Original Article

Prevalence of *Trypanosoma cruzi* infection in blood donors in El Salvador between 2001 and 2011

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Abstract

Introduction: El Salvador is regarded as a highly endemic country for Chagas disease, as evidenced by the relatively high estimated positive serology rate for *Trypanosoma cruzi* among blood donors. This study aimed to identify the factors contributing to this high rate by analyzing changes in *T. cruzi* seroprevalence.

Methodology: Secondary data were collected from 31 blood banks operated by the Ministry of Health, the Red Cross, the Institute of Salvadoran Social Security, and the Military Hospital. The data were analyzed to determine the number of cases of *T. cruzi* seropositivity, and the average prevalence of seropositivity by province. Simple linear regression was performed to identify trends in *T. cruzi* seropositivity.

Results: Analysis of the 885,187 blood samples collected between 2001 and 2011 revealed 21,693 cases of transfusion-related infections, with a significant reduction of *T. cruzi* seropositivity from 3.7% in 2001 to 1.7% in 2011, reflecting a 54% decrease over the course of a decade (R^2 = 89.6%, $p > 0.001$). *T. cruzi* seroprevalence decreased in San Salvador, Santa Ana, Sonsonate, and Cuscatlán. In contrast, seroprevalence remained high with no decrease in Ahuachapán and San Vicente, and consistently low in the remainder of the country.

Conclusions: Although the national prevalence of *T. cruzi* among blood donors has decreased, it remains high in the provinces of Ahuachapán and San Vicente. Strengthening vector control activities and developing an approach for the systematic follow-up of prospective blood donors with positive serology for *T. cruzi* are required, especially in areas with high seropositivity.

Key words: blood banks; Chagas disease; El Salvador; *Trypanosoma cruzi; Rhodnius prolixus; Triatoma dimidiata.*

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Introduction

Chagas disease is a parasitic disease caused by infection with the *Trypanosoma cruzi* parasite. In El Salvador, Chagas disease was first identified in 1913 [1], four years after its description in Brazil [2]. In the years since, the Southern Cone region has experienced remarkable success in decreasing *T. cruzi* transmission [3-5]. Stimulated by this achievement, the Central American Initiative for Chagas Disease Control (Iniciativa de los Países de América Central para el Control de la Transmisión Vectorial, Transfusional y la Atención Médica de la Enfermedad de Chagas, or IPCA) was launched in 1997 to achieve three main objectives: reduction in the rate of domiciliary infestation by *Triatoma dimidiata*, a major vector of Chagas disease in Central America; elimination of *Rhodnius prolixus*, another major vector; and prevention of the transmission of *T. cruzi* through blood transfusions [6].

In El Salvador, elimination of *R. prolixus* was confirmed officially in 2010 by the IPCA [7-9]. However, El Salvador continues to be regarded as a country highly endemic for Chagas disease, as the national estimated positive serology rate for *T. cruzi* among blood donors is higher than that in other endemic countries in Central America. In 2005, this rate was found to be 2.42% in El Salvador, compared to 1.40% in Honduras and 0.01% in Guatemala [10].

This study aimed to identify the possible factors contributing to this high rate by analyzing changes in *T. cruzi* seroprevalence by province and performing trend analysis for seroprevalence. Our findings reveal important insights that will assist in improving the technical and administrative processes of blood donation throughout El Salvador.

Methodology

Study area

El Salvador is a Central American country of 5.8 million inhabitants divided into 5 regions and 14 provinces. The national blood bank network (Red Nacional de Bancos de Sangre de El Salvador) consists of 31 blood banks operated by four different institutions: the Ministry of Health (Ministerio de Salud de El Salvador or MINSAL), the Red Cross, the Institute of Salvadoran Social Security (ISSS), and the Military Hospital. In 1992, the government of El Salvador launched the Safe Blood Program throughout the blood bank network to establish mandatory screening for the following five pathogenic agents: *T. cruzi,* hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV), and syphilis. By 1996, serological screening for these pathogens had reached 100% [11]. In the years since, the four agencies under the national blood bank network have been responsible for maintaining quality standards by clarifying the roles and responsibilities of each blood bank [12,13].

Data sources

The data examined in this study were secondary data collected from anonymous blood donors that had been summarized in terms of total number of donors and prevalence of serologic markers for *T. cruzi.* The national data were collected from MINSAL, the Red Cross, the ISSS, and the Military Hospital and the provincial data from MINSAL alone. Although this study aimed to investigate the serological findings for all the provinces over the entire study period, this aim could not be completely fulfilled because of two interruptions to the collection of primary data by MINSAL. First, as MINSAL had not provided serological screening services in La Libertad over the entire study period, it had collected no data regarding this province; thus, this province could not be investigated in the present study. Second, as MINSAL had suspended the operation of blood banks in Chalatenango and Cuscatlán in 2008 and 2011, respectively, data regarding these provinces during these years were unavailable. Moreover, specific demographic data, such as those regarding exact age (as opposed to age group), rural–urban classification, and marital status of all the blood donors and those infected with *T. cruzi* were not contained in the summarized screening results provided by the four institutions.

Diagnostic methods

Since 2001, all blood donated at the 31 blood banks in El Salvador operated by the four institutions has been screened for *T. cruzi* antibody using enzyme-linked immunosorbent assay (ELISA; Chagatest ELISA Recombinant, Version 3.0, Wiener Lab, Rosario, Argentina). Prior to 2001, different methods had been utilized, with MINSAL and the Red Cross having conducted all *T. cruzi* testing by in-house indirect hemagglutination assay (IHA) and parasite lysate ELISA (Orgenics, Yavne, Israel) until 2000, and subsequently by in-house indirect immunofluorescence assay (IFA) for six months in 2001. Unfortunately, no data regarding the methods and reagents used by the ISSS and the Military Hospital in serological testing before 2001 were available.

Currently, clinical confirmation of Chagas disease in samples identified as reactive requires positive results for two of the following serological tests: the Chagas ELISA test, IFA, and/or IHA (Chagatest HAI, Wiener Lab, Rosario, Argentina) [14]. All samples found to be positive by the Red Cross, ISSS, and the Military Hospital are sent to MINSAL for confirmation. MINSAL has also conducted periodic internal and external evaluations since 1994 by sending blood specimens once a year to the World Health Organization (WHO) Collaborating Center for Quality Control of Serology in Blood Banks (Fundação Pró-Sangue, Hemocentro de São Paulo) in Brazil.

In 2001, selection criteria for blood donation (in addition to the previously established criterion of age between 18 and 65 years) were introduced to exclude potential donors at risk of Chagas disease during the pre-evaluation process [13]. Whether potential donors met all the selection criteria has been evaluated since 2001 by administration of a questionnaire containing items assessing the risk of Chagas disease.

Data analysis

The percentages of prospective blood donors positive for *T. cruzi* and four other pathogens were calculated by analysis of the nationwide blood screening results conducted by MINSAL, the Red Cross, the ISSS, and the Military Hospital.

Seroprevalence by province was calculated by analysis of data collected from MINSAL alone. Because the sensitivity of the serological testing conducted by MINSAL and the Red Cross prior to 2001 was low and the methodologies utilized by the ISSS and the Military Hospital prior to 2001 were unclear, only data collected from 2001 and after were included in the analysis.

To verify the tendency toward seropositivity for *T. cruzi* during the study period, a graph was drawn illustrating the simple linear regression results at a significance level of 5%. Prevalence was calculated and tables and figures were prepared using Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using the Statistical Package for the Social Sciences version 20 (IBM, SPSS Corp., Chicago, IL, USA).

Ethical considerations

This study analyzed secondary data that did not contain any personal information, and thus maintained the privacy of all potential donors. The study was conducted according to the ethical standards and had been approved by the Ministry of Health.

Results

Analysis of the 885,187 blood samples collected by MINSAL, the Red Cross, the ISSS, and the Military Hospital between 2001 and 2011 revealed 21,693 cases of transfusion-related infections (Table 1). The absolute number of donors increased progressively from 72,245 in 2001 to 91,543 in 2011. Analysis of the partial data regarding sex and age available for the 1,538 cases of *T. cruzi* infection detected in 2011 revealed that 843 (68%) were male patients and 397 (32%) were female patients, and that 822 (66.3%) were 15–44 years of age, 262 (21.1%) were 45–64 years of age, and 112 (9.0%) were 65 years of age and over.

Figure 1 shows the change of the prevalence for five pathogenic agents: *T. cruzi,* hepatitis B virus, hepatitis C virus, HIV, and syphilis in blood samples collected from prospective donors. The *T. cruzi* prevalence was markedly higher than that of the other four agents over the study period. At the same time, the serological positivity of *T. cruzi* among blood donors decreased steadily over the study period, from 3.7% in 2001 to 1.7% in 2011, reflecting a 54% reduction in positivity over the course of a decade (R^2 = 89.6%, $p > 0.001$).

Table 2 presents the total number of blood donors, number of *T. cruzi*-positive donors, average *T. cruzi* seroprevalence, and the results of correlation and linear regression analysis of trends in seroprevalence by province. In total, MINSAL conducted testing of 483,526 (55.0%) samples collected by the national blood bank network, among which it detected 12,365 serologically positive cases of *T. cruzi*, thus yielding a mean prevalence of 2.6% during the study period. Of the seropositive cases, 56.8% of the cases were noted in the San Salvador Metropolitan area, followed by 36.0% in the Western region, 5.1% in the Paracentral region, 1.9% in the Eastern region, and 0.2% in the Central region.

Figure 1. Changes in the prevalence of *Trypanosoma cruzi*, hepatitis B virus, hepatitis C virus, HIV, and syphilis among blood donors in El Salvador from 2001 to 2011. Source: Blood Bank, Central Laboratory of the Ministry of Health of El Salvador

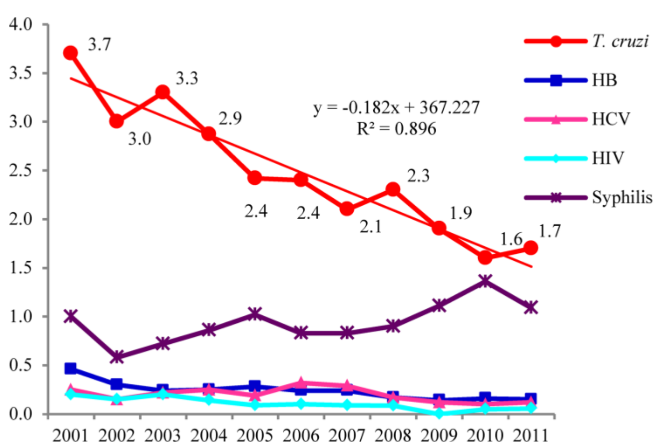

Figure 2. Average prevalence of *Trypanosoma cruzi* among blood donors at blood banks operated by the Ministry of Health of El Salvador by region from 2001 to 2011. Source: Blood Bank, Central Laboratory of the Ministry of Health of El Salvador

Table 1. Number of blood donors, number of *Trypanosoma cruzi*-positive donors, and *T. cruzi* prevalence in El Salvador from 2001 to 2011.

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Total
No. of blood donors	72,245	73,594	76,141	79,783	80,142	80,460	81,756	81,922	82,757	84,844	91,543	885,187
No. of blood donors positives for *T. cruzi*	2,673	2,208	2,513	2,288	1,942	1,946	1,711	1,905	1,600	1,369	1,538	21,693
Seroprevalence (%)	3.7	3.0	3.3	2.9	2.4	2.4	2.1	2.3	1.9	1.6	1.7	2.5

Source: Blood Bank, Central Laboratory of the Ministry of Health of El Salvador

Table 2. Number of blood donors, number of *Trypanosoma cruzi*-positive donors, average *T. cruzi* seroprevalence, and results of correlation and linear regression analysis of trends of seroprevalence by province from 2001 to 2011

	Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	No. of blood donors	No. of positive donors	Average prevalence	Linear regression	
																R^2	*p*
Western	Ahuachapan	0.2	3.0	7.5	5.5	5.6	5.1	6.2	6.1	5.0	4.2	6.4	9,894	483	4.9	0.210	0.156
	Santa Ana	9.2	6.6	6.5	5.1	4.2	1.8	2.8	4.5	2.9	2.2	2.0	63,826	2,791	4.4	0.742	< 0.001
	Sonsonate	4.3	7.8	6.7	3.9	7.9	2.1	2.0	2.2	3.4	2.7	2.9	30,636	1,178	3.8	0.388	0.040
Central	La Libertad	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Chalatenango	0.3	0.4	0.6	1.2	0.0	0.2	0.0	0.0	n/a	n/a	n/a	5,489	25	0.5	0.123	0.441
Metropolitan	San Salvador	5.5	2.7	3.8	2.7	2.2	2.7	2.5	2.2	1.7	1.6	1.2	270,386	7,019	2.6	0.704	0.001
Paracentral	Cuscatlan	5.6	2.6	4.6	3.8	0.0	0.0	1.3	0.0	0.0	0.0	n/a	1,416	36	2.5	0.686	0.003
	La Paz	0.7	4.9	0.0	1.4	0.3	1.4	0.9	1.8	2.0	1.2	1.3	14,153	180	1.3	0.011	0.764
	Cabañas	0.0	1.3	0.0	1.5	1.2	0.8	0.0	0.0	0.0	0.0	0.0	1,768	9	0.5	0.205	0.162
	San Vicente	7.0	4.5	0.0	3.5	4.3	0.9	3.3	5.5	4.6	2.0	4.2	10,681	405	3.8	0.012	0.753
Eastern	Usulutan	0.0	0.2	0.0	0.1	0.0	0.1	0.2	0.4	0.4	0.4	0.1	12,732	21	0.2	0.378	0.044
	San Miguel	0.6	0.1	0.3	0.3	0.0	0.2	0.1	0.6	1.0	0.3	0.5	53,326	200	0.4	0.103	0335
	Morazan	0.0	0.0	0.0	0.0	0.0	1.0	0.4	1.8	0.0	0.0	0.0	2,774	7	0.3	0.042	0.546
	La Union	0.0	0.0	0.7	1.3	0.0	0.0	0.0	0.0	0.1	0.0	0.0	6,445	11	0.2	0.098	0.348
MINSAL Total	No. of blood donors	43,354	42,555	41,867	43,441	42,699	43,715	44,542	43,954	42,977	45,725	48,697					
	No. of positive donors	2,129	1,349	1,531	1,236	981	903	940	1,030	830	704	722	483,526				
	Average prevalence	4.9	3.2	3.7	2.8	2.3	2.1	2.1	2.3	1.9	1.5	1.5		12,365	2.6	0.802	< 0.001
	Coverage of MINSAL (%)	60.0	57.8	55.0	54.4	53.3	54.3	54.5	53.7	51.9	53.9	53.2	55.0				

Source: Blood Bank, Central Laboratory of the Ministry of Health of El Salvador

However, the average prevalence of *T. cruzi* was higher in the three western provinces of Ahuchapán (4.9%), Santa Ana (4.4%), Sonsonate (3.8%), and San Vicente (3.8%) in the Paracentral region (Table 2, Figure 2). Although a large number of cases of *T. cruzi* seropositivity were detected in San Salvador, the prevalence decreased significantly over the study period (R^2 = 70.4%, p = 0.001). Similarly, the prevalence significantly decreased in Santa Ana (R^2 = 74.2%, p < 0.001), Sonsonate (R^2 = 38.8%, p = 0.040), and Cuscatlán (R^2 = 68.6%, p = 0.003). In contrast, the prevalence remained high with no decrease in Ahuachapán (R^2 = 21.0%, p = 0.156) and San Vicente (R^2 = 1.2%, p = 0.753), and remained consistently low in the remainder of the country.

Discussion

This study reviewed the data collected during the screening of prospective blood donors in El Salvador between 2001 and 2011 to assess the current epidemiological profile of blood donors in the nation. According to the Pan American Health Organization (PAHO), in 2005, an estimated 232,000 El Salvadorans (4.0% of the total population) were infected with Chagas disease and an additional 2.7 million were considered to be at risk [10]. Additionally, PAHO estimated that the seroprevalence among blood donors in El Salvador was higher (2.42%) than that in the other endemic countries in Central America. Clarifying these data, this study found that the percentage of blood donors positive for *T. cruzi* was highest in 2001 (3.7%) and decreased progressively over the study period such that by 2011 it had fallen to 1.7%, a rate on par with the national prevalence of the neighboring countries of Guatemala (1.75%) [15] and Honduras (1.6%) [16].

The first epidemiological study conducted between 1955 and 1957 aimed to determine the *T. cruzi* seroprevalence in the nine provinces via serological analysis and the extent of triatomine infestation (*i.e.*, the infestation index) in 14 provinces via entomological survey of 137 rural communities [17,18]. This study found the seroprevalence to be 14.2% among adults and 19.9% among children under 15 years of age. A subsequent study in 1972 found that 20.5% of adults living in rural areas had been diagnosed as *T. cruzi* positive by the indirect immunofluorescent test (IFI) [19]. In a nationwide epidemiological study of 14 provinces conducted between 1999 and 2000, *T. cruzi* seroprevalence (as detected by ELISA) was found to be 0.29% in 8,433 children under 15 years of age and 2.3% in 3,149 adults [20]. Comparison of these results with the results of the current study reveals that the prevalence was lower in 1999–2000 than in 2001, which was found to be 3.7% in this study. In the most recent epidemiological survey, which analyzed data collected from 2,663 children residing in eight provinces in 2008, the seroprevalence was found to be 1.11% [21], revealing a gradual decline in serologic response for *T. cruzi* in the rural child and adult population in the areas studied.

Serologic surveys of El Salvadoran blood banks have also yielded important findings. Testing of 1,000 blood donors in 1963 at the Rosales Hospital, the sole tertiary general hospital in El Salvador, revealed a 5.3% positive response, while hemagglutination testing of sera from 537 donors at the Maternity Hospital in San Salvador in 1970 revealed an 8.7% positive response [22]. Analysis of data collected between 1992 and 1995 revealed a mean *T. cruzi* seroprevalence of 2.4% among 45,552 blood donors tested by in-house IHA at the MINSAL reference laboratory [23].

The decreasing prevalence of *T. cruzi* among blood donors may be associated with the elimination of *R. prolixus*, an important exotic vector for trypanosomiasis. In the 1955–1957 study described above, a high rate (26.3%) of infection with two species of triatomine was identified in the 137 communities surveyed. Specifically, of the 4,871 triatomine bugs detected, 2,525 (51.8%) corresponded to *T. dimidiata* and 2,346 (48.2%) to *R. prolixus*; 19% of triatomines were infected with *T. cruzi*, and 28.9% were infected with either *Trypanosoma rangeli* or with both *T. cruzi* and *T. rangeli* [17]. However, *R. prolixus* was last detected in El Salvador in 1976 [18] and officially declared by the PAHO to have been eliminated from the country in 2010 [7-9].

Two factors that may have contributed to the elimination of *R. prolixus* in El Salvador have been urbanization and environmental improvements. The increase in the urbanization rate from 32.9% in 1971 [24] to 75% in 2013 [25] may be related to the reduction in the percentage of the population living in straw huts, a natural habitat for *R. prolixus*, from 32.9% in 1971 to 0.27% in 2007 [26]. As is well known, *T. cruzi* infection in humans has been more prevalent among rural dwellers than among urban inhabitants. Nevertheless, it is important to note that high rates of *T. dimidiata* infection were recorded in semi-urban areas in 1967, as well as in small urban areas of the provinces of Sonsonate and Cuscatlán (28.0% and 86.7%, respectively) [22].

Another possible factor in the elimination of *R. prolixus* is the implementation of nationwide vector control activities. It is well known that significant decreases in *T. cruzi* seroprevalence and mortality due to Chagas disease resulted from the implementation of vector control programs in the Southern Cone region [27-30]. Similarly, the operation of a strong national malaria control program in El Salvador since the 1970s, which has resulted in a decrease in the number of malaria cases (last recorded as 15 per year in 2011) [31], might have contributed to a reduction in *R. prolixus* infection as a secondary positive effect. In comparison, Guatemala and Honduras continue to report a high number of malaria cases, having reported between 6,814 and 7,591 cases annually in 2011. Despite the elimination of *R. prolixus* in El Salvador, continuous vector control activities are still required because of the high prevalence of *T. dimidiata*, a sylvatic vector of Chagas disease, throughout the nation. To achieve this aim, vector control activities have been continued under the National Chagas Disease Control Program. Launched within the IPCA framework with support of the Japan International Cooperation Agency (JICA) in 2003, this program initially targeted the 3 western provinces of El Salvador. By 2008, the program had expanded to 7 provinces, and by 2010, to all 14 provinces.

The selection of donors using the questionnaire introduced in 2001 may also have contributed to the reduced seroprevalence of *T. cruzi*. Specifically, it may have deterred those who had been bitten by the triatomine bug and those who were diagnosed with Chagas from donating blood. However, the results of previous studies have suggested that the use of this type of questionnaire is of limited value [5,32-34]. In the case of El Salvador, it is difficult to evaluate the degree of its impact because the questionnaire was introduced in the first year of the observation period of this study.

Analysis of the *T. cruzi* screening results by province (Table 2) reveals a high prevalence of serological positivity in the three provinces of the Western region. The results were similar to those of a previous study of 455 El Salvadorans who had been diagnosed with heart disease at the national tertiary hospital in 2010 [35]. Specifically, the study results indicated a significant association between *T. cruzi* seropositivity (16.7%) and residence in the Western region during childhood and youth. Moreover, an analysis of blood screening results between 1992 and 1995 according to residence revealed a higher prevalence in the provinces of the Western region (6.2%) than in the Metropolitan (2.0%) and Eastern regions (0.3%) [23]. However, the prevalence among blood donors cannot be used as an indicator of current epidemiological status because no data about when the seropositive individuals had become infected are available. While it is likely that they had become infected during childhood, especially those residing in rural areas, no data are available with which to determine whether infection of those residing in the three western provinces had occurred prior to the elimination of *R. prolixus* from the country or prior to the implementation of the vector control program in 2003. Moreover, review of the data could not explain the reason for the higher positive serology prevalence in the San Vicente province in the Paracentral region. Nevertheless, it is likely to be associated with the province having the highest dispersion index (100%) and rate of infection with *T. dimidiata* (41.6%) among all 14 provinces between 1999 and 2000, although no subject in the study was seropositive for *T. cruzi* [20]. To confirm this association and evaluate the impact of Chagas disease control activities, especially in the Western region, a serological study targeting children under five years of age is recommended.

The main limitation of this study was its use of secondary data collected from prospective blood donors – who may not be representative of the national population – rather than primary data collected from a representative sample of the population. For example, people considered to be at risk were excluded during the pre-evaluation process using the questionnaire in addition to those who were excluded owing to their poor health status or those who had died due to Chagas disease. Moreover, provincial data were available only from the blood banks operated by MINSAL, and not from the other three institutions involved in national blood donation and screening. Furthermore, the MINSAL data did not include specific demographic data whose analysis, coupled with knowledge regarding *T. cruzi* infection through transfusion, could have provided more sensitive results. Nevertheless, we believe that these data were useful, as previous studies of Latin American countries using similar data [2,36,37] suggested that the prevalence of *T. cruzi* antibodies in blood donors is a good predictor of prevalence on a national scale, and that the findings provide important insights into the current epidemiology of Chagas disease in El Salvador.

The results of this study highlight the need for the evaluation of current epidemiological status, especially in the Western region and San Vicente province, where a high seroprevalence was detected. Conducting a serological study targeting children under the age of five years along with an entomological study to analyze the extent of triatomine infestation will assist in the estimation of risk of transmission in these areas. Furthermore, providing immediate treatment to potential donors who report having Chagas disease or who are found to be *T. cruzi* seropositive at blood banks is required, along with serological follow-up of patients after completion of drug therapy to confirm that the results have become negative. MINSAL should consider incorporation of these procedures into the control and monitoring activities of the current Chagas disease program as highly beneficial means of improving health care throughout the country.

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