

Prevalence of Chagas Disease in the Latin American–born Population of Los Angeles

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(See the Editorial Commentary by Manne-Goehler on pages 1189-90.)

Background. According to an estimate from the Centers for Disease Control and Prevention (CDC), Chagas disease (CD) may affect 1.31% of Latin American immigrants in the United States, with >300 000 cases. However, there is a lack of real-world data to support this estimate. Little is known about the actual prevalence of this neglected tropical disease in the United States, and the bulk of those infected are undiagnosed.

Methods. From April 2008 to May 2014, we screened 4,755 Latin American–born residents of Los Angeles County. Blood samples were tested for serologic evidence of CD. We collected demographic data and assessed the impact of established risk factors on CD diagnosis, including sex, country of origin, housing materials, family history of CD, and awareness of CD.

Results. There were 59 cases of CD, for an overall prevalence of 1.24%. Prevalence was highest among Salvadorans (3.45%). Of the 3,182 Mexican respondents, those from Oaxaca (4.65%) and Zacatecas (2.2%) had the highest CD prevalence. Salvadoran origin (aOR = 6.2; 95% CI = 2.8–13.5; $P < .001$), prior knowledge of CD (aOR = 2.4; 95% CI = 1.0–5.8; $P = .047$), and exposure to all 3 at-risk housing types (adobe, mud, and thatched roof) (aOR = 2.5; 95% CI = 1.0–6.4; $P = .048$) were associated with positive diagnosis.

Conclusions. In the largest screening of CD in the United States to date outside of blood banks, we found a CD prevalence of 1.24%. This implies >30 000 people infected in Los Angeles County alone, making CD an important public health concern. Efficient, targeted surveillance of CD may accelerate diagnosis and identify candidates for early treatment.

Keywords. Chagas disease; *Trypanosoma cruzi*; prevalence; neglected tropical diseases; screening.

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, affects over 6 million people worldwide, claims 7,000 lives annually, and causes the greatest burden of disability-adjusted life years of any parasitic disease in Latin America [1, 2]. Through immigration, CD has emerged as a public health concern in nonendemic countries including the United States, Spain, Italy, Japan, and Australia. Annual global healthcare costs from CD are estimated at \$627 million, of which \$118 million are spent in the United States [3].

Current data on the prevalence of CD in the United States are based on indirect estimates or limited information from testing of blood donations. Utilizing immigration totals and the World Health Organization (WHO) prevalence estimates for *T. cruzi*

infection for Latin American countries in 2005, the Centers for Disease Control and Prevention (CDC) estimated an overall prevalence of 1.31% for Latin American immigrants in the United States, entailing 300 167 individuals with the disease, 30–45 000 prevalent cases of cardiomyopathy, and 63–315 new congenital infections annually [4]. A recent study using the WHO's 2010 updated prevalence rates estimated 326–347 000 Latin American immigrants with CD in the United States [5]. Nonetheless, there is insufficient epidemiological data to confirm these estimates. Because CD has a decades-long chronic indeterminate form where symptoms are absent, the vast majority of people with CD are unaware of their infection, and in the United States, routine screening generally does not occur outside of blood and organ donations [6]. Screening of the blood supply in Los Angeles found levels of seropositive samples ranging from 1/9,850 in 1996 to 1 in 1,993 in 2006 [7, 8]. However, blood donors may not represent all socioeconomic strata [9]. Prior research with immigrant populations suggests blood donor testing underestimates the actual prevalence of CD, which is concentrated among marginalized populations with low access to healthcare [10, 11]. The present study assesses CD prevalence and identifies important risk factors using a community-level screening program of Latin American immigrants living in the Los Angeles area.

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METHODS

Setting

Research took place in Los Angeles County, California, which has the largest population of Latin American immigrants of any metropolitan area in the United States at nearly 2.5 million [12]. The top 2 countries of birth for this population are Mexico (79%) and El Salvador (12%). In Los Angeles County in 2010, nearly half of Latin American immigrants lacked health insurance, and 56% had not completed high school; median income for working adults was \$24 000, or less than 40% of that for white non-Hispanics [12]. Screening was coordinated by the Center of Excellence for Chagas Disease (CECD) at Olive View-UCLA Medical Center, one of few providers in the United States offering comprehensive care for Chagas disease. The CECD conducts ongoing outreach in the Latino community of Los Angeles in partnership with local churches and other organizations.

Participants and Procedures

From April 2008 to May 2014, CECD staff and volunteers participated in 89 health fairs in 36 sites including churches (n = 27) and other community organizations (n = 9). Latin American immigrants residing in Los Angeles County aged 18–60 years were eligible for participation in the study. We did not test adults older than 60 because there is limited evidence for benefit of antitrypanosomal treatment in this age group. Ethical approval was obtained from the Institutional Review Board of the Olive View-UCLA Education and Research Institute. Participants provided written informed consent prior to enrollment in the study.

Data collection

Study participants provided a blood sample and basic demographic information, including education and place of birth. The first 3,013 study participants also filled out a questionnaire on knowledge/awareness of Chagas disease; the results for the first 2,677 of these respondents are described elsewhere [13]. In the current study, we utilize the survey items to identify potential risk factors for CD. Blood samples were tested for presence of *T. cruzi* antibodies; positive diagnosis was determined by concordance on 2 tests. Initial testing was performed using an enzyme-linked immunosorbent assay (ELISA; Goldfinch Diagnostics, Inc., Iowa City, IA, sensitivity=100%, specificity=99.4% [14]; or Chagatest ELISA v. 3.0, Wiener Lab Group, Rosario, Argentina, sensitivity=99.3%, specificity=99.6%) [15, 16]. Positive results on ELISA testing were confirmed with a Chagas radioimmune precipitation assay (RIPA, sensitivity and specificity=100%) [15] to detect *T. cruzi* antigen-specific immunoglobulin G (IgG) antibodies (performed by University of Iowa) or an immunofluorescence antibody assay (IFA, sensitivity=94%, specificity=94.9%) [17] to fixed *T. cruzi* epimastigotes (performed by CDC). If the first 2 results were discordant,

an additional sample was drawn and confirmed at CDC with an immunoblot assay using trypomastigote excreted-secreted antigens (sensitivity and specificity=100%) [18, 19].

Statistical Analysis

Baseline characteristics were reported as means for continuous variables and proportions for categorical variables and analyzed using the Student t-test or χ^2 test as appropriate. We calculated Pearson correlation coefficients for associations between variables. CD risk factors were assessed using a multivariable logistic regression model in a stepwise backward selection process to calculate odds ratios (ORs) and 95% confidence intervals (CIs). We removed variables with cell sizes <5 but retained known risk factors for CD even if they were not significant in the univariate analysis. Associations were considered significant at a 2-sided alpha of 5%. Statistical calculations were performed in IBM SPSS Statistics version 23.0 (Armonk, NY: IBM Corp).

RESULTS

Sample Characteristics

We screened 4,755 adults who were born in Latin America and resided in Los Angeles County (Table 1). The majority of

Table 1. Sex, Education Level, and Country of Origin of Study Participants in Los Angeles County, April 2008 to May 2014

	N	%
Sex		
Male	1614	34.5
Female	3067	65.5
Age range (years)		
18–30	488	10.3
31–40	1199	25.4
41–50	1715	36.3
51–60	1319	27.0
Education		
<High school	3104	66.8
≥High school	1541	33.2
Country of origin		
Mexico	3182	66.9
El Salvador	811	17.1
Guatemala	475	9.9
Other	287	6.0
Residence in Latin America		
Rural	595	20.0
Urban	2386	80.0
Housing type in Latin America		
Adobe	2329	79.7
Mud	1016	34.4
Thatched roof	407	13.6
Number of housing risk factors		
0	386	14.0
1	1502	53.2
2	692	24.5
3	231	8.2

respondents (65.5%) were female, likely reflecting higher participation of women in church-related and health-related activities [20]. Most participants (3182, 66.9%) were born in Mexico; others were from El Salvador (811, 17.1%) and Guatemala (475, 9.9%). The remaining 287 participants came from 14 other Latin American countries. The breakdown of national origins in the sample resembles that of the Latin American-born population in Los Angeles County [12].

We assessed socioeconomic risk factors for CD, including education level, and asked respondents if they had ever lived in a rural area of Latin America or in housing made of materials susceptible to triatomine infestation (mud, adobe, or thatched roof). To ensure accurate categorization, we showed respondents pictures of each type of house and used terminology common in Mexico and Central America. Although only 20% of respondents had lived in a rural area of Latin America, 79.7% had previously lived in an adobe home, 34.4% in a mud home, and 13.6% in a home with a thatched roof. Although 14% of respondents had not lived in a house with any of these characteristics, 53.2% had lived in homes with only 1 of these characteristics, 24.5% with 2 and 8.2% with all 3. Two-thirds of respondents had less than a high school education.

Screening Results

There were 59 confirmed cases of CD, for a prevalence of 1.24% (95% CI = 0.93–1.55%) (Table 2). Mean years of age was not significantly different between participants who tested positive (median = 47 years, range = 24–59) or negative (median = 44 years, range = 18–60, $P = .370$). There was not a significant difference in the mean number of years CD-positive (24.8 years, range = 5–47) and CD-negative respondents (23.2 years, range = 0.5–60) had lived in their countries of origin ($P = .229$). Compared to negative participants, more CD-positive respondents fell within the 41–50 age range (45.8 vs. 36.2%), but the differences between age categories and a test for trend ($P = .435$) were not statistically significant, nor was the slightly higher prevalence among females (1.30%) compared to males (1.18%). Figure 1 shows the geographical distribution of positive participants in Los Angeles County, which reflects the reach of the CECD's screening efforts rather than population-level dynamics of CD. As expected, positive cases clustered near the CECD in the San Fernando Valley of northern Los Angeles, where 85 of the 89 health fairs conducted by the CECD and its partner organizations occurred. The highest number of positive participants came from El Salvador (28), followed by Mexico (25), Guatemala, (3), Honduras (2), and Argentina (1) (Table 2, Figure 2). Prevalence was substantially higher among Salvadorans (3.45%) and participants without a high school education (1.45%) (Table 2). We stratified the 3,182 Mexican respondents by state of origin and compared states using a χ^2 test; the highest prevalence was in respondents from Oaxaca (4/86, 4.65%, $P = .004$) and Zacatecas (5/226, 2.20%, $P = .028$).

Risk Factors for Positive CD Diagnosis: Univariate Analysis

We assessed socioeconomic and clinical risk factors for CD among the 3,013 survey respondents (Table 2). A greater percentage of respondents with CD than negative participants had previously lived in a house with a thatched roof (31.4 vs. 13.4%, $P = .002$) or had lived in all 3 types of housing (24.2 vs. 8.0%, $P = .001$). The percentage who had lived in a rural area of Latin America was notably higher for people with CD (30.6 vs. 19.8%), but the difference was not significant. For clinical risk factors, prior cardiac ($n = 4$) or CD diagnosis ($n = 6$) and family history of sudden death ($n = 2$) or CD ($n = 2$) were reported by few CD-positive respondents; of these, prior CD diagnosis was statistically significant. Compared to CD-negative respondents, a significantly higher proportion of positive participants had previously heard of CD (36.1 vs. 12.8%, $P < .001$). In an analysis of pairwise associations, most study variables were weakly correlated ($r < 0.2$); the strongest correlation was between mud and thatched roof housing (Pearson correlation coefficient = 0.27; 95% CI = 0.23–0.32; $P < .001$). Thatch and mud are often used in conjunction to construct homes in rural Latin America. Seropositive respondents had lived only slightly longer in Latin America ($P = .229$) and spent nearly as many years in the United States (19.3) as the seronegative group (20.3, $P = .505$).

Multivariable Analysis of CD Risk Factors

We gauged the impact of CD risk factors through multivariable logistic regression (Table 3). Salvadorans had a much higher odds of CD compared to other countries of origin in the sample (aOR = 6.2; 95% CI = 2.8–13.5; $P < .001$). Knowledge of the existence of CD (aOR = 2.4; 95% CI = 1.0–5.8; $P = .047$) was associated with *T. cruzi* infection. Moreover, people who had lived in all 3 types of at-risk housing (adobe, mud, and thatched roof) were more likely to have a positive diagnosis (aOR = 2.5; 95% CI = 1.0–6.4; $P = .048$). Not completing a high school education (aOR = 2.1; 95% CI = 0.9–5.2; $P = .092$) or having lived in thatched roof housing (aOR = 2.0; 95% CI = 0.9–4.4; $P = .099$) were not significant in the adjusted model. People who had lived in a rural area of Latin America (aOR = 1.3; 95% CI = 0.6–3.0; $P = .503$) remembered being bitten by triatomines (aOR = 1.3; 95% CI = 0.6–2.9; $P = .523$) or had a prior diagnosis of cardiac problems (aOR = 1.7; 95% CI = 0.5–5.6; $P = .389$) did not have a significantly higher odds of CD.

DISCUSSION

This is the first large-scale study outside of blood banks to assess the prevalence of CD in a US population of Latin American immigrants. The CD prevalence in our study (1.24%) was notably similar to that predicted by CDC's immigration-based estimate using country of origin prevalence data (1.31%) [4]. If the prevalence found in our investigation were projected to all 2.5 million Latin American-born residents of Los Angeles County, it would signify >30 000

Table 2. Prevalence of Chagas disease by sex, country of origin, and survey risk factors for study participants in Los Angeles County, April 2008 to May 2014

	Chagas+ N = 59	Chagas – N = 4696	Chagas Prevalence (95% CI)	P value
Total	59/4755 (1.24)	4696/4755 (98.76)	1.24 (0.93–1.55)	...
Demographic variables				
Median age, years (range)	47 (24–59)	44 (18–60)370
Age range				
18–30	3/59 (5.1)	485/4662 (10.4)	0.61 (0.0–1.30)	.277
31–40	13/59 (22.0)	1186/4662 (25.4)	1.08 (0.49–1.67)	.550
41–50	27/59 (45.8)	1688/4662 (36.2)	1.57 (0.98–2.16)	.129
51–60	16/59 (27.1)	1303/4662 (27.9)	1.21 (0.62–1.80)	.888
Mean years lived in Latin America	24.8	23.2229
Mean years lived in the US	19.3	20.3505
Sex				
Males	19/59 (32.2)	1595/4622 (34.5)	1.18 (0.65–1.71)	.711
Females	40/59 (67.8)	3027/4622 (65.5)	1.30 (0.90–1.70)	
Education				
<High school	45/59 (76.3)	3059/4586 (66.7)	1.45 (1.03–1.87)	.121
≥High school	14/59 (23.7)	1527/4586 (33.3)	0.90 (0.43–1.37)	
Country of origin				
Mexico	25/59 (42.4)	3157/4696 (67.2)	0.79 (0.48–1.10)	<.001
Oaxaca	4/25 (16.0) ^a	82/3157 (2.6) ^a	4.65 (0.20–9.10)	.004
Zacatecas	5/25 (20.0) ^a	221/3157 (7.0) ^a	2.20 (0.29–4.11)	.028
Jalisco	5/25 (20.0) ^a	792/3157 (25.1) ^a	0.63 (0.08–1.18)	.650
Other	11/25 (44.0)	2062/3157 (65.3) ^a	0.53 (0.22–0.84)	.026
El Salvador	28 (47.5)	783/4696 (16.7)	3.45 (2.19–4.71)	<.001
Guatemala	3 (5.1)	472/4696 (10.1)	0.63 (0.0–1.34)	.275
Other	3 (5.1)	284/4696 (5.0)	1.04 (0.0–2.21)	.758
Socioeconomic risk factors				
Lived in rural area/farm	11/36 (30.6)	584/2945 (19.8)	1.85 (0.77–2.93)	.110
Housing type in Latin America				
Thatched roof	11/35 (31.4)	396/2947 (13.4)	2.70 (1.13–4.27)	.002
Mud	15/35 (42.9)	1001/2918 (34.3)	1.47 (0.73–2.21)	.290
Adobe	30/35 (85.7)	2299/2888 (79.6)	1.28 (0.82–1.74)	.372
Number of housing risk factors				
0	3/33 (9.1)	393/2788 (14.1)	0.77 (0.0–1.63)	.411
1	17/33 (51.5)	1485/2788 (53.3)	1.13 (0.60–1.66)	.516
2	5/33 (15.2)	687/2788 (24.6)	0.72 (0.09–1.35)	.446
3	8/33 (24.2)	223/2788 (8.0)	3.46 (1.10–5.82)	.001
Clinical risk factors				
Remembers triatomine bites	11/36 (30.6)	774/2932 (26.4)	1.40 (0.58–2.22)	.574
Prior cardiac diagnosis	4/36 (11.1)	177/2946 (6.0)	2.21 (0.07–4.35)	.275
Prior Chagas disease diagnosis	6/36 (16.7)	6/2961 (0.2)	50.0 (21.7–78.3)	<.001
Family history sudden death	2/36 (5.6)	293/2915 (10.1)	0.68 (0.0–1.62)	.371
Family history heart disease	12/35 (34.3)	769/2853 (27.0)	1.54 (0.68–2.4)	.332
Family history Chagas disease	2/35 (6.5)	29/2822 (1.0)	6.45 (0.0–15.1)	.054
Heard of Chagas disease	13/36 (36.1)	381/2969 (12.8)	3.30 (1.54–5.06)	<.001

^a percentages based on Mexican participants only.
Abbreviation: CI, Confidence Interval.

people infected. Importantly, this exceeds the prevalence found in blood donations of Latin Americans living in the United States (0.13–0.5%) [21]. This suggests blood donor screening underreports the actual prevalence of CD in immigrants, much as was found in prior research in Europe [10]. Our screening took place through community organizations rather than healthcare institutions, enabling the study to reach a Latin American immigrant population that is historically underserved and has limited access to health insurance and medical services [22].

Salvadorans were 6.2 times more likely to test positive for CD than other Latin Americans. The prevalence of CD in Salvadorans in our study was 3.45%, nearly 3 times that of the WHO's most recent estimate of the prevalence of *T. cruzi* infection in El Salvador [1]. Our sample consists of adults only, whereas the WHO estimate includes all age strata, which may explain the difference. Additionally, the higher prevalence in our sample could reflect its regional or socioeconomic composition, but the possibility that current estimates for El Salvador are underreported should not be discounted. Similarly, an

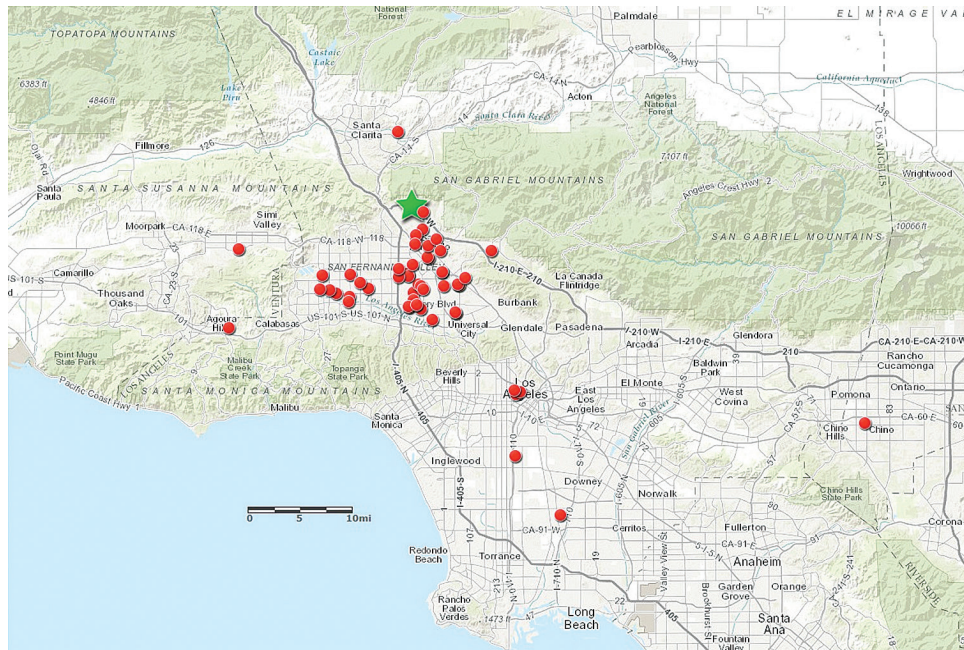


Figure 1. Chagas disease positive cases in Los Angeles county and proximity to the CECD, April 2008 to May 2014. ● = positive case, ★ = CECD. Abbreviation: CECD, Center of Excellence for Chagas Disease.

investigation in El Salvador's Sonsonate province determined 3.6% of 797 women of childbearing age were *T. cruzi* positive [23]. Other evidence suggests high levels of infestation by the vector *Triatoma dimidiata* persist in some communities, which elevates rates of acute infection and seroprevalence in children [24–26]. More epidemiological studies are needed to assess current rates and patterns of *T. cruzi* infection in El Salvador [25].

On the other hand, the prevalence of CD among Mexican immigrants in the present study (0.79%) is nearly identical to the estimate put forth by the WHO (0.78%) [1] and higher than that detected in screening of the blood supply (0.38%) [27]. We observed significantly higher seroprevalence among respondents from Oaxaca and Zacatecas. Similarly, previous research has indicated a high risk of *T. cruzi* infection for the state of Oaxaca.



Figure 2. Countries of origin of *Trypanosoma cruzi* positive cases in Los Angeles, 2008–2014*. * One case from Argentina is not shown.

Table 3. Multivariable analysis of selected risk factors and odds of positive diagnosis of study participants, Los Angeles County, April 2008 to May 2014

Risk Factor	Adjusted OR (95% CI)	P value
Salvadoran origin	6.2 (2.8–13.5)	<.001
Female sex	1.2 (0.6–2.6)	.619
Lived in thatched roof house	2.0 (0.9–4.4)	.099
All 3 housing risk factors	2.5 (1.0–6.4)	.048
Lived in rural area/farm	1.3 (0.6–3.0)	.503
Heard of Chagas disease	2.4 (1.0–5.8)	.047
< High school education	2.1 (0.9–5.2)	.092
Recalls triatomine bites	1.3 (0.6–2.9)	.523
Prior cardiac diagnosis	1.7 (0.5–5.6)	.389

Abbreviations: CI, confidence interval; OR, odds ratio.

A review of published data from Mexico calculated pooled prevalence estimates of 17.7% for the state of Oaxaca and 5.6% for Zacatecas, based on prior serological and clinical studies [28]. Médecins Sans Frontières/Doctors Without Borders reported a seroprevalence range of 4–12% in Oaxaca [29].

Besides country of origin, we analyzed potential socioeconomic risk factors for CD including prior housing conditions. In a study of women of childbearing age in El Salvador, illiteracy, anemia, age, lack of education, and awareness of CD were significantly associated with being seropositive [23]. Similarly, we found prior knowledge of CD was related to being seropositive. This might reflect greater exposure of individuals in endemic areas to campaigns and interventions targeting CD but could also stem from participation bias because knowledge of the disease may strengthen participants' inclination to seek diagnosis. Although prevalence of CD in our sample was higher among people who did not complete high school, the difference was not significant. We did not detect a significantly higher prevalence of CD among people who had lived in rural areas, who comprised only 20% of the sample. However, the majority had lived in adobe houses, which are common in low-income or peripheral areas of many Latin American cities. Prior research has demonstrated a link between *T. cruzi* infection and housing construction [30–32]. In this study, living in all 3 at-risk housing types (adobe, mud, and thatched roof) in Latin America was significantly associated with CD diagnosis.

Sex, age, prior history of heart problems, and recollection of triatomine bites were not significant risk factors. Although prevalence of *T. cruzi* infection tends to increase with age in endemic areas, we did not observe a significant trend based on age categories. This is likely due to the impact of immigration; once individuals moved to the United States, their period of high exposure to the vector ended; thus levels of infection were relatively similar across the over 30 age categories, whereas the lower prevalence observed in people under 30 may reflect the impact of vector control programs. Recall of triatomine bites did not have predictive value in our study, perhaps because triatomines are nocturnal and feed on blood while hosts are asleep, so the bites may

go unnoticed. In a study of blood donors in the United States, those who recalled being bitten by triatomines or who had lived in a rural area of Latin America showed a higher risk of CD, but seronegative respondents in the sample included non-Latinos [21]. In contrast, our study focuses exclusively on Latin American immigrants. We did not detect an association between personal or family history of heart conditions and being seropositive, and we did not observe significant differences based on sex.

Screening for CD has the potential to significantly improve outcomes for *T. cruzi*-infected patients. One study conducted in patients with minimal cardiac involvement showed reduced disease progression after treatment with benznidazole [33], whereas another study of chronic CD patients with no cardiac involvement reported a smaller proportion of patients who underwent trypanocidal therapy suffered disease progression compared with untreated patients [34]. Importantly, the BENEFIT trial did not identify an advantage for antitrypanosomal therapy for patients who already had developed moderate to severe cardiomyopathy, underscoring the need to treat CD patients early [35]. In our study area, CD has been associated with higher mortality. In a study of Los Angeles patients with nonischemic cardiomyopathy who had resided in Latin America for at least 12 months, the prevalence of CD was 19%, and the risk of death or heart transplant was significantly higher in this group (hazard ratio = 4.46) [36].

Major gaps persist in current knowledge of CD in the United States. Further epidemiological studies are needed in other states with potentially large populations with CD, including Texas, Florida, New Jersey, Illinois, and Virginia [5]. There is a lack of data on the incidence of autochthonous transmission, a risk throughout the southern United States, and on prevalence in high-risk groups, such as migrant workers. Pooling epidemiological data from multiple sites could provide key insights for national screening and treatment guidelines and programs. Finally, there is a need for treatment studies with US populations with CD, as current research, including recent clinical trials [35, 37], has occurred almost exclusively in Latin America and Europe.

This study's results support targeted screening of US Latin American immigrant patients for CD. Early diagnosis and treatment of CD could considerably reduce morbidity, mortality, and costs related to treatment of cardiomyopathy ensuing in the advanced stages of the disease. For US Latin American immigrants, country of origin, knowledge of the existence of CD, and prior residence in certain types of at-risk housing may help identify whom to screen for CD so that timely treatment can be provided.

Limitations

The study has several limitations. Participants represent a convenience sample who attended health fairs at churches and community organizations that are mostly near the CECD. Data collection took place solely in Los Angeles County, so our results may not be generalizable to other counties and states. We only

gathered data on a limited number of variables and did not collect information on income, occupation, risk for congenital transmission, and other factors which could influence CD prevalence. Because we only screened respondents under 60 and prevalence levels of *T. cruzi* infection are higher in older adults [38], the actual prevalence of CD in the underlying adult population could be greater. However, we did not observe a significant association between age and CD prevalence; emigration out of endemic areas may tend to dilute this effect. Due to the possibility of autochthonous and congenital transmission in the United States [39, 40], the risk of CD is not restricted to Latin American immigrants.

Notes

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Potential Conflicts of Interest. The authors have no conflicts of interest to report. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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