chagasic infection in humans in Brazil

Five articles reported that there are several main sources of risk for the development of chagasic infection directly related to the Brazilian population. The presence of triatomines in residences is considered one of the major risk factors for Chagas disease in humans as well as close contact between humans and animals in endemic areas, which may be an abundant source of blood for triatomines, maintaining the peridomestic cycle of T. cruzi. Precarious conditions of dwelling, health and subsistence infrastructure in the ecosystems, where vectors are abundant are preponderant factors for the establishment of Chagas disease.

Aspects such as low monthly family income and agriculture as a profession are also associated with Chagas disease, as well as the process of migration from rural areas to urban areas. In addition, a family history of Chagas disease is an
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<th>Article title</th>
<th>Author</th>
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<th>Journal</th>
<th>Type of study</th>
<th>Objective</th>
<th>Results/Conclusions</th>
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<tr>
<td>Prevalence of Trypanosoma cruzi antibodies in blood donors from the Sao Paulo State, Brazil, between 2012 and 2014</td>
<td>Slavov et al.</td>
<td>2017</td>
<td>The Journal of Infection in Developing Countries</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To examine the prevalence of anti-T. cruzi IgM/IgG antibodies in blood donors from the western part of Sao Paulo State in the period between 2012 and 2014.</td>
<td>The confirmed overall T. cruzi seroprevalence among blood donors was 0.10%, which can be considered low. The discordance obtained for T. cruzi prevalence by serologic and immunofluorescence methods demonstrates that more specific routine diagnoses are needed to diminish the cost of assays and loss to blood supply as all seropositive blood bags are immediately discarded.</td>
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<td>Distribution of serological screening markers at a large hematology and hemotherapy center in Minas Gerais, Southeastern Brazil.</td>
<td>Silva et al.</td>
<td>2016</td>
<td>Brazilian Journal of Hematology and Hemotherapy</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To assess the distribution of serological markers in blood donors at the blood banks of the Fundação Centro de Hematologia e Hemoterapia de Minas Gerais, Brazil, between January 2006 and December 2012.</td>
<td>Approximately 78.9% of the donors were considered eligible for the study after clinical screening. Data on the profile of serological ineligibility by the blood banks of the Fundação Hemominas highlight the particularities of each region thereby contributing to measures for health surveillance and helping the blood donation network in donor selection procedures aimed at improving blood transfusion safety.</td>
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<td>Prevalence of Chagas disease among blood donor candidates in Triangulo Mineiro, Minas Gerais State, Brazil.</td>
<td>Lopes et al.</td>
<td>2015</td>
<td>Journal of Sao Paulo Institute of Tropical Medicine</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To analyze the serological profile of blood donors in blood banks of Hemominas hematology center, in Ituiutaba, Minas Gerais.</td>
<td>Analysis of data showed no significant difference between genders and there was a positive correlation between increasing age and the percentage of seropositive patients for Chagas disease. Therefore, adopting strategies that allow the safe identification of donors with positive serology for Chagas disease is essential to reduce indeterminate serological results.</td>
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<td>Antibody levels correlate with detection of Trypanosoma cruzi DNA by sensitive PCR assays in seropositive blood donors and possible resolution of infection over time.</td>
<td>Sabino et al.</td>
<td>2013</td>
<td>Transfusion</td>
<td>Descriptive quantitative study:</td>
<td>To compare the results obtained by two laboratories using different PCR protocols on coded sets of samples collected from seropositive blood donors from Brazil, Honduras and the US.</td>
<td>Among seropositive donors, PCR-positive rates varied by country for the BSRI laboratory: Brazil (57%), Honduras (32%) and the US (14%). For all three countries, persistent DNA positivity correlated with higher ELISA S/CO values, suggesting that high-level seroreactivity reflects chronic parasitemia.</td>
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<td>Prevalence of Chagas disease in blood donors at the Uberaba Regional Blood</td>
<td>Lima et al.</td>
<td>2012</td>
<td>Journal of the Brazilian Society of Tropical</td>
<td>Descriptive</td>
<td>To verify the tendency of ineligibility and describe the epidemiologic profile of donors.</td>
<td>Among the serum positive-donors, there was a significant predominance among those aged 30 years or over. This study affirmed the importance of systematically combating the vector, resulting in a drop of the contamination risk factor due to blood transfusion and in the improvement of the quality of hemotherapy practices.</td>
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<td>Center, Brazil, from 1995 to 2009</td>
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<td>Medicine</td>
<td>quantitative study: retrospective.</td>
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<td>Control of transfusional transmission</td>
<td>Moraes-Souza and Silva</td>
<td>2011</td>
<td>Journal of the Brazilian Society of Tropical</td>
<td>Qualitative study.</td>
<td>To evaluate the control of transfusion-related transmission of Chagas disease.</td>
<td>The highly favorable results of combating the vector and donor serological coverage reduced the prevalence of seropositivity to 0.2% and 1.3%, respectively, in Brazil and Latin America, and the annual transmission rate due to blood transfusion in Brazil dropped from 20,000 to 13 in four decades. The study showed that the control strategies for vector and transfusion transmission of Chagas disease are effective.</td>
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<td>New challenges and the future of control</td>
<td>Silveira</td>
<td>2011</td>
<td>Journal of the Brazilian Society of Tropical</td>
<td>Qualitative study.</td>
<td>To evaluate the challenges and perspectives for the control of the Chagas disease.</td>
<td>The epidemiological situation of Chagas disease was altered as a result of control actions. However, transmission, related to the enzootic cycle, such as extra-domiciliary vectorial transmission, in addition to oral transmission, became relevant in the number of human cases of T. cruzi infection.</td>
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<td>Seropositivity for Chagas disease among blood donors in Araraquara, São</td>
<td>Ferreira et al.</td>
<td>2011</td>
<td>Journal of the Brazilian Society of Tropical</td>
<td>Descriptive</td>
<td>To evaluate the seropositivity rates among blood donors in Araraquara, between January 2004 and December 2008.</td>
<td>Positive serology was diagnosed in 0.04% of 4951 blood donations. The age of the seropositive subject was between 51 and 60 years old. The low rate of positive donors may reduce the risk of transfusion transmission of Chagas disease</td>
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<td>Paulo State, from 2004 to 2008</td>
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<td>Medicine</td>
<td>quantitative study: retrospective.</td>
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<td>Enhanced classification of Chagas serological results and epidemiological</td>
<td>Sabino et al.</td>
<td>2010</td>
<td>Transfusion</td>
<td>Descriptive</td>
<td>To describe the Chagas serological patterns obtained by testing at 3 large blood centers during 2007 and 2008 as part of the REDS-II International study in Brazil.</td>
<td>In 2007–8, 877 of 615,433 donations were discarded due to Chagas assay reactivity. The study proposed a classification algorithm that may have practical importance for donor counseling and epidemiological analyses of T. cruzi seroreactive donors.</td>
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<td>characteristics of seropositive donors at 3 large blood centers in Brazil</td>
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<td>Socioepidemiological screening of serologically ineligible blood donors due to Chagas disease for the definition of inconclusive cases</td>
<td>Silva et al.⁹</td>
<td>2010</td>
<td>Memories of the Oswaldo Cruz Institute</td>
<td>Descriptive quantitative study.</td>
<td>To describe the sociodemographic and epidemiological characteristics of blood donors with non-negative serology for Trypanosoma cruzi to determine possible risk factors associated with serological ineligibility.</td>
<td>The frequency of serological ineligibility was 0.28%, with a predominance of inconclusive reactions (52%) and seropositivity among first-time donors, donors older than 30 years, females, donors from risk areas and subjects living in rural areas, who had contact with the triatome vector and with a family history of Chagas disease. The results identified the population most affected by T. cruzi.</td>
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<td>The prevalence of chagasic infection among blood donors in the State of Pernambuco, Brazil</td>
<td>Melo et al.⁴</td>
<td>2009</td>
<td>Brazilian Journal of Hematology and Hemotherapy</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To analyze the profile of the blood donors of HemoCentro de Pernambuco (Hemope), who presented reactivity for Chagas disease from 2002 to 2007.</td>
<td>A prevalence of 0.17% was found for Chagas disease and 6.89% of the discarded bags were due to this reactivity. Most donors were men (p-value &lt;0.0001). The age group of 18–30 years gave the lowest number of reactive serologies (20.21%). Epidemiological studies evaluated the risk of transmission of the disease by blood transfusion and the effectiveness of vector control measures.</td>
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<td>Anti-Trypanosoma cruzi antibody detection in blood donors in the Southern Brazil</td>
<td>Araújo et al.¹⁷</td>
<td>2008</td>
<td>Brazilian Journal of Infectious Diseases</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To evaluate the positivity for T. cruzi in blood samples of donor candidates in southern Brazil.</td>
<td>Of 4482 samples collected in 2004 and 2005, the reactivity for anti-T. cruzi was 0.96%. Among those, 21 cases were confirmed; most were female, with little schooling and mean age of 47.2% years old. More than one diagnostic technique must be used to obtain more reliable and conclusive results.</td>
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<td>The epidemiologic profile and prevalence of cardiopathy in Trypanosoma cruzi infected blood donor candidates, Londrina, Paraná, Brazil</td>
<td>Marques et al.³⁰</td>
<td>2005</td>
<td>Journal of São Paulo Institute of Tropical Medicine</td>
<td>Descriptive quantitative study.</td>
<td>To set goals for the improvement of services and to assess the possibility of decentralization, by determining the patients’ characteristics.</td>
<td>The profile found was: young (mean age 42.95 years), male, Caucasian, low level of schooling, low family income, agricultural worker, from rural areas and the vector as the main mechanism of transmission. This study emphasizes the importance of expanding medical services to areas with a greater prevalence of infected individuals.</td>
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<td>On the possibility of autochthonous Chagas disease in Roraima, Amazon Region, Brazil, 2000–2001</td>
<td>Moura et al. 12</td>
<td>2005</td>
<td>Journal of São Paulo Institute of Tropical Medicine</td>
<td>Descriptive quantitative study.</td>
<td>To investigate the possibility of the occurrence of autochthonous cycle of Chagas disease in Roraima.</td>
<td>Natural triatomine infection was not found in intestinal contents. The presence of anti-T. cruzi antibodies was verified in 25 individuals (1.4% out of 1821, all &gt;15-year-old, 20 migrants). Results show that Chagas disease is not endemic in the areas studied. However, all elements of the transmission cycle are present, demanding adequate and continuous vigilance.</td>
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<td>The discarding of blood units and the prevalence of infectious diseases in donors at the Pro-Blood Foundation/Blood Center of São Paulo, São Paulo, Brazil</td>
<td>Salles et al. 12</td>
<td>2003</td>
<td>Pan American Journal of Public Health</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To analyze the changes in the proportion of blood units discarded from 1991 thru 2001 at the Pro-Blood Foundation/Blood Center of São Paulo, and to determine the prevalence of infectious diseases among donors at the Blood Center in November 2001</td>
<td>A significant decrease in discard was found from 1991 (20%) to 2001 (9%). The decrease in discard and the prevalence of infectious diseases among donors in 2001 reflect the increase in the percentage of repeat donors in this blood bank.</td>
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<td>Chagasic infection prevalence in blood donors for the Hemocentro Regional de Iguatu</td>
<td>Sobreira et al. 14</td>
<td>2001</td>
<td>Journal of the Brazilian Society of Tropical Medicine</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To evaluate the frequency of T. cruzi infection among blood donors at the Hemocentro Regional de Iguatu, Ceará (1996–1997) using the Enzyme Linked Immune Sorbent Assay (ELISA) and Hemagglutination Passive Reverse (HPR) tests.</td>
<td>Of the 3232 donors analyzed, 61 were seropositive for chagasic infection, with the majority of donors being in the 18- to 30-year age group, men and from rural areas. The results showed that the use of two or more different tests by blood banks prevents transfusion associated Chagas disease.</td>
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<td>Trypanosoma cruzi infection in blood donors</td>
<td>Bonamett et al. 15</td>
<td>1998</td>
<td>Revista de Saúde Pública</td>
<td>Descriptive quantitative study: retrospective.</td>
<td>To evaluate seropositivity for T. cruzi infection in blood donors and to compare this rate with those found in blood Banks in 1958 and 1975.</td>
<td>The seroprevalence rate found was 1.3%. A trend of temporal decrease of the positivity rate of the serological tests for the diagnosis of T. cruzi infection was detected in the blood banks of the city in the years 1958, 1975 and 1995.</td>
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Table 1 – (Continued)

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<tr>
<td>Chagas disease: an algorithm from donor screening and positive donor counseling</td>
<td>Hamerschlag et al.</td>
<td>1997</td>
<td>Journal of the Brazilian Society of Tropical Medicine</td>
<td>Descriptive quantitative study.</td>
<td>To evaluate ELISA methodology and to propose an algorithm for blood banks to be applied to Chagas disease.</td>
<td>The sensitivity of the screening by Indirect Immunofluorescence Assay (IFA) and the 3 different ELISA tests was 100%. For Chagas disease, ELISA seems to be the best test for blood screening. It was possible to propose an algorithm to screen samples and confirm donor results at the blood bank.</td>
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important factor since it is a good indicator of previous coexistence with the triatomine vector. The possibility of congenital transmission should be considered in cases where mothers are carriers of Chagas disease.⁹

The risk of Chagas disease transmission by blood transfusion is dependent on factors such as the presence of the parasite in the blood or transfused component, type and number of infected products transfused, immunological status of the recipient, level of donor serological coverage, and sensitivity of the serological tests used in donor selection. After transmission, the chagasic infection in the recipient is generally asymptomatic with rare acute forms being manifested mainly in immunosuppressed patients.⁵

On the other hand, other mechanisms directly dependent on the enzootic cycle of transmission have become more important in Brazil, such as extra-domiciliar vectorial transmission in some cases associated with extractive activities, vector home transmission by visitors and also by ingestion, with an increasing number of cases of acute Chagas disease especially in the Amazon region.¹⁰

**Diagnosis of Chagas disease in blood banks**

According to the literature, the methodology applied for the screening of Chagas disease in blood donors should employ tests that are simple, specific, sensitive and low cost, and each donation should be submitted to at least two tests using different methods. The methods designed to detect *T. cruzi* antigens have low sensitivity because the parasitemia level is low or non-existent in individuals with chronic infections. The classical assays such as complement fixation, indirect immunofluorescence assay and reverse passive hemagglutination assay were commonly used in blood banks.¹¹

However, with the growing development of biotechnology, the serological tests used in blood banks were redesigned to have high sensitivity. Consequently, these tests improved blood transfusion quality and there was an improvement in specificity. Thus, there was a decrease in blood collection indexes from serological screening tests.¹²

The laboratory diagnosis is challenging both in the acute and chronic phases of *T. cruzi* infection despite the development of new techniques. The diagnosis is usually based on serological assays, as the direct detection of parasites is difficult even in modern molecular techniques such as polymerase chain reaction (PCR) because of low levels or absence of parasitemia. The proof of the diagnosis of Chagas disease is difficult, because of the lack of widely available and validated confirmatory tests and thus a large amount of inconclusive blood bank results are generated.¹³

The kits used for the diagnosis of Chagas disease use *T. cruzi* antigens obtained from strains and various forms of the parasite. The antibodies that react with these antigens can react with antigens from other pathologies such as *Leishmania* sp. conferring cross-reactions. This decreases the specificity of diagnostic tests for Chagas disease, which may be the cause of the inconclusive results found. This problem would be solved if the tests used specific antigens present in the most diverse strains and forms of *T. cruzi*.²

In the face of the evidence that most of these inconclusive reactions translate into failed serological tests, these tests cause many healthy individuals to be considered as having a serious illness, which causes social and psychological difficulties to the excluded donor. In addition, it causes unnecessary discard of blood units and significant financial losses for the country. In this regard, studies are needed to find new measures to improve the accuracy of serological tests, which would consequently reduce the unnecessary disposal of blood bags.⁴

**Epidemiological profile of Chagas disease in blood donors in Brazil**

According to the detailed analysis of the articles, one of the most significant considerations is that the majority of blood donors seropositive for Chagas disease in Brazil present a specific epidemiological profile. They are generally men, over 30 years old, with a low level of schooling, low income and coming mainly from rural areas and living in urban centers (Figure 1).¹⁰-¹³,15-16

A study performed in the state of Ceará corroborated with the data presented, where it is noticed that the male contribution to the reactivity for Chagas disease was significantly higher than women.¹⁴ Similar results were found in studies conducted in the states of Minas Gerais, São Paulo, Paraná and Pernambuco.¹²,13 However, according to the National Health Foundation, there is no positive correlation between donor gender and reactive serology for the disease because it affects both men and women alike.³,⁵

However, other studies have shown that this predominance of the male gender is the result of cultural differences in the practice of blood donation, which is commonly attributed to
Epidemiological profile of Chagas disease in blood donors in Brazil

Living in urban centers
From rural areas
Low income
Low level of schooling
Male
Over 30 years of age

Figure 1 – Epidemiological profile of Chagas disease in blood donors in Brazil.

men as excellent donors. On the other hand, two studies showed that females presented a higher prevalence of T. cruzi reactivity that was directly associated with the habit of female farmers sleeping close to the clay walls of their homes, thus increasing exposure to triatomines.

Another approach to the prevalence of chagasic infection in blood donors in the state of Pernambuco suggests that the reduction in infection levels in younger individuals is a reflection of the measures for vector control in the state. Young donors are less infected because of vector control measures and older people stop to donate blood because of their age.

Some studies point to the fact that some of the contaminated individuals are rural farmers and probably contracted the infection directly from infected insects. However, it should be remembered that a large number of individuals in the urban zone are migrants from rural areas in search of a better life.

According to a study conducted in the state of Sao Paulo, the current T. cruzi prevalence in this region is probably due to two events: immigrant influx from endemic areas with vectorial transmission and the presence of residual undiagnosed asymptomatic cases before the eradication of the vectorial transmission. In recent years, due to the increased immigration of chronically infected individuals from endemic regions, an increase of the T. cruzi seroprevalence in non-endemic areas such as the state of São Paulo is expected.

Other aspects identified were the prevalence of low monthly family income and low level of schooling among blood donors infected with T. cruzi. Analysis of the studies revealed that people with Chagas disease are usually individuals with low professional qualifications and incomplete education who work in places that require more physical effort and do not offer good working conditions.

Nevertheless, there has been a great decrease in the occurrence of new cases of Chagas disease in recent decades. This was possibly due to epidemiological surveillance, improvement in income and housing conditions, the supply of electricity and access to education and healthcare. Although, these improvements were not as significant in the northern semi-arid region of the state of Minas Gerais, considered one of the poorest regions of Brazil, where the socioeconomic conditions of the local population are still precarious and of great concern, mainly in the rural zone. These conditions may account for the re-emergence of Chagas disease in this region, as its spatial distribution is coincident with that of poor populations and the disease is directly related to socioeconomic conditions.

Conclusion

In this study, the results found with the analysis of articles contribute to new reflections on this theme. The study showed that the risk factors associated with chagasic infection in the Brazilian population are quite heterogeneous. It also emphasized the need for the development of more specific and practical serological and confirmatory tests for the proper diagnosis of Chagas disease among blood donors thus eliminating inconclusive results. In addition, the evaluation of the literature allowed the identification of the epidemiological profile of blood donors in Brazil infected by T. cruzi, which is characterized in general by men, subjects older than 30, with a low level of schooling, low income and coming mainly from rural areas.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES


