Original Article

ELISA seroprevalence of *Trypanosoma cruzi* in a cohort of heart disease patients

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Abstract

Introduction: Chagas disease is the most important parasitic disease in El Salvador and Latin America. The disease has two phases: acute and chronic, with an intermediate unspecified phase. The chronic phase develops in 30% of infected persons and its most common manifestation is cardiac disease. This study aimed to obtain reference data for the prevalence of *T. cruzi* seropositivity in a cohort of cardiac patients.

Methodology: A cross-sectional study involving consecutive heart disease patients consulting at the National Hospital Rosales was conducted over a six-month period. Congenital heart disease patients were excluded. A survey, file review, and ELISA serological test were conducted for each subject.

Results: Out of 455 subjects, 76 were seropositive for *T. cruzi*, giving a seropositivity prevalence of 16.7%. The average age of the patients was 58.09 years and the female:male ratio was 2.79:1.

No significant difference was found between *T. cruzi* seropositive and seronegative patients in terms of age and gender. No association between *T. cruzi* serological status and either area of residence or seeing vectors in the house was found. However, living in the Salvadoran western region during childhood was significantly associated with seropositivity ($p = 0.003$). Other factors associated with *T. cruzi* seropositivity included: clinical diagnosis of cardiac Chagas disease; electrocardiographic (ECG) findings of a permanent pacemaker, all atrioventricular (AV) blocks and right bundle branch block; and grade III-IV radiological cardiomegaly.

Conclusions: *T. cruzi* seropositivity prevalence was found to be 16.7% among heart disease patients in a public reference hospital.

Key words: Chagas disease; heart disease; prevalence; seropositivity; serology

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Introduction

Chagas disease, also known as "American trypanosomiasis", "poverty disease" and "promoter of poverty", is the fourth most important vector-transmitted parasitic disease in Latin America [1], accounting for 662,000 disability-adjusted life years (DALYs) per annum [2]. Since its identification in 1909, this disease had been confined to Latin America until it emerged in the United States and Europe in the last few decades due to the high immigration rate from endemic regions [3-5].

In 1956, 31 tripanosomiasis cases were serologically confirmed in El Salvador presuming an approximate Chagas cardiac disease (CCD) prevalence of 4.03% based on finite data [6]. Several subsequent Salvadoran studies [7] revealed higher prevalence rates of *Trypanosoma cruzi* (*T. cruzi*) infection in rural residents with different positivity rates obtained by xenodiagnosis (3.8% to 18.4%) and complement fixation tests (17.3% to 46.7%). Moreover, the acute phase of the disease was similarly observed in children and young adults, both *Triatoma dimidiate* and *Rhodniusprolixus* vectors were found in the country, and electrocardiographic (ECG) and thoracic radiological (X-ray) changes were less common and milder than those reported in South American countries [7]. In 1998, *T. cruzi* seroprevalence in El Salvador was estimated to be 14.7 per 100,000 among blood donors [8], then 3.37% among the Salvadoran population in 2005 [4]. Furthermore, the national serological screenings of *T. cruzi* were first conducted in the late 1990s, reporting an average index of 0.36%, 0.28%, and 2.1% among children younger than 7 years old, 7- to 14-year-old children, and individuals older than 14 years old, respectively [9].

Chagas disease is described as having two distinctive phases, the acute phase and the chronic phase [10], with an intermediate period known as the

indeterminate phase, when patients are asymptomatic but parasite carriers. It has been estimated that approximately 30% of infected persons evolve toward the chronic phase, which may include pathological involvement/damage to the heart, esophagus and/or colon [11]. Cardiac damage is the most severe and frequent outcome [10]. Chagas cardiac disease (CCD) is also the most common cause of heart disease in Latin America and, in endemic areas, it is the first cause of cardiovascular death in subjects between 30 and 50 years old [4].

Serological testing is considered the gold standard for chronic patient screening, and a sensitivity of 95% for the enzyme-linked immunosorbent assay (ELISA) has been reported [12-14]. For those cases when the serological test is inconclusive, promising tests with high sensitivity and reproducibility, such as trypomastigote excreted-secreted antigens(TESA) blot and PCR, are being investigated [13,14].

Through the last decade, efforts have been made in El Salvador to eradicate the vector, and studies have been conducted therefore to estimate the prevalence of *T. cruzi* seropositivity in the asymptomatic population, blood donors, and inhabitants of endemic areas [9], but not in the chronic Chagas patients. This study aimed to estimate the seroprevalence of *T. cruzi* in a cohort of cardiac disease patients in El Salvador.

Methodology

Study population

This cross-sectional study included 539 patients consulting in the cardiology ward at the National Hospital Rosales (NHR), the reference third-level hospital in the public health system of El Salvador. Enrolled patients were diagnosed with heart disease and arrhythmias by a cardiologist regardless of its etiology. They were either seeking a first or a subsequent consultation, or consulting during a previously planned and specified period of time. Patients previously diagnosed with Chagas cardiac disease (CCD) were also recruited. Congenital heart disease was the only exclusion criterion.

During a six-month period spanning from May to October 2010, an educational lecture on Chagas disease and its vector was delivered in the cardiology consultation waiting area by the recruiting personnel, after which all consecutive patients meeting the inclusion criteria were invited to participate in the study. Those patients who agreed to participate signed an informed consent form and filled out a survey. Samples of 5 cu mm of blood were drawn from the participating patients at the hospital laboratory, and their files were checked for clinical, ECG and thoracic X-ray data.

The study was approved by the Research Ethics Committee at the National Hospital Rosales, and a written informed consent was obtained from each participant prior to any data collection.

Serological testing

All samples were processed in batches of ten at the serology area of the hospital laboratory and tested using Chagatest ELISA recombinant v.3.0 third generation (Wiener Laboratories, Rosario, Argentina) by technicians trained in serological testing. The samples underwent six automated washes using Erba Lisa Wash (Erba Diagnostic, Manheim, Germany), then were read using Erba Lisa Scan II version 2.10 (Erba Diagnostic Manheim, Germany) with a primary 450 nm-filter and a secondary 630 nm-filter. The processor had been previously calibrated, verified, and programmed by specialized technicians and the laboratory professionals were trained to use both the washer and the processor. The readings, made independently by these two trained laboratory professionals, were assessed as either “positive” or “negative”.

Statistical analysis

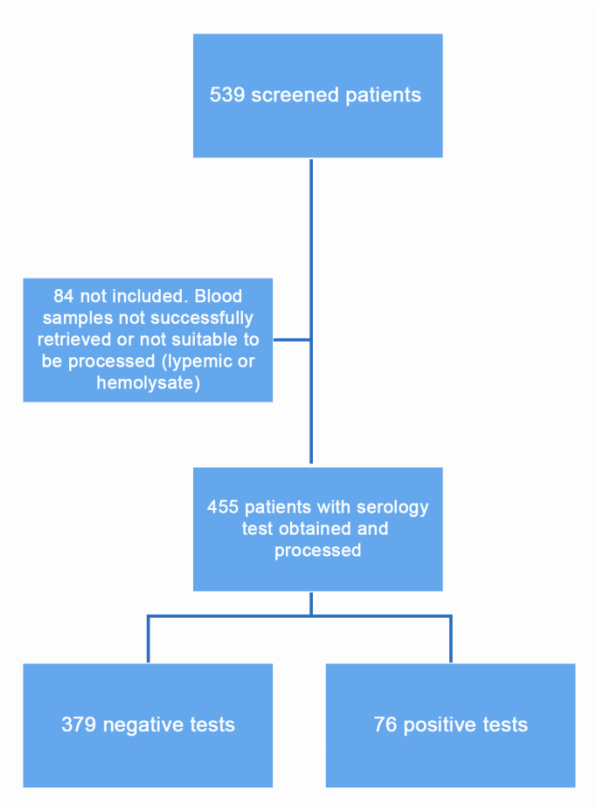
A sample size of 297 was calculated with 98% confidence intervals considering the approximately annual 5,000 consultations at the NHR cardiology ward and the last reported *T. cruzi* national seroprevalence of 3.3%. The authors decided to include 500 consecutive patients rather than random sampling.

Data were processed using IBM SPSS Statistics version 16.0 (IBM SPSS, Chicago, USA). Descriptive statistics were used, categorical and parametric variables were analyzed using chi square test, and means were compared using Student's t-test. Association was considered statistically significant when a p value < 0.05 was obtained. Missing data were withdrawn from the analysis.

Results

Only 455 patients were finally included in the study as 79 patients withdrew their consent prior to venipuncture or after first venipuncture failure, and 5 patients' sera were lipemic or hemolysed (Figure). Seventy-six of 455 patients showed positive serological results for *T. cruzi*, obtaining a prevalence of 16.7% (IC 95% 13.49- 20.34).

Figure. Cardiac disease patients enrolled in *T. cruzi* seroprevalence study in El Salvador



Socio-demographic characteristics

The mean age of the patients was 58.09 years (SD 16.65), with a female:male ratio of 2.79 to 1. No significant difference was found between *T. cruzi* seropositive and seronegative patients in terms of either age or gender [Table 1].

Geographical area and type of housing construction

Patients were asked to provide information about their residence area during childhood and youth and their current place of residence. Twelve subjects reported immigrating from other Central American countries. A significant association was found between *T. cruzi* seropositivity and residing in the western region of the country during childhood and youth ($p = 0.003$).Otherwise, area of residence was not statistically associated with *T. cruzi* serological status ($p = 0.150$) [Table 2]. A significant association was found between *T. cruzi* seronegativity and mixed housing construction using bricks and cement during childhood ($p = 0.002$) [Table 3].

Background of patients

The majority of the patients (328 of 455; 72.08%) remembered seeing vectors in their houses during their childhood, though no association was found between this fact and *T. cruzi* serological status ($p = 0.36$). Prior to our study, 37 patients (8.13%) had been serologically tested for *T. cruzi* infection, and 32 of them were diagnosed with Chagas disease by their cardiologists.

Previous diagnosis of Chagas cardiac disease and T. cruzi *antiparasite medication*

Our study identified 47 seropositive patients who had not been tested for Chagas cardiac disease before. In particular, 32 patients were previously diagnosed with Chagas cardiac disease (CCD) by their cardiologists, among whom 29 patients were *T. cruzi* seropositive in the present study and three patients were seronegative. Fifteen of 29 seropositive patients had been medicated with an antiparasite (Nifurtimox), while the three seronegative patients had been treated with either Nifurtimox for three years (2 patients) or Benznidazole for six months (1 patient) prior to this study.

Four patients not previously diagnosed with CCD had been treated with an antiparasite medication at some time in their lifetime. Our study revealed that two of these patients were seropositive and the other two were seronegative.

Clinical cardiological diagnosis

Table 4 illustrates the relationship between the established clinical diagnoses of our cohort and seropositivity. Grouping them into different diagnoses, we found an association between being seropositive and having Chagas cardiac disease, while being seronegative was associated with mixed heart disease (ischemic and hypertensive; $p = 0.0016$) and tachyarrhythmia ($p = 0.001$) [Table 4].

Electrocardiographic findings

Grouping electrocardiographic findings, we found an association between seropositivity and having a permanent implanted pacemaker, various types of atrioventricular (AV) block (with or without pacemakers), and right bundle branch block ($p = 0.004$) [Table 5].

Table 1. Sociodemographic characteristics and *T. cruzi* serological status of heart disease patients consulting at the National Hospital Rosales (NHR), El Salvador (n = 455)

Sociodemographic characteristics	**Number of seronegative patients**	**Number of seropositive patients**	**p value***
Sex Female Male Female:male ratio	 280 99 2.82:1	 55 21 2.61:1	0.336
Age (mean) SD†	58.42 16.99	56.41 14.85	0.785

* No significant difference was found between *T. cruzi* seropositive and seronegative patients in terms of either age or gender.
† SD = standard deviation

Table 2. Current and past residence areas and *T. cruzi* serological status of heart disease patients consulting at the National Hospital Rosales (NHR), El Salvador (n = 455)*

Residence area during childhood	**Number of seronegative patients (%)**	**Number of seropositive patients (%)**	**Total**
Western region†	32 (8.44)	16 (21.05)	48
Central region	199 (52.51)	42 (55.26)	241
Para central region	80 (21.11)	8 (10.53)	88
Eastern region	58 (15.3)	8 (10.53)	66
Other Central American countries	10 (2.64)	2 (2.63)	12
Total	379	76	455
Current residence area	**Number of seronegative patients (%)**	**Number of seropositive patients (%)**	**Total**
Western region	19 (5.01)	9 (11.84)	28
Central region	298 (78.63)	56 (73.68)	354
Para central region	40 (10.55)	7 (9.21)	47
Eastern region	14 (3.69)	3 (3.95)	17
Other Central American countries	8 (2.11)	1 (1.32)	9
Total	**379**	**76**	**455**

* Residence area was not statistically associated with *T. cruzi* serological status (p = 0.150)
† *T. cruzi* seropositivity was significantly associated with residing in the western region of the country during childhood and youth (p = 0.003)

Table 3. Type of housing constructions during childhood and youth and *T. cruzi* serological status of heart disease patients consulting at the National Hospital Rosales (NHR), El Salvador (n = 455)

Type of housing construction	**Number of seronegative patients (%)**	**Number of seropositive patients (%)**	**Total**
Adobe/Bahareque	265 (69.92)	54 (71.05)	319
Mixed construction (bricks and cement)*	72 (19)	5 (6.58)	77
Aluminium sheets	4 (1.05)	1 (1.32)	5
Mud/soil	12 (3.17)	10 (13.16)	22
Palm tree leaf	4 (1.05)	1 (1.32)	5
Wood	5 (1.32)	3 (3.95)	8
Missing data (patients don't remember)	17 (4.49)	2 (2.63)	19
Total	379	76	455

* *T. cruzi* seronegativity was significantly associated with mixed housing construction using bricks and cement during childhood (p = 0.002)

Table 4. Clinical diagnoses and *T. cruzi* serological status of heart disease patients consulting at the National Hospital Rosales (NHR), El Salvador (n = 455)*****

Clinical diagnosis	Number of seronegative patients (%)	Number of seropositive patients (%)	Total
Tachyarrithmia	49 (12.93)	1 (1.32)	50
Valvulopathy	30 (7.92)	3 (3.95)	33
Hypertensive cardiopathy	83 (21.9)	10 (13.16)	93
Mixed cardiopathy	111 (29.29)	11 (14.47)	122
Myocardiopathy	16 (4.22)	8 (10.53)	24
Chagas cardiac disease	3 (0.8)	29 (38.16)	32
Bradyarrythmia	22 (5.8)	8 (10.53)	30
Ischemic cardiopathy	48 (12.66)	3 (3.95)	51
Congestive cardiac failure	2 (0.53)	1 (1.32)	3
Complete atrioventricular (AV) block	5 (1.32)	1 (1.32)	6
Pre-excitement syndrome	1 (0.26)	0 (0)	1
Sinus disease	1 (0.26)	1 (1.32)	2
Others	4 (1.06)	0 (0)	4
Missing data	4 (1.06)	0 (0)	4
Total	379	76	455

***** *T. cruzi* seropositivity was significantly associated with Chagas cardiac disease; while seronegativity was significantly associated with mixed heart disease (ischemic and hypertensive; p = 0.0016) and tachyarrhythmia (p = 0.001).

Table 5. Electrocardiographic (ECG) findings and *T. cruzi* serological status of heart disease patients consulting at the National Hospital Rosales (NHR), El Salvador (n = 455)*****

ECG finding	Number of seronegative patients (%)	Number of seropositive patients (%)	Total
Normal	166 (43.8)	18 (23.68)	184
All arrhythmias except atrioventricular (AV) block	43 (11.35)	10 (13.16)	53
Permanent pacemaker	13 (3.43)	10 (13.16)	23
Complete atrioventricular (AV) block without pacemaker	2 (0.53)	1 (1.32)	3
Second degree AV block	4 (1.06)	1 (1.32)	5
Right bundle block	31 (8.18)	15 (19.74)	46
Left bundle block	35 (9.23)	8 (10.53)	43
Ischemic cardiopathy	21 (5.54)	5 (6.58)	26
Biventricular enlargement	3 (0.8)	0 (0)	3
Left ventricular enlargement	21 (5.54)	3 (3.95)	24
Right ventricular enlargement	1 (0.26)	0 (0)	1
Right auricular enlargement	1 (0.26)	0 (0)	1
No ECG reading	38 (10.03)	5 (6.58)	43
Total	379	76	455

***** *T. cruzi* seropositivity was significantly associated with having implanted a permanent pacemaker, all kinds of atrioventricular (AV) block (with or without pacemakers), and right bundle branch block (p = 0.004)

Thoracic X-rays

Grade III-IV radiological cardiomegaly was significantly associated with *T. cruzi* seropositivity (p = 0.02).

Discussion

The present cross-sectional study was conducted to determine ELISA seroprevalence of *T. cruzi* in a targeted unexplored group of patients with heart disease. The cardiology ward at the National Hospital Rosales (NHR), a third-level public reference hospital, is accessed by patients from all over the country, mostly from the lower income groups, from both urban and rural areas. Recently, many epidemiological studies have been conducted in El Salvador aiming at eradicating this disease, but none of them investigated the seroprevalence of *T. cruzi* in heart disease patients, mostly because it is not included in the heart disease diagnostic tests; hence the chronic infection prevalence has never being approached.

The present study found that ELISA seroprevalence of *T. cruzi* was 16.7% in a group of patients with heart disease. With the limitations of a cross-sectional study, we cannot argue any cause-effect relationship between the *T. cruzi* seropositivity and heart disease. Moreover, as we used only one serological test for screening, we never intended to diagnose Chagas cardiac disease. Rather, the authors infer that the prevalence found in this study could be somehow related to the fact that the NHR, as the public reference hospital, cares for the majority of the Salvadoran population, especially patients from the lowest income levels who live exposed to the vector of this disease.

The rate of *T. cruzi* seropositivity prevalence in our cohort of heart disease patients is relatively high in comparison to that of some other population groups, such as blood donors and asymptomatic inhabitants in highly infested vector areas [3,8,9]. This fact should also call attention to the possibility of a higher *T. cruzi* infection rate in the Salvadoran population in the last decades than the rates published previously [3,8,9].

Most of the respondents replied yes to the questions regarding exposure to factors associated with parasite infection, such as living in a rural area, living in a house built with natural materials such as soil, wood and palm tree leaves that are likely to attract vectors, and seeing vectors in their houses (72.08%); however, prevalence was not directly related to this fact. It is necessary to note that only 37 subjects (8.13%) had been previously screened for Chagas once during their lifetime as part of a public health policy, even though a large majority of our subjects were females, had been pregnant more than once, and therefore were considered a high-risk group due to the potential for vertical infection transmission.

Although all of El Salvador has been considered as endemic for Chagas disease, the subjects who reported living in the western region during their childhood were mainly associated with *T. cruzi* seropositivityin our study. With its predominant coconut palm flora and hot weather, this area of the country provides an ideal environment for the vector as previously reported [9]. The data about housing construction is also consistent with current knowledge about vectors infesting most houses not built with cement and bricks but with more natural materials such as soil, wood and palm tree leaves.

Our study group was comprised of patients with a wide range of clinical cardiology diagnoses. The two most common diagnoses associated with seropositivity were Chagas cardiac disease and heart disease requiring permanent implanted pacemakers. This finding was consistent with the association of seropositivity and ECG readings, such as complete AV blocks with or without pacemaker, and the right bundle branch block, an alleged pathognomonic characteristic of CCD.

We acknowledge that the study design has some limitations, such as utilizing only one type of serological testing, and retrieving data from patients' files. Enrolling patients regardless of their ECG and thoracic X-ray findings led to variable readings as well as missing values. In spite of these limitations, our findings are consistent with those of previous studies, particularly investigations illustrating the damage caused by Chagas cardiac disease to the electrical cardiac system, mainly the big bundles, such as the AV and the right bundle.

Conclusion

We believe that vector eradication is a good primary preventive measure against Chagas disease; however, efforts should also be made in research to assess the disease evolution, physiopathology, and treatment. Chronic patients must be recognized as a public health problem, not only for the impact Chagas disease has on mortality and the quality of life, but also because of its high cost and burden to the health system. Better established research protocols with sufficient funding should be conducted to measure chronic infection and its relation to cardiac damage.

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