Prevalence of Chagas Heart Disease in a Region Endemic for *Trypanosoma Cruzi*

Evidence From a Central Bolivian Community

Jessica E. Yager*, Daniel F. Lozano Beltran†, Faustino Torrico†, Robert H. Gilman‡,§,‖, Caryn Bern¶

Brooklyn, New York, USA; Cochabamba, Bolivia; Lima, Peru; Baltimore, Maryland, USA; and San Francisco, California, USA

ABSTRACT

Background: Though the incidence of new *Trypanosoma cruzi* infections has decreased significantly in endemic regions in the Americas, medical professionals continue to encounter a high burden of resulting Chagas disease among infected adults. The current prevalence of Chagas heart disease in a community setting is not known; nor is it known how recent insecticide vector control measures may have impacted the progression of cardiac disease in an infected population.

Objectives: We sought to determine the current prevalence of *T. cruzi* infection and associated Chagas heart disease in a Bolivian community endemic for *T. cruzi*.

Methods: Nested within a community serosurvey in rural and periurban communities in central Bolivia, we performed a cross-sectional cardiac substudy to evaluate adults for historical, clinical, and electrocardiographic evidence of cardiac disease. All adults between the ages of 20 and 60 years old with *T. cruzi* infection and those with a clinical history, physical exam, or electrocardiogram consistent with cardiac abnormalities were also scheduled for echocardiography.

Results: Of the 604 cardiac substudy participants with definitive serology results, 183 were seropositive for infection with *T. cruzi* (30.3%). Participants who were seropositive for *T. cruzi* infection were more likely to have conduction system defects (1.6% vs. 0% for complete right bundle branch block and 10.4% vs. 1.9% for any bundle branch block; $p = 0.008$ and $p < 0.001$, respectively). However, there was no statistically significant difference in the prevalence of bradycardia among seropositive versus seronegative participants. Echocardiogram findings were not consistent with a high burden of Chagas cardiomyopathy: valvulopathies were the most common abnormality, and few participants were found to have low ejection fraction or left ventricular dilatation. No participants had significant heart failure.

Conclusions: Though almost one-third of adults in the community were seropositive for *T. cruzi* infection, few had evidence of Chagas heart disease.

Chagas disease, caused by the parasite *Trypanosoma cruzi*, remains an important cause of morbidity and mortality in Central and South America [1–3]. Vector-borne transmission occurs when the feces from the infected triatomine insect is inoculated through the bite wound or intact mucosa of the mammalian host. Since 1991, affected countries in the Southern Cone have implemented insecticide campaigns with the goal of reducing the vector burden and interrupting transmission [4]. Nevertheless, an estimated 8 million to 10 million people are currently living with Chagas disease [2,5,6].

Infection with *T. cruzi* often goes undetected. The acute phase lasts 4 to 8 weeks and generally results in only nonspecific signs and symptoms. Infection persists in the absence of effective treatment, and untreated patients enter the chronic phase of infection. Approximately 20% to 30% of those infected subsequently develop cardiac or, less commonly, digestive disease years or decades after infection. Common manifestations of Chagas cardiomyopathy include atrioventricular and bundle branch blocks, arrhythmias, and dilated cardiomyopathy, eventually leading to congestive heart failure [1,7,8].

In central Bolivia, an area that remains endemic for *T. cruzi*, the primary livelihood is subsistence farming. Most farmers continue to live in adobe houses with mud floors, structures that easily accommodate triatomine bugs. In 2003, the Bolivian national control program instituted large-scale insecticide campaigns to reduce triatomine infestation. We conducted a serosurvey of all community residents older than 2 years of age to determine the

Drs. Yager and Lozano Beltran received research funding from the Fogarty International Clinical Research Fellows Program of the Fogarty International Center, National Institutes of Health. Dr. Torrico reports no relationships that could be construed as a conflict of interest.
From the *Division of Infectious Diseases, Department of Medicine, State University of New York Downstate Medical Center, Brooklyn, New York, USA; †Centro Universitario de Medicina Tropical, Facultad de Medicina y CEADES Salud y Medio Ambiente, Universidad Mayor de San Simon, Cochabamba, Bolivia; ‡Asociacion Benefica PRISMA, Lima, Peru; §Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru; ‖Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; and the ¶Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California, USA. Correspondence: J. E. Yager (jessica.yager@downstate.edu).

GLOBAL HEART

VOL. 10, NO. 3, 2015
ISSN 2211-8160/$36.00.
http://dx.doi.org/10.1016/j.gheart.2015.07.002

community-wide prevalence of *T. cruzi* infection, and a nested study of cardiac disease in adults 20 to 60 years old.

METHODS

Ethics statement

The protocol was approved by the institutional review boards of CEADES, Asociación Benéficia PRISMA, and Johns Hopkins Bloomberg School of Public Health. All adult participants provided written informed consent, with separate forms for the serosurvey and the cardiac substudy. Written informed consent was obtained from the parent or guardian on behalf of children <18 years old, and children aged 7 to 17 years provided written assent.

Study setting and serosurvey participants

The study was conducted in Punata, a province of Cochabamba Department with a population of 47,735 inhabitants at the time of the 2001 census. Participants were recruited between January 2009 and May 2010. Communities were selected to include rural and periurban populations living in the catchment area of Punata Hospital, located in the central city (also called Punata). Field-workers invited all residents 2 years or older to participate. All participating individuals provided a blood sample for serologic testing. Testing was also offered for children younger than 2 years whose mothers were found to have *T. cruzi* infection, based on the risk of congenital transmission.

Adult cardiac substudy

All permanent community residents in the age range of 20 to 60 years who participated in the serosurvey were asked to participate in the nested cardiac substudy. Adults older than 60 years were excluded based on previous evidence that rates of Chagas heart disease peaked in adults ages 30 to 50 years old [9–12]. They were also excluded to reduce the number of age-related nonspecific cardiac findings on electrocardiograms (ECG) and echocardiograms.

Laboratory methods

Serum samples were evaluated for antibodies to *T. cruzi* using at least 2 of 3 commercially available assays: a rapid immunochromatographic screening test based on recombinant *T. cruzi* antigens (STAT-PAK, Chembio Diagnostic Systems, Inc., Medford, New York); an epimastigote lysate enzyme-linked immunosorbent assay (Chagatek, bioMérieux, Buenos Aires, Argentina); and an indirect hemagglutination assay (IHA—Chagatest, Wiener Laboratorios, Rosario, Argentina). All tests were performed and interpreted following the manufacturers' guidelines. Individuals were considered to have *T. cruzi* infection if they had 2 positive serologic tests. Twelve individuals participating in the nested cardiac substudy had inconclusive serologic results based on conflicting or indeterminate assay results; these individuals were excluded from analysis.

Cardiac study procedures

All substudy participants underwent a focused history and physical exam and an ECG and gave 1 blood sample. All individuals who were positive for infection with *T. cruzi* and all those with any evidence of cardiac abnormalities by history, physical exam, or ECG (including bradycardia with a heart rate <60 beats/min) were scheduled to undergo echocardiogram. Echocardiograms were performed using a portable Esaote Caris machine (Genoa, Italy). A cardiologist read all ECG and performed and interpreted all echocardiograms. ECG were coded and interpreted according to established criteria [13]. The cardiologist was blinded for all ECG interpretations; study participants occasionally revealed their *T. cruzi* infection status to the cardiologist during their echocardiogram rendering echocardiogram blinding incomplete.

Statistical analysis

We evaluated associations between infection with *T. cruzi* and cardiac lesions using odds ratios with 95% confidence intervals (CI) and 2-tailed Fisher exact test with an alpha of 0.05, as well as multivariate logistic regression models. Logistic regression models included age and serostatus as predictor covariates. Because incomplete right bundle branch block (RBBB) has been shown to be a highly nonspecific finding and can be a normal variant in healthy adults [14], all analyses evaluating combined conduction system defects excluded incomplete right bundle branch blocks. Data were collected and managed using Microsoft Access (Redmond, WA, USA); statistical analysis was performed using Stata (version 11.0, IBM, Armonk, NY, USA).

RESULTS

Study population

A total of 1,380 individuals participated in the *T. cruzi* serosurvey; 36 individuals (2.6%) were excluded due to inconclusive serology results, yielding a survey population of 1,344 (Table 1). The mean age of all participants in the community serosurvey was 31.29 ± 21.83 years. The mean age of the participants in the cardiac substudy was 39.19 ± 12.16 years, and among those who received an echocardiogram was 39.89 ± 12.26 years. Seroprevalence increased with increasing age in all decades until the seventh decade, with a maximum seroprevalence of 56.88% among study participants between the ages of 60 and 69 years. Rates were lower among study participants in their 70s and 80s but with confidence intervals that overlapped with those in younger age groups. Twenty-one of 52 participants between the ages of 70 and 79 years were positive for *T. cruzi* infection, yielding a seroprevalence of 40.38% (95% CI: 27.31 to 54.87). Of those participants between the ages of 80 and 89 years, 7 of 18 were positive for *T. cruzi* infection, yielding a seroprevalence of 38.89% (95% CI: 18.26 to 63.86) (Figure 1).

Cardiac disease according to *T. cruzi* infection

Of 632 eligible adults, 604 (95.6%) participated in the cardiac substudy; 183 (30.3%) had confirmed *T. cruzi* infection. Three seropositive participants (1.6%) had complete RBBB on ECG (1.6%) compared with none of the seronegative participants (p = 0.008) (Table 2). Nineteen *T. cruzi*–infected participants had any conduction system defect, compared with 8 seronegative participants (10.4% vs. 1.9%, p < 0.001). The percentages with bradycardia were similar among those with and without *T. cruzi* infection (3.8% vs. 4.7%, respectively, for heart rate <50 beats/min).

The prevalence of RBBB was highest in seropositive individuals older than 40 years, whereas bifasicular blocks were read more commonly in younger seropositive participants (Figure 2). The prevalence of any conduction system defect among both seropositive and seronegative individuals was highest among those between the ages of 20 and 29 years: 23.1% and 2.6% among seropositive and seronegative individuals, respectively. In multivariable logistic regression models adjusted for sex and age (by decade), bifasicular block and any conduction system defect were significantly associated with *T. cruzi* infection (odds ratio for bifasicular block 8.19, 95% CI: 1.55 to 43.17, p = 0.02; any conduction system defect 7.36, 95% CI: 2.97 to 18.6, p < 0.001).

Per study protocol, 434 participants were scheduled for echocardiograms, but only 201 participants (46%; 78 seropositive, 123 seronegative) underwent the examination (Table 3). Participants who missed an echocardiogram were contacted by the study team by phone or by home visit to reschedule the appointment. Those who missed repeat appointments often cited the need to work and the lack of any symptomatic illness as the reasons for skipping the echocardiogram. One seronegative participant had an uninterpretable study due to body habitus; the echocardiogram was excluded from analysis. There was no sex difference between seropositive participants who did and did not present for echocardiogram; seronegative women were more likely to miss their appointment than seronegative men were. Left ventricular dilation (defined as left ventricular end-diastolic diameter > 57 mm) was found in 4 seropositive and 3 seronegative participants (Table 4). Seven seropositive participants had left atrial dilation (defined as >40 mm) compared with only 2 uninfected participants (9.0% vs. 1.6%, p = 0.014). One seronegative participant had an ejection fraction of 42%; 2 seropositive and 2 seronegative participants had slightly depressed ejection fractions between 51% and 54%. There was no difference in the distribution of ejection fractions by infection status (mean 68 ± 0.16% for seropositives vs. 68 ± 0.16% for seronegatives; p = 0.959 by Student *t* test). Sixteen participants had valvulopathies not related to Chagas disease: 12 aortic sclerosis; 2 mitral prolapse; 1 mitral stenosis; and 1 mitral insufficiency. There was no significant difference in rates of valvular disease among participants with and without *T. cruzi* infection. None of the participants had an apical aneurysm or an intracavitary thrombus.

TABLE 1. Baseline characteristics of participants in community serosurvey with conclusive serologic data

	Community (n = 1,344)*		Cardiology Substudy (n = 604)		Completed Echocardiogram (n = 200)	
		Seropositive		Seropositive		Seropositive
Age, yrs						
<20	540	30 (5.6)	—	—	—	—
20–29	189	28 (14.8)	179	26 (14.5)	53	10 (18.9)
30–39	126	39 (31.0)	118	39 (33.1)	43	15 (34.9)
40–49	159	52 (32.7)	152	47 (30.9)	47	22 (46.8)
50–60	158	73 (46.2)	155	71 (45.8)	57	31 (54.4)
>60	172	87 (50.6)	—	—	—	—
Female	818	187 (22.9)	387	107 (27.6)	112	46 (41.1)
Male	526	122 (23.2)	217	76 (35)	88	32 (36.4)
Rural	966	251 (25.1)	456	146 (32.0)	137	58 (42.3)
Periurban	378	58 (15.3)	148	37 (25.0)	63	20 (31.8)

Values are n or n (%).

*Thirty-six individuals were excluded due to inconclusive serology results.

DISCUSSION

Our study was one of the first in decades to evaluate the community burden of *T. cruzi* infection and Chagas heart disease in the inter-Andean valleys of Bolivia. Housing improvement programs began in Cochabamba in the 1990s, and household insecticide application was instituted on a large scale by the Bolivian National Chagas Disease Control Program between 2000 and 2004 (personal communication, F. Torrico, 2009) [15]. Our data support the success of these efforts. We found a much lower prevalence of *T. cruzi* infection than a study conducted in Cochabamba Department in 1988 did (74% in 1988 vs. 23% in our data) and a strikingly lower prevalence in children (38% in 1988 vs. 5.6% in our data) [12].

Although our age range and case definitions varied from those used in the 1988 study, it is interesting to note

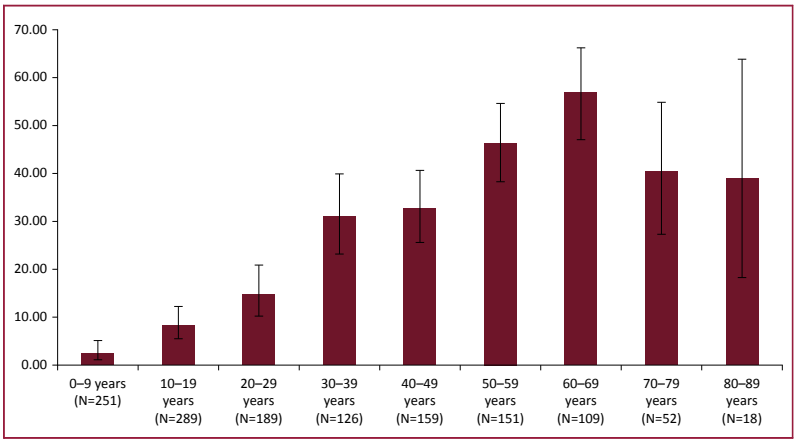

FIGURE 1. Prevalence of *Trypanosoma cruzi* infection in Punata, Bolivia community serosurvey, by age.

TABLE 2. ECG findings* in individuals with and without *T. cruzi* infection

	Seropositive (n = 183)	Seronegative (n = 421)	Odds Ratio	95% Confidence Interval	p Value
Heart rate rhythm					
Heart rate <60 beats/min	63 (34.4)	128 (30.4)	1.202	0.815–1.764	0.329
Heart rate <50 beats/min	7 (3.8)	20 (4.8)	0.797	0.280–2.009	0.613
Mean heart rate	62.65 ± 9.4	62.66 ± 10.6			
Atrial fibrillation/flutter	0	1 (0.2)	0.0		1.000†
Ventricular arrhythmia (PVC)	1 (0.5)	0	0.0		0.303†
Bundle branch blocks					
RBBB (complete)	3 (1.6)	0	0.0		0.027†
LAFB	7 (3.8)	5 (1.2)	3.309	0.888–13.378	0.052†
LPFB	3 (1.6)	1 (0.2)	7.000	0.556–368.280	0.085†
Bifasicular block	6 (3.3)	2 (0.5)	7.102	1.250–72.337	0.006
Any BBB‡	19 (10.4)	8 (1.9)	5.981	2.432–16.061	<0.001
Incomplete RBBB	13 (7.1)	29 (6.9)	1.034	0.481–2.112	0.924
Other abnormalities					
1° AV block	4 (2.2)	4 (1.0)	2.330	0.428–12.633	0.253†
ST-T changes	13 (7.1)	13 (3.09)	2.400	1.001–5.739	0.025
Low voltage QRS	0	1 (0.2)	0.0		1.000†
Normal ECG	88 (48.1)	228 (54.2)			0.171

Values are n (%) or mean ± SD, unless otherwise indicated.

AV, atrioventricular; BBB, bundle branch block; ECG, electrocardiogram; LAFB, left anterior fascicular block; LPFB: left posterior fascicular block; LVH, left ventricular hypertrophy; PVC, premature ventricular contraction; RBBB: right bundle branch block; *T. cruzi, Trypanosoma cruzi*.

*No participant was found to have LVH or an abnormal initial portion of their QRS complex on ECG.

†By 2-tailed Fisher exact test.

‡Any complete bundle branch block (RBBB, LAFB, LPFB, bifasicular block).

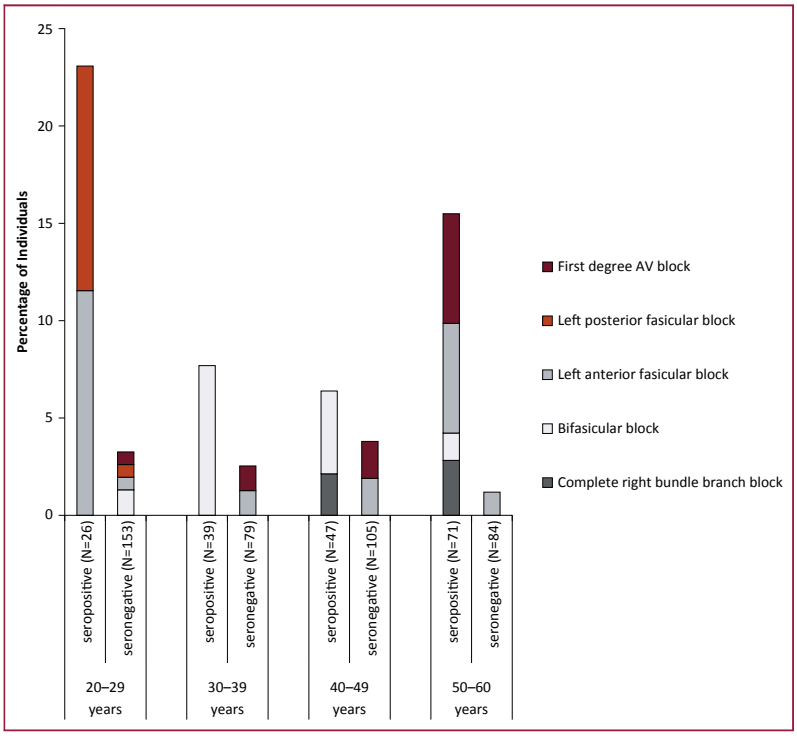

FIGURE 2. Electrocardiogram findings by age and *Trypanosoma cruzi* serostatus. AV, atrioventricular.

that we also found a lower rate of bundle branch blocks among infected participants than the 1988 study did (20% in 1988 vs. 10.4% in our data), and we found no participants with clinically evident congestive heart failure (compared with 9% in the 1988 data) [12]. A similar study restricted to Bolivian women of childbearing age in the 1980s also showed higher rates of conduction system abnormalities than we found [11]. Nevertheless, *T. cruzi* infection is still a significant risk factor for conduction system defects in our data. These findings provide an optimistic picture for the future, because bundle branch blocks [10], decreased ejection fraction [16,17], left and right ventricular dysfunction [18,19], and abnormal diastolic function [20,21] have all been associated with increased mortality in individuals with Chagas disease. Evidence from mouse models [22,23] and communities [24] suggest that continued vector-borne exposure to *T. cruzi* may increase the risk of Chagas cardiomyopathy. Failure to down-regulate the anti–*T. cruzi* inflammatory immune response is widely accepted as an important driver of Chagas cardiomyopathy development and progression [25,26]. Vector-borne parasite exposure is hypothesized to maintain sustained antigen exposure and consequent higher chronic inflammatory immune responses, contributing to Chagas cardiomyopathy. The lower rate of clinical disease in our study may reflect the better vector control over the previous decades and decreased exposure to the

TABLE 3. Comparing those who did and did not complete echocardiograms, by serostatus, in those who were scheduled for study per protocol

	Chagas Seropositive		Chagas Seronegative	
	Received Echo (n = 78)	No Echo (n = 105)	Received Echo (n = 123)	No Echo (n = 128)
Mean age, yrs	44.0 ± 11.2	43.4 ± 11.6	37.3 ± 12.2	36.6 ± 11.9
Female	46 (59.0)	61 (58.1)	67 (54.5)	85 (66.4)
Heart rate <60 beats/min	28 (35.9)	35 (33.3)	67 (54.5)	60 (46.9)
Heart rate <50 beats/min	4 (5.1)	3 (2.9)	7 (5.7)	13 (10.2)
Bifasicular block	3 (3.9)	3 (2.9)	2 (1.6)	—
Any conduction system defect	6 (7.7)	13 (12.4)	6 (4.9)	2 (1.6)

Values are mean ± SD or n (%).
Echo, echocardiogram.

parasite. However, further data are needed to better understand this possible association.

Our observation of an association between *T. cruzi* infection and left atrial dilation is intriguing but its clinical significance is unclear. Previous studies have found left atrial dilation associated with RBBB in infected individuals, perhaps indicating early manifestations of cardiac involvement [27–29]. Furthermore, left atrial dilation has been linked to increased risk of sudden cardiac death in those with known Chagas cardiomyopathy [20,21]. The most common abnormalities found on echocardiogram were valvulopathies, most commonly isolated aortic sclerosis in participants between 50 and 60 years old. This finding was consistent with previous studies and is most consistent with age-related changes [30]. We anticipated seeing a greater burden of valvular disease associated with known or suspected rheumatic heart disease, which most commonly causes mitral stenosis; it is not common to have aortic disease resulting from rheumatic heart disease without concomitant mitral involvement [31,32]. We found no association between valvulopathies and *T. cruzi* infection.

Study limitations

First, it is possible that those individuals who were in poor health did not present to our mobile clinic to participate in the study, and we therefore underestimated the cardiac disease burden. Second, we excluded adults over age 60 years to minimize nonspecific cardiac disease such as paroxysmal atrial fibrillation. Although the work of Lima-Costa et al. [33,34] indicates that Chagas may be a greater problem in elderly patients than was previously recognized, previous studies have found the majority of cardiac disease developing in adults between 30 and 50 years old [9–12]. It is possible, however, that our exclusion of adults over age 60 reduced the amount of Chagas heart disease detected. Finally, our echocardiogram findings may have been affected by the low rates of echocardiogram completion. However, consensus among Chagas experts indicates that the rate of

TABLE 4. Echocardiography findings* in individuals with and without *T. cruzi* infection

	Seropositive (n = 78)	Seronegative (n = 122)	Odds Ratio	95% Confidence Interval	p Value
LV end-diastolic diameter	4.58 ± 0.94	4.42 ± 0.79			0.207
LVEF	0.68 ± 0.16	0.68 ± 0.12			0.959
LA diameter	3.00 ± 0.84	2.84 ± 0.68			0.149
LV dilation	4 (5.1)	3 (2.4)	2.144	0.351–14.989	0.435†
LAD	7 (9.0)	2 (1.6)	5.915	1.077–59.355	0.030†
EF <55%	2 (2.6)	3 (2.5)	1.044	0.853–9.329	1.000†
Any valvulopathy	8 (10.3)	8 (6.5)	1.629	0.506–5.215	—
Early systolic dysfunction	2 (2.6)	1 (0.8)	3.184	0.162–189.36	0.562†
LVH	62 (79.5)	96 (78.7)	1.049	0.495–2.274	0.892

Values are mean ± SD or n (%), unless otherwise indicated.
EF, ejection fraction; LA, left atrium; LAD, left atrial dilation; LV, left ventricle; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy.
*No participant was found to have an apical aneurysm, an intracavitary thrombus, or diffuse wall motion abnormalities on echocardiogram.
†By 2-tailed Fisher exact test.

clinically significant Chagas heart disease is rare in the absence of ECG abnormalities [35].

CONCLUSIONS

Our study found low rates of Chagas cardiomyopathy in a community endemic for *T. cruzi*, possibly related to the effects of widespread vector control efforts over the previous 20 years. More research is needed to better understand who, among those infected with *T. cruzi*, is at increased risk of progression to associated disease.

ACKNOWLEDGMENTS

The authors would like to thank Drs. René Ugarte, Marco Solano Mercado, and Gimena Rojas Delgadillo for their assistance in the laboratory and in the field. They would additionally like to thank all the members of the field team who collected data and specimens for the community study, and the members of CEADES Salud y Medio Ambiente who assisted with data collection and management.

REFERENCES

1. Hidron AI, Gilman RH, Justiniano J, et al., for the Chagas Disease Working Group in Peru and Bolivia. Chagas cardiomyopathy in the context of the chronic disease transition. PLoS Negl Trop Dis 2010;4:e688.
2. Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010;375:1388–402.
3. Dias JC, Silveira AC, Schofield CJ. The impact of Chagas disease control in Latin America: a review. Mem Inst Oswaldo Cruz 2002;97:603–12.
4. Dias JC. Southern Cone Initiative for the elimination of domestic populations of Triatoma infestans and the interruption of transfusional Chagas disease: historical aspects, present situation, and perspectives. Mem Inst Oswaldo Cruz 2007;102(Suppl 1):11–8.
5. Bowman NM, Kawai V, Levy MZ, et al. Chagas disease transmission in periurban communities of Arequipa, Peru. Clin Infect Dis 2008;46:1822–8.
6. WHO Expert Committee. Control of Chagas disease. World Health Organ Tech Rep Ser 2002;905:i–vi. 1—109, back cover.
7. Teixeira AR, Nascimento RJ, Sturm NR. Evolution and pathology in Chagas disease—a review. Mem Inst Oswaldo Cruz 2006;101:463–91.
8. Coura JR. Chagas disease: what is known and what is needed—a background article. Mem Inst Oswaldo Cruz 2007;102(Suppl 1):113–22.
9. Maguire JH, Mott KE, Lehman JS, et al. Relationship of electrocardiographic abnormalities and seropositivity to *Trypanosoma cruzi* within a rural community in northeast Brazil. Am Heart J 1983;105:287–94.
10. Maguire JH, Hoff R, Sherlock I, et al. Cardiac morbidity and mortality due to Chagas' disease: prospective electrocardiographic study of a Brazilian community. Circulation 1987;75:1140–5.
11. Weinke T, Ueberreiter K, Alexander M. Cardiac morbidity due to Chagas' disease in a rural community in Bolivia. Epidemiol Infect 1988;101:655–60.
12. Pless M, Juranek D, Kozarsky P, Steurer F, Tapia G, Bermudez H. The epidemiology of Chagas' disease in a hyperendemic area of Cochabamba, Bolivia: a clinical study including electrocardiography, seroreactivity to *Trypanosoma cruzi*, xenodiagnosis, and domiciliary triatomine distribution. Am J Trop Med Hyg 1992;47:539–46.
13. Lázzari JO, Pereira M, Antunes CM, et al. Diagnostic electrocardiography in epidemiological studies of Chagas' disease: multicenter evaluation of a standardized method. Rev Panam Salud Publica 1988;4:317–30.
14. Le VV, Wheeler MT, Mandic S, et al. Addition of the electrocardiogram to the preparticipation examination of college athletes. Clin J Sport Med 2010;20:98–105.
15. Analisis De La Situacion de Salud Cochabamba 2005/2006. Cochabamba, Bolivia: Ministerio de Salud y Deportes, Prefectura del Departamento de Cochabamba, Servicio Departamentald de Salud; 2006.
16. Viotti RJ, Vigliano C, Laucella S, et al. Value of echocardiography for diagnosis and prognosis of chronic Chagas disease cardiomyopathy without heart failure. Heart 2004;90:655–60.
17. Riberio AL, Cavalvanti PS, Lombardi F, Nunes Mdo C, Barros MV, Rocha MO. Prognostic value of signal-averaged electrocardiogram in Chagas disease. J Cardiovasc Electrophysiol 2008;19:502–9.
18. Rassi A, Little WC, Xavier SS, et al. Development and validation of a risk score for predicting death in Chagas' heart disease. N Engl J Med 2006;355:799–808.
19. Nunes Mdo C, de Barbosa Mde M, Brum VA, Rocha MO. Morpho-functional characteristics of the right ventricle in Chagas' dilated cardiomyopathy. Int J Cardiol 2004;94:79–85.
20. Bestetti RB, Dalbo CM, Arruda CA, Correia Filho D, Freitas OC. Predictors of sudden cardiac death for patients with Chagas' disease: a hospital-derived cohort study. Cardiology 1996;87:481–7.
21. Nunes MC, Barbosa MM, Ribeiro AL, Colosimo EA, Rocha MO. Left atrial volume provides independent prognostic value in patients with Chagas cardiomyopathy. J Am Soc Echocardiogr 2009;22:82–8.
22. Bustamante JM, Rivarola HW, Fernández AR, et al. *Trypanosoma cruzi* reinfections in mice determine the severity of cardiac damage. Int J Parasitol 2002;32:889–96.
23. Andrade SG, Campos RF, Sobral KS, Magalhães JB, Guedes RS, Guerreiro ML. Reinfections with strains of *Trypanosoma cruzi*, of different biodemes as a factor of aggravation of myocarditis and myositis in mice. Rev Soc Bras Med Trop 2006;39:1–8.
24. Acquatella H, Catalioti F, Gomez-Mancebo JR, Davalos V, Villalobos L. Long-term control of Chagas disease in Venezuela: effects on serologic findings, electrocardiographic abnormalities, and clinical outcome. Circulation 1987;76:556–62.
25. Dutra WO, Gollob KJ. Current concepts in immunoregulation and pathology of human Chagas disease. Curr Opin Infect Dis 2008;21:287–92.
26. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simoes MV. Pathogenesis of chronic Chagas heart disease. Circulation 2007;115:1109–23.
27. Parada H, Carrasco H, Guerrero L, Molina C, Checos R, Martinez O. Clinical and paraclinical differences between chronic Chagas' cardiomyopathy and primary dilated cardiomyopathies [in Spanish]. Arq Bras Cardiol 1989;53:99–104.
28. Rossi MA. Microvascular changes as a cause of chronic cardiomyopathy in Chagas' disease. Am Heart J 1990;120:233–6.
29. Mengel JO, Rossi MA. Chronic chagasic myocarditis pathogenesis: dependence on autoimmune and microvascular factors. Am Heart J 1992;124:1052–7.
30. Coffey S, Cox B, Williams MJ. The prevalence, incidence, progression, and risks of aortic valve sclerosis: a systematic review and meta-analysis. J Am Coll Cardiol 2014;63:2852–61.
31. Ravisha MS, Tullu MS, Kamat JR. Rheumatic fever and rheumatic heart disease: clinical profile of 550 cases in India. Arch Med Res 2003;34:382–7.
32. Czarny MJ, Resar JR. Diagnosis and management of valvular aortic stenosis. Clin Med Insights Cardiol 2014;8(Suppl 1):15–24.
33. Lima-Costa MFF, Barreto SM, Guerra HL. Chagas' disease among older adults: branches or mainstream of the present burden of *Trypanosoma cruzi* infection? Int J Epidemiol 2002;31:688–9.
34. Lima-Costa MF, Peixoto SV, Ribeiro AL. Chagas disease and mortality in old age as an emerging issue: 10 year follow-up of the Bambuí population-based cohort study (Brazil). Int J Cardiol 2010;145:362–3.
35. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of Chagas disease in the United States: a systematic review. JAMA 2007;298:2171–81.