Trypanosoma cruzi Parasitemia in US Blood Donors with Serologic Evidence of Infection

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Trypanosoma cruzi infection (which causes Chagas disease) is typically undiagnosed and persists if untreated. We sought to affirm that T. cruzi–seropositive US blood donors have persistent infection with demonstrable parasitemia long after acquisition of infection. Fifty-two previously identified seropositive donors (positive by 2 methods) provided up to 3 blood specimens for testing by polymerase chain reaction (PCR) and hemoculture; most participants (67%) provided only 1 specimen for testing by polymerase chain reaction (PCR) and hemoculture; most participants (67%) provided only 1 specimen. When evaluated ~2 decades after immigration, 33 donors (63%) had PCR evidence of parasitemia; 3 also had culture-confirmed infection. This affirmation that bloodstream parasites are detectable—and potentially transmissible—decades after immigration strengthens the rationale for donor screening.

Chagas disease (American trypanosomiasis) is caused by infection with the protozoan parasite Trypanosoma cruzi [1–5]. In 1909, the Brazilian clinician Carlos Chagas published his initial descriptions of the disease, its etiologic agent, and triatomine insect vectors [6]. Although vectorborne transmission is confined to the Americas (and principally to rural regions of Latin America), other routes of transmission (e.g., by blood transfusion or organ transplantation) are not geographically restricted.

The general principle that untreated infection persists for decades, if not for life, is exemplified by the course in Chagas’s first patient, Berenice [6]. In 1909, when she was 2 years old, Chagas was asked to evaluate her for malaria. He examined her blood by light microscopy, detected circulating trypomastigotes (not malaria parasites), and noted that she had patent parasitemia only during the initial phase of infection. The acute phase usually resolves within several weeks or months and merges into the chronic phase, as parasite-specific antibodies appear and the parasite seemingly disappears. The estimated lifetime risk of developing cardiac or other sequelae is ≥20%–30% [3]. In 1961 and 1978, when Berenice was rediscovered and reevaluated, no sequelae were found, but subpatent parasitemia was detected by xenodiagnosis [6].

Long-term persistence of infection and large-scale migration from rural Latin America translate into the potential for nonvectorial transmission in other regions [1–5]. Estimates of the number of infected US immigrants range from tens of thousands to well over 100,000 [1, 2]. The ramifications for blood safety have been assessed through various means, including seroprevalence studies. In a study conducted by the American Red Cross (ARC), the estimated T. cruzi–seropositivity rate among blood donors in metropolitan Los Angeles rose from 1/9900 in 1996 to 1/5400 in 1998 [7, 8].

Nationwide projections have suggested that hundreds of potentially infective blood products are transfused annually, but only 5 US transfusion-associated cases of infection have been documented in more than a decade [5]. This discordance raised the question of whether the seroreactivity noted in blood-donor studies was a manifestation of prior infection. To strengthen the rationale for donor screening, we sought to affirm that seropositive donors have persistent infection with demonstrable parasitemia long after immigration and acquisition of infection.

Methods. The investigation was conducted November 1997–October 2000 in ARC’s Southern California Blood Services Region (Los Angeles and Orange Counties). Eligible subjects included 147 T. cruzi–seropositive donors (positive by 2 methods) who were identified during 1994–1998 in a study that has been described elsewhere [7, 8]; they had been counseled about their results, given written materials about Chagas disease, referred to their clinicians for medical care, and deferred indefinitely from donating blood.
ARC staff followed standard operating procedures to find and recruit these deferred donors for the investigation described here. The protocol was approved by the institutional review boards of the ARC and the Centers for Disease Control and Prevention. Participants provided written informed consent, were interviewed for epidemiologic characterization, and were evaluated by polymerase chain reaction (PCR) and hemoculture for *T. cruzi* parasitemia. Three blood specimens, obtained at ≥6-month intervals, were requested, unless a specimen tested positive by both methods. Specimens were transported to the ARC’s regional laboratory for initial processing and thereafter (at 4°C) to ARC’s Holland Laboratory in Maryland.

For PCR, ~10 mL of EDTA blood was mixed (1:1) with a solution containing 6 mol/L guanidine-HCl/0.2 mol/L EDTA [9]. At the Holland Laboratory, 1 mL of lysate was boiled for 5 min to shear *T. cruzi* kinetoplast DNA [10]; DNA was extracted by phenol/chloroform procedures or with an Isoquick nucleic acid extraction kit (MicroProbe); and PCR amplifications were conducted as described elsewhere [9]. Minicircle primers S35 and S36 were used to yield 330-bp fragments from the variable region; PCR products were analyzed by gel electrophoresis. For hemoculture [11], ~30 mL of heparinized blood was centrifuged (800 g for 30 min), and theuffy coat and red-cell pellet were resuspended in a tube containing 25 mL of liver-infusion-tryptose (LIT) medium. At the Holland Laboratory, the culture tube was centrifuged (4,000 g for 30 min); theuffy coat, the upper half of the red-cell pellet, and the lower half of the pellet were placed in 3 separate tubes containing 6 mL of LIT medium; and the tubes were incubated at 26°C, mixed by inversion every 2–3 days, and monitored by light microscopy until parasites were noted or 16 weeks had elapsed.

![Figure 1](https://academic.oup.com/jid/article-abstract/198/4/609/833527)
Participants were interviewed in English or Spanish with a questionnaire that addressed age, sex, educational level, blood transfusions, birthplace for subject and mother, year of immigration, and lifetime residence and travel history, which included housing conditions and recall of triatomine bugs (a photograph was shown). Clinical evaluations and management were beyond the scope of the study.

Univariate analyses were conducted for descriptive purposes. Two-tailed \( P \) values were calculated using the \( \chi^2 \) test or (when appropriate) Fisher’s exact test for binary variables and the Wilcoxon 2-sample test for continuous variables. Statistical significance was defined as \( P < 0.05 \).

**Results.** Fifty-two (35%) of 147 eligible seropositive donors enrolled in the study. A larger proportion of eligible donors who had been prospectively (vs. retrospectively) identified could be tracked down and recruited: 23 (59%) of 39 whose index donation occurred in 1998 vs. 29 (27%) of 108 who had donated earlier (\( P < 0.001 \)).

The 52 participants provided up to 3 blood specimens for testing by PCR and hemoculture, most (67%) of whom provided only 1 sample (figure 1). Overall, 33 donors (63%) had evidence of parasitemia: all 33 had at least 1 PCR-positive specimen, 3 of whom also had culture-confirmed infection. Eight (73%) of 11 donors who had evidence of parasitemia and were evaluated more than once had >1 positive specimen: 7 donors had >1 PCR-positive specimen; and 1 donor had different specimens that were positive by PCR (the first) and culture (the third). Five persons had consistently positive PCR results, including 3 who were evaluated twice and 2 who were evaluated thrice. Only 1 (5%) of 19 donors without evidence of parasitemia was evaluated thrice.

Epidemiologic data were available for 51 donors (98%), most (80%) of whom were born in Mexico (\( n = 25 \)) or El Salvador (\( n = 16 \)) (table 1). The median intervals from immigration to enrollment for donors with vs. without demonstrable parasitemia were 17 years (range, 3-38 years) vs. 22 years (range, 9-37 years) (\( P = 0.09 \)). Donors from 8 (of 10) countries had evidence of parasitemia. The 3 culture-positive donors were from El Salvador, Argentina, and Colombia; their ages at enrollment ranged from 42 to 62 years, and they had immigrated from 9 to 37 years earlier. The 5 donors with consistently positive PCR results included 3 from Mexico, 1 from El Salvador, and 1 from Ecuador, with a median interval from immigration to enrollment of 15 years.

**Discussion.** The general principle that untreated *T. cruzi* infection persists indefinitely is based on various types of laboratory and clinical evidence [1, 3, 12]. Much of the knowledge about the natural history of the infection in ostensibly healthy adults is based on observations in particular settings in South America. As reflected in our study population, the Latin American immigrants in the United States represent the full spectrum of *T. cruzi*–endemic regions, but the bulk are from Mexico and Central America (table 1) [1, 2]. In the context of a paucity of documented US transfusion–associated cases and the diversity encompassed by Chagas disease, it was important to affirm, as we did, that the principle of persistent infection applies under scenarios pertinent to US immigrants and transfusion medicine.

The seropositive donors we evaluated for parasitemia enrolled in the study ~2 decades, on average, after immigration. For the study group as a whole, the prior probability of infection was high. The participants had been identified as being seropositive in an investigation in which they were selected for testing on the basis of a risk-factor question and their donation testing positive by 2 methods, including radioimmunoprecipitation assay [7, 8]. The epidemiologic plausibility of infection was solidified during an interview on enrollment in this study (table 1). In general, even the subset of donors who visited *T. cruzi*–endemic regions after immigration had nominal risk for reexposure (data not shown).

Our main findings were that a substantial proportion (63%) of the 52 participants had PCR evidence of parasitemia; that several also had hemoculture-confirmed infection; that some of the relatively few donors who provided >1 blood specimen had consistently positive PCR results, whereas others had variable results; and that the aggregate epidemiologic profiles of donors with vs. without evidence of parasitemia were comparable in univariate analyses.

Our focus was on affirming the qualitative principle of persistent infection, not on determining the precise prevalence of infection or parasitemia among these or other seropositive US donors. We do not have sufficient evidence to conclude that all 52 seropositive participants had persistent infection, nor can we exclude the possibility of false-positive results for some donors or specimens. However, the observed prevalence of parasitemia probably underestimates the true prevalence of chronic infection: suboptimal sensitivity (i.e., true- or false-negative PCR and culture data for finite volumes of blood collected at random points in time) likely played a larger role than suboptimal specificity of the serologic and molecular data. Most participants (67%) provided only 1 blood specimen (vs. 3), probably in part because of the long interval (>6 months) between the requested blood draws.

During the chronic phase of infection, fluctuating, low-level parasitemia is characteristic, which underscores the utility of serial testing of large-volume specimens by sensitive methods. The numbers and volumes of specimens needed to have a reasonable probability of detecting parasitemia vary among infected persons, *T. cruzi* strains and settings, and detection methods [12–15]. Specimen volumes represent a small portion of the total blood volume in an adult and usually do not approximate the volumes that are transfused. Even under optimal circumstances, undoubtedly infected persons can have consistently negative results for long periods. Variable results for serial specimens may reflect fluctuations in parasite density or low parasitemias that,
by chance, are intermittently detected. Although donors with consistently demonstrable parasitemia might have a greater transmission potential, it is prudent to assume that low inocula could cause infection.

The higher positivity rate by PCR compared with hemoculture (63% vs. 6%) supports the consensus view that molecular tools generally are more sensitive than conventional parasitologic methods for detecting low parasitemias [9, 12–14]. Reliance on hemoculture data alone may underestimate the prevalence of infection and the potential for bloodborne transmission: for a culture to be positive, the entire parasite must be present in an aliquot and must be sufficiently viable to convert into replicative culture forms. Optimal handling (e.g., rapid and minimal processing) is vital for specimens with scarce parasites.

In our study, all of the specimens were handled by staff in 2 states, and some cultures might have been differentially compromised (e.g., by delayed processing and inadvertent freezing during transport). Nevertheless, the observed positivity rate of 6% is noteworthy. Culture positivity constitutes parasitologic confirmation in the classic sense, and the culture-positive donors were from 2 continents, were not young adults, and were not recent immigrants. Therefore, these donors and culture results constitute proof of principle.

Our findings—which underscore the essentially lifelong potential for bloodborne transmission—strengthen the scientific basis for developing and licensing intervention measures: in De-
December 2006, the Food and Drug Administration licensed an assay for testing US blood donations for antibodies to T. cruzi [4]. Our findings also remain pertinent during the evolving implementation phase of control strategies, in part because of persistent skepticism that ostensibly healthy, long-term US residents can transmit T. cruzi to others and may have or develop severe sequelae of Chagas disease. Donor testing has the potential not only to decrease the risk for secondary transmission but also to lead to secondary and tertiary prevention of morbidity and mortality in persons whose infection would have remained undetected [3]. Thus, US donor testing may be lifesaving for recipients and donors, as well as for others found to be infected, as the general level of awareness about Chagas disease increases.

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