Original Report

Antibodies to *Trypanosoma cruzi* among Blood Donors in Buenos Aires, Argentina

Jorgelina L. Blejer, MD;* María C. Saguier, MD;* and Horacio J. Salamone, MD*

ABSTRACT

Objectives: The aim of this work was to study the prevalence of anti-*Trypanosoma cruzi* in the blood donor population in Buenos Aires, to compare the relative sensitivity and specificity of the two screening tests used and to confirm the results with a third assay.

Material and Methods: Between May 1995 and July 1999, 64,887 blood donor consecutive samples were screened with the following commercial tests: indirect hemagglutination (IHA) (Polychaco, Buenos Aires, Argentina) and enzyme-linked immunosorbent assay (ELISA) (40,222 with Chagatek, Organon Teknika, Buenos Aires, Argentina, and 24,665 with Chagas EIA, Abbott, São Paulo, Brazil). Repeatedly reactive samples in one or both tests were analyzed with a third method: dot blot (Bio Chagas, Gador, Buenos Aires, Argentina) or particle agglutination (Serodia, Fujirebio, Tokyo, Japan). Sera that reacted in at least two tests were considered positive.

Results: The seroprevalence was 2.66% (1744 samples were reactive for one or both screening tests), and 1.46% (949 samples) were confirmed positive. The ELISAs proved to be more sensitive (relative sensitivity: 99.67–99.71%), whereas 192 samples (0.47%) were IHA false-negatives (relative sensitivity: 79.77%). Relative specificity for EIA was 98.47–99.23% and for IHA 99.85%.

Conclusions: Results suggest the need of performing two screening tests for Chagas disease in blood banks from endemic areas and the importance of a third confirmatory assay to avoid unnecessary medical counseling.

Key Words: *blood donors, Chagas disease, serologic tests, transfusion-transmitted disease*

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*Transfusion Medicine Division and Clinical Research Department, Institute of Cardiology and Cardiovascular Surgery, Favaloro Foundation, Buenos Aires, Argentina.

Address correspondence to Dr. Jorgelina Blejer, Sección Medicina Transfusional, Fundación Favaloro, Av. Belgrano 1746 - 2° Piso, 1093 Buenos Aires, Argentina. E-mail: jblejer@ffavaloro.org.

Chagas disease is endemic in Latin America. Its agent, *Trypanosoma cruzi*, is transmitted mainly through the feces of infected triatomid bugs. Once infection occurs, the parasite may produce an acute disease, that will naturally resolve, and the infected host will remain asymptomatic for decades before chronic manifestations emerge.[1]

Blood transfusion is the second most common means of infection in endemic areas.[2,3] The risk of infection via transfusion of a contaminated blood unit is in the range of 12% to 25%.[4] Consequently, the challenge for blood banks is to identify and exclude chronic, asymptomatic carriers of the parasite without negatively affecting the blood supply.

In Latin America, the prevalence of *T.cruzi*-infected blood in blood banks is as high as 62% in Bolivia and around 2 to 3% in major cities like Caracas, Venezuela, and Buenos Aires, Argentina.[2]

Current methods for diagnosing *T.cruzi* infection are based on detection of antibodies to the parasite, because conventional parasitologic methods are difficult to perform and have a sensitivity of 30 to 50%.[5] Because none of the available test kits for detecting antibodies to *T.cruzi* is highly sensitive,[6] it has been mandated in Argentina that all blood donations must be screened by two assays based on different methods or different antigen preparations.

The assays are not sufficiently specific, especially in areas of low prevalence of infection.[7] This is primarily because the antigen preparations currently employed are derived from parasite extracts and contain epitopes that may be detected by serum antibodies of patients with other infections.[8,9]

The sensitivity and specificity of serologic tests are high when evaluated with well-characterized sera, compared with negative serum from persons with no history of exposure to the Chagas parasite. However, when combinations of serologic tests are used on samples obtained from epidemiologic surveys or blood bank screening in endemic areas, discrepant results are commonly detected.[7,10] Consequently, the Pan American Health Organization suggests the use of at least two methods for the diagnosis of the disease.[7]

Table 1. Reactivity for Chagas Disease in Each Test or Combination of Tests among 64,887 Blood Units

Pattern Number (Assay)	Group A		Group B	
	Number (%)	DS/CO* < 2 (%)	Number (%)	DS/CO < 2 (%)
1 (EIA)	308 (0.760)	292 (94.0)	377 (1.500)	233 (61.8)
2 (IHA)	71 (0.180)		23 (0.090)	
3 (EIA+IHA)	28 (0.070)	18 (64.0)	16 (0.006)	10 (62.5)
4 (EIA+3rd)	138 (0.340)	105 (76.1)	54 (0.220)	37 (68.5)
5 (IHA+3rd)	2 (0.005)		1 (0.004)	
6 (EIA+IHA+3rd)	436 (1.080)	67 (15.4)	274 (1.110)	22 (8.0)

DS/CO = donor sample optical density/cutoff optical density.

The goal of this work was to study the prevalence of anti-*T. cruzi* in the blood donor population, to compare the relative sensitivity and specificity of the two screening tests used, and to confirm the results with a third assay.

MATERIALS AND METHODS

Study Population

Between May 1995 and July 1999, 64,887 consecutive volunteer blood donors were studied.

Serologic Tests

Donations were screened with two commercial tests:

1. Indirect hemagglutination assay (IHA) (Polychaco, Buenos Aires, Argentina) and
2. Enzyme-linked immunosorbent assay (ELISA) (40,222 with Chagatek, Organon Teknika, Buenos Aires, Argentina: group A; 24,665 with Chagas EIA, Abbott, São Paulo, Brazil: group B). The presence of antibody to *T. cruzi* was determined by relating the optical density (OD) of the specimen to the cutoff value (donor sample OD/cutoff OD).

Each assay was performed according to the respective manufacturer's instructions.

All initially reactive samples were tested again. If a sample was repeatably reactive (RR) in any of the tests, the unit was discarded and the sample was assayed with a third method:

1. Dot blot (DB) (Bio Chagas, Gador, Buenos Aires, Argentina) which uses recombinant antigens. This EIA is carried out on test strips consisting of a plastic backing covered with a nitrocellulose membrane to which a mixture of *T. cruzi* antigens has been applied as a horizontal line. This test has shown a sensitivity of 99.6% when tested with samples IHA-, immunofluorescent assay (IFA) and enzyme immunoassay (EIA)-positive (299 of 300 samples) and a specificity of 99.1% (347/350 samples),[11] or
2. Particle agglutination (PA) (Serodia, Fujirebio, Tokyo, Japan), which uses gelatin particle carriers .

Sera that reacted in at least two tests were considered positive and when reacted in only one test were considered discordant.

Statistical Analysis

Seroprevalence percentages were calculated as the mean, standard error (SE), and confidence interval (CI) at 95%.

RESULTS

The seroprevalence was 2.66% (1,728/64,887 samples were reactive for one or both screening tests, SE = 0.0685, CI = 2.185-2.566; $P < 0.05$) and 1.46% (949/64,887 sera that reacted in at least 2 of 3 tests, SE = 0.0471; CI = 1.5578-1.8780; $P < 0.05$) were confirmed positive. There were six groups with different patterns (Table 1).

The sera considered positive (at least 2 of 3 assays reactive) were: 44 samples (0.07%) from pattern 3 (EIA and IHA reactive), 192 samples (0.29%) from pattern 4 (EIA and third assay reactive), 3 samples (0.0046%) from pattern 5 (IHA and third assay reactive), and 710 samples (1.09%) from pattern 6 (EIA, IHA, and third assay reactive).

It is remarkable that 525 of 685 samples in pattern 1 (false-positive EIA) had a weak EIA result (donor sample OD/cutoff OD < 2). The percentages are 94% (292 samples) and 61.8% (233 samples) for EIA in group A and B (Organon and Abbott), respectively. In pattern 3 (false-negative third assay) 28 samples (63.63%) and in pattern 4 (false-negative IHA) 142 samples (73.9%) had a weak result. On the other hand, in pattern 6 (three assays reactive) 621 samples (87.46%) had an EIA strong result (donor sample OD/cutoff OD > 2).

As shown in Table 2, both EIAs proved to be more sensitive (99.67% and 99.71% relative sensitivity for tests

Table 2. Diagnostic Performance of the Three Assays Used To Screen Donated Blood Samples

	Reactive	*Confirmed Positive**	*False Negative*	*False Positive*	*Relative Sensitivity*	*Relative Specificity*
EIA (A)	910	602	2	308	99.67	99.23
EIA (B)	721	344	1	377	99.71	98.47
IHA	851	757	192	94	79.77	99.85

*At least 2 of 3 tests reactive.

A and B, respectively) in the studied blood donor population, whereas 192 samples were IHA false-negative (79.77% relative sensitivity). The relative specificity results were 99.23%, 98.47%, and 99.85% for EIA test A, EIA test B, and IHA, respectively.

DISCUSSION

Serologic tests are the most reliable and practical procedures for identifying chronically Chagas disease-infected individuals. In general, the sensitivities and specificities of the commercial serologic tests for *T. cruzi* are high when they are evaluated with well-characterized sera, but discrepancies are common when combinations of serologic tests are used in epidemiologic surveys or blood bank screening.[6,7,12]

In the particular case of Chagas disease, no serologic gold standard for the definition of the disease exists, since cross-reactivity of *T. cruzi* antigens with antibodies raised against other coendemic parasites (*Leishmania* and *Trypanosoma rangeli*) is frequent.[13]

None of the methods for diagnosis of Chagas disease can be regarded as 100% safe in blood bank screenings. Furthermore, with the low prevalence of infected donors, one has to expect low positive predictive values, leading to a high false-positive rate of results that must be confirmed by other methods.[14] Contradictory results have been obtained by different methods and laboratories, probably owing to the use of different strains of *T. cruzi* and different antigenic fractions and procedures, causing variations in sensitivity and specificity.[15]

The prevalence of blood donors with anti-*T. cruzi* at the blood bank was 2.66%. Of all tested sera, 1.09% were positive in the three tests, 0.37% in two tests, and a 1.2% reacted in only one test. That means that the confirmed seroprevalence is 1.46% (at least 2 tests reactive).

The seroprevalence obtained in our blood bank service is lower than that reported by Schmuñis and colleagues.[16] They have reported a 3.6 to 4.9% incidence of positive donors between 1995 and 1997 in Argentina. The reason for this discrepancy could be the different blood donor populations. They have analyzed the data from the whole country, including rural and urban areas, and the majority of the blood donors who donate in our service live in Buenos Aires, a major city.

Both EIAs were more sensitive but less specific than IHA. Other studies reported satisfactory results in the relative sensitivities and specificities from different commercial EIAs and a lower sensitivity for IHA.[10,13,17,18]

Saéz-Alquézar et al, evaluating the performance of Brazilian blood banks in testing for Chagas disease, noted that 42.1% of the enrolled blood banks reported errors, showing that the use of more than one technique is still necessary in the screening of anti-*T. cruzi*. Blood banks using only IHA were responsible for 49 of the 64 errors, which would imply that this assay has a low performance.[19]

The ideal diagnostic test must have high sensitivity and high specificity. In most cases, however, increased sensitivity can be obtained at the expense of specificity, leading to an increased proportion of false-positive results, as occurred with both EIAs used in the present study.

Other authors have described a variable number of cases reacting at about the cutoff value for positivity in the presently used serologic tests.[20] In the present study, results show that one could predict a false-positive result if the OD value is low and the sample is reactive in one test.

Owing to socioeconomic factors, the migration of infected people from the areas in which the disease is endemic to the urban centers is frequent, and blood transfusion has become an important means of infection.[13] The intensive immigration to the United States, Canada, Europe, and Australia opened the possibility of Chagas disease spreading to new frontiers. In fact, there are several reports of transfusion-transmitted Chagas in North America and Europe.[21-24] Furthermore, since Chagas disease is usually asymptomatic and rarely recognized by American and European physicians, other transfusion-related cases may have occurred and not been detected. Several studies have reported the presence of serologic markers for *T. cruzi* in Latin American immigrants in the United States and Europe[25,26]; and recent papers have determined that the seroprevalence of *T. cruzi* in American blood donor populations with lower levels of risk ranges from 0.01% to 0.2%.[10,27,28] These studies primarily involved populations with high numbers of at-risk blood donors, but it was also reported that blood donors seropositive for *T. cruzi* are present in populations with low to moderate risk.[29]

Blood screening has not been implemented in the United States, in part because no test for blood bank screening has been licensed by the U.S. Food and Drug Administration (FDA). Many sensitive and specific assays have been described, such as radioimmunoprecipitation assay (RIPA), polymerase chain reaction (PCR), and chemiluminescent EIA,[30-33] which have been shown to be sensitive but are not feasible for blood bank screening. Recently, it has been reported that a new immunoblot assay, INNO-LIA, is a reliable assay for the serologic confirmation of Chagas disease, showing a specificity of 98.1 to 99.3% and a sensitivity of 99.4 to 100%.[34,35]

Results of the present study suggest the need of performing two screening tests for Chagas disease in blood banks from endemic areas and the importance of a third confirmatory assay to avoid unnecessary medical counseling. In countries with a low prevalence of anti-*T. cruzi*, like the United States, an initial screening identification of *T. cruzi*-positive donors with an EIA could be used along with a supplemental or confirmatory test, including PCR, Western blot, RIPA, or LIA to allow the reentry of initially false-positive blood donors.

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Correction

Changes over Time in the Epidemiology of Diarrhea and Malnutrition among Children in an Urban Brazilian Shantytown, 1989 to 1996

In *International Journal of Infectious Diseases* Volume 4, Number 4, 2000 "Changes over Time in the Epidemiology of Diarrhea and Malnutrition among Children in an Urban Brazilian Shantytown, 1989 to 1996" by Sean R. Moore, MS; Aldo A. M. Lima, MD, PhD; John B. Schorling, MD; Manuel S. Barboza Jr, MD; Alberto M. Soares, PhD; and Richard L. Guerrant, MD, page 179, the first sentence of the results section of the abstract should have read as follows; please note these changes in your copy.

Results: Declines in both age-adjusted attack rates (6.0 episodes/child-year in study year 3 [1991] to 2.5 episodes per child-year in study year 8 [1996]) and days of diarrhea per child-year (30.8 days/child-year in year 3 to 8.5 days/child-year in year 8) were correlated with yearly improvements in mean nutritional status ($R^2 = 0.84$; $P < 0.05$), for mean length-for-age with mean number of episodes/child-year.