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Major Article

Analysis of the seroprevalence of and factors associated with Chagas disease in an endemic area in northeastern Brazil

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Abstract

Introduction: Chagas disease (CD) is currently considered a neglected disease; hence, identifying the factors associated with its high prevalence is essential. This study aimed to identify the seroprevalence of and the possible factors associated with CD in inhabitants of the City of Limoeiro do Norte, northeastern Brazil. **Methods:** Between April and November 2013, blood collection was conducted and a semi-structured questionnaire was administered. Blood samples that showed positive or possible serology for anti-*Trypanosoma cruzi* antibodies based on indirect immunofluorescence, hemagglutination indirect, and an enzyme-linked immunosorbent assay were analyzed. Associations between CD positivity and the study variables were analyzed using prevalence ratios (PR) with 95% confidence intervals (CI). **Results:** A total of 812 individuals were analyzed, of which *T. cruzi* seropositivity was determined in 4.2% (34 individuals). Sociodemographic variables showing a significant association with *T. cruzi* positivity included age >50 years (PR = 27.6; 95% CI = 6.66-114.4), elementary level education (PR = 5.15; 95% CI = 1.83-14.47), and retirement (PR = 7.25; 95% CI = 3.72-14.14). Positivity for *T. cruzi* was 6.17 times higher in those who had a history of living in rammed earth houses compared with those who did not (95% CI = 2.19-17.37). There was no evidence of vertical transmission in the individuals studied. Among the individuals infected with *T. cruzi*, the majority reported having a comorbidity ($p < 0.01$). **Conclusions:** This study demonstrated the seroprevalence of CD and identified factors associated with a high prevalence of CD.

Keywords: Chagas disease. Seroepidemiological investigation. *Trypanosoma cruzi*. cross-sectional study.

INTRODUCTION

Chagas disease (CD) remains an important health problem in Latin America and is recognized by the World Health Organization as one of the 13 neglected tropical diseases[1]. There are 7-8 million infected people worldwide, with 75-90 million people at potential risk of infection and >10,000 deaths/year[2,3,4]. In Brazil, 2-3 million people are infected with Trypanosoma cruzi, which generates a high social and financial cost, as well as the need for continuous surveillance[5,6].

Historically, the results of at least two National Serological Investigations, which were performed from 1975-1980 and 2001-2008, have been published in the literature[7,8,9].

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In 1921, the presence of infected triatomine bugs was first described for the cities of Cariri and Quixadá[10]. However, the first cases of CD (also in Cariri) were not diagnosed until 1942[11,12]. Since the time of identification of these early cases until the official institution of the Campaign for Chagas Disease Control (CCDCh) in 1975, several studies were performed and suggested the requirement for the initially affected cities to implement regular control activities[13].

A serological study conducted in 1967 in the State of Ceará showed a prevalence of CD infection of 14.8%[13]. More recently, 16.7% of samples from the City of Limoeiro do Norte, which were analyzed using the complement fixation reaction, were seropositive for CD[13]. Seroepidemiological investigations conducted in Ceará in the 2000s showed that the CD prevalence in isolated cities ranged from 3.1% in Jaguaruana to 5.8% in Acopiara. Furthermore, triatomines were determined to be positive for the parasite *T. cruzi* in Limoeiro do Norte, at the same time[14,15,16,17,18].

In 2006, an outbreak of acute CD involving eight people in the City of Redenção and probable oral transmission was reported[19]. In the following decade, the prevalence decreased significantly across the state, reaching 0.02% according to an investigation of children from 2001-2008[9]. With a reduction in the prevalence of cases, the natural infection of triatomine, and the gradual decrease in the actions of the Control Program, the City **of** Limoeiro do Norte showed the highest rate of infection of captured triatomines (5%) among the cities served by the Control Program of Chagas Disease of the Regional Laboratory of Endemics of Limoeiro do Norte, Ceará from 2009-2011[18]. In a 2011 pilot study in Sapé, a region of the city, the prevalence of CD in humans was shown to be 2.6%[20].

Currently, there is the possibility of a gradual reduction in the entomological surveillance activities performed by the CCDCh due to the reallocation of agents to the dengue control program and before the initiation of active transmission of CD, as anticipated in recent reports. This study aimed to identify the seroprevalence of CD and the factors associated with CD prevalence in the City of Limoeiro do Norte, Northeastern Brazil.

METHODS

Ethical considerations

Following Resolution no. 466 of December 12, 2012, the research was submitted and approved by the Research Ethics Committee of the Federal University of Ceará (approval number: 237,128; CAAE: 13298513.5.0000.5054).

Design and study area

A cross-sectional study and seroepidemiological investigation was conducted in the City of Limoeiro do Norte, northeastern Brazil. To calculate the sample size, the CD prevalence was estimated to be 5%, considering an error of ±3.5% with a 95% confidence interval (CI). Following these parameters, and adding a possible loss of 10%, a minimum sample size of 799 people was estimated using StatCalc software 3.5.1.

Samples were collected from participants in health centers to ensure the health and safety of the patients. The seven health units with the largest number of enrollments were considered for inclusion in the study (Antônio Holanda de Oliveira, Luís Alves de Freitas, Santa Luzia, João XXIII, Cabeça Preta, Arraial, and Center).

Material collection

During visits by healthcare agents, individuals were informed about the study and were invited to participate. At the health centers, participants received information about the research and signed informed consent forms. For each participant, we collected 4.5mL of blood and administered a semi-structured questionnaire, which was applied between April and November 2013. The semi-structured questionnaire comprised 32 questions related to the sociodemographic characteristics of individuals, general data, and risk factors for CD such as age, sex, schooling, place of birth, place of origin, knowledge about the transmitter bug (triatomine), possession of animals, types and characteristics of housing, a history of blood transfusion or donation, and a family history of CD. Additionally, we investigated other data (e.g., doctor's appointments) related to the use of healthcare services and comorbidities reported by the participants.

Criteria for inclusion and exclusion

Individuals of both sexes (>2 years-of-age) who resided in the study area, signed the consent form, and permitted blood collection were included in the study. In the case of minors, their guardians signed consent forms and literate children signed assent forms. Individual were excluded if insufficient blood samples to perform the necessary examinations were obtained.

Biological sample

Blood samples were collected via venipuncture without anticoagulant in tubes containing separation gel. Blood samples were centrifuged at 2,500 rotations/minute for 8 minutes. After centrifugation, the supernatant (serum) was aspirated into a prelabeled 2-mL microtube, packed in a cooler with ice, and transported to the Laboratory for Research on Chagas Disease (LPDC) of the Department of Clinical and Toxicological Analysis of the Federal University of Ceará. The samples were stored in a freezer at -20°C until serological testing was completed.

Serological tests

For the serological analysis conducted at the LPDC, enzyme-linked immunosorbent assays (ELISAs) were performed. Briefly, the tests were conducted at a dilution of 1:25, according to the recommendation of the Wama diagnóstica® kit of ELISA for CD. Sera showing positivity or possible serology for anti-*T. cruzi* antibodies were forwarded to the Central Public Health Laboratory of Ceará, where they were analyzed using three different methods: indirect immunofluorescence (IIF), hemagglutination inhibition (HAI), and ELISA. Positive results in at least two of the three serological testing methods were considered positive[21].

Non-reactive sera were released as negative, based on Ordinance 2,712/2013, which redefines the technical regulation of hemotherapy procedures and allows blood units that are non-reactive for anti-*T. cruzi* antibodies in a high-sensitivity test, such as an ELISA, to be released.

Participants with seronegative results received the results of the laboratory tests in their residences during scheduled visits by healthcare agents. Individuals with positive results received their test results with proper information on the disease and were forwarded to a physician for evaluation (clinical, laboratorial, and epidemiological) at the Walter Cantídio University Hospital.

Statistical analysis

The analyses were performed using GraphPad Prism 5 and the Epi info software, version 3.5.4. Associations between *T. cruzi* positivity and the study variables were determined using Fisher's chi-squared or exact test and are shown as prevalence ratios (PR) with 95% CIs. $P<0.05$ was considered statistically significant.

RESULTS

Characterization of the study population

Blood samples were collected from 812 individuals at seven healthcare units (**Table 1**). The average age of the patients was 40.8 years, ranging from 2 to 86 years. Additional characteristics of the participants were as follows: 557 (68.6%) were female, 383 (47.2%) were aged <39 years, 759 (94.4%) had a family income of <2 times the minimum wage, 481 (59.3%) attended elementary school, and 527 (64.9%) were from Limoeiro do Norte. Among the registered occupations, agriculture was the most cited [203 (25%) individuals]. We interviewed 493 women (>15 years of age), of which 144 (29.2%) reported having had at least one abortion.

Regarding family history of CD, 243 (30%) respondents had a relative with a CD diagnosis. Among these participants, 27 (11.5%) responded that the mother had the disease. In relation to their previous residences, 402 (54%) participants resided in rammed earth houses. Regarding blood transfusions, 123 (15.1%) reported having made at least one donation and 59 (7.3%) claimed to have received blood at least once in their lives.

Most respondents reported the presence of insects and/or rodents in their residences [641 (79.3%) individuals] and were able to accurately identify the triatomine [547 (67.7%) individuals].

TABLE 1

Number of participants according to health unit.

Health unit	Participants	
	number	percentage
Santa Luzia	65	8.0
Antônio Holanda de Oliveira	67	8.2
João XXIII	116	14.3
Cabeça Preta	124	15.3
Luís Alves de Freitas	135	16.6
Center	151	18.6
Arraial	154	19.0
Total	812	100.0

Serological analysis of anti-*Trypanosoma cruzi* antibodies

The results of the serological tests showed that 33 individuals were seropositive for CD and one was of an indeterminate serology. The participant with indeterminate results was considered positive in this study, as she had a current positive IIF test result, as well as positive results from prior ELISA and IIF testing of a blood sample from a reference laboratory in 2012. Furthermore, she reported during a subsequent interview that she had already received etiological treatment. Therefore, the prevalence of CD was of 4.2% (34/812) in the studied area (**Table 2**).

In the study population, seropositive individuals were detected in all health units. Four (11.8%) infected individuals reported having made a previous blood donation, although no additional information was obtained in order to elucidate the destination of these donations, and whether or not they were rejected.

Analysis of factors associated with Chagas Disease

Most confirmed cases were female (73.5%), with an average age of 59.5 years (age range, 45-78 years). There was a significant difference associated with an age of ≥50 years compared with an age of <50 years ($p < 0.01$), while there was no significant difference between the sexes ($p = 0.53$) (**Table 3**).

Positivity for CD was 5.15 times higher among individuals who had a partial elementary school level education compared with those who had completed elementary school (95% CI = 1.83-14.47; $p < 0.01$). Despite the prevalence of infection being higher among individuals from Limoeiro do Norte compared with individuals from other cities of Ceará and other states, this difference was not significant (PR = 1.13; 95% CI = 0.56-2.29; $p = 0.73$). Conversely, the prevalence of positive serology among retirees was 7.2 times higher compared with other professions, which was significant (95% CI = 3.72-14.14; $p < 0.01$) (**Table 3**).

We analyzed the results of the findings from 493 women (>15 years-of-age); positivity for CD was 2.63 times higher for women who had undergone an abortion compared with those who had not (95% CI = 1.23-5.62; $p = 0.01$).

The prevalence of seropositivity among individuals who had resided in rammed-earth houses in the past was 6.17 times higher compared with those who had never lived in this type of housing (95% CI = 2.19-17.37; $p < 0.01$; **Table 4**). No significant difference in infection was observed with regard to the current housing conditions of the participants. Moreover, no association was found with respect to the type of house ($p = 0.58$), having electric power ($p = 0.08$), having running water ($p = 0.52$), or the number of bathrooms in the residences ($p = 0.21$).

Knowledge of the triatomine was not significantly associated with CD, although it is important to note that the study participants who were aged >30 years were 83% more likely to identify the triatomine, as compared with those aged <30 years (95% CI = 1.58-2.12; $p < 0.01$).

In this study, positivity for CD was 4.61 times higher among those who visited a physician >2 times/year (95% CI = 1.79-11.87; $p < 0.01$); 2.16 times higher among those who underwent testing with some frequency (95% CI = 1.08-4.33; $p = 0.03$); 4.7 times higher among those who had a comorbidity (95% CI = 2.07-10.67; $p < 0.01$); and 3.92 times higher among those who reported having hypertension (95% CI = 2.04-7.53; $p < 0.01$) (**Table 5**).

DISCUSSION

In this study, the prevalence of CD in Limoeiro do Norte was 4.2%, which was higher than the latest indexes reported for Northeastern Brazil. A fact that contributes to this finding is the higher sensitivity of the diagnostic tests used in this study compared with those used previously, such as in the national investigation of 1975-1980[7,22].

TABLE 2

Sociodemographic and epidemiological aspects of positive cases for Chagas disease in Limoeiro do Norte, Ceará, Brazil.

	Serological confirmation			Sociodemographic data				Epidemiological aspects		
Id	**ELISA**	**IIF**	**HAI**	**Sex**	**Age**	**School**	**Income**	**Fam hist CD**	**Resided REH**	**Presence of insects**
1	RS	RS	NRS	F	57	CHS	Up to 2 MW	No	NI	Yes
2	RS	RS	RS	F	78	I	Up to 2 MW	No	Yes	Yes
3	RS	RS	RS	F	58	IES	Up to 2 MW	No	Yes	No
4	RS	IND	RS	F	45	IES	< 1 MW	Yes	Yes	Yes
5	RS	RS	NRS	F	63	IES	Up to 2 MW	NI	Yes	No
6	RS	RS	RS	F	54	IES	Up to 2 MW	Yes	Yes	Yes
7	RS	RS	RS	M	53	IES	Up to 2 MW	Yes	Yes	Yes
8	RS	RS	---	F	55	IES	Up to 2 MW	No	Yes	Yes
9	RS	---	RS	M	71	IES	Up to 2 MW	No	Yes	No
10	RS	RS	---	F	55	I	Up to 2 MW	Yes	Yes	Yes
11	RS	---	RS	F	63	IES	Up to 2 MW	Yes	No	Yes
12	RS	---	RS	F	66	I	Up to 2 MW	No	Yes	Yes
13	RS	RS	RS	M	68	IES	Up to 2 MW	Yes	No	Yes
14	RS	RS	---	F	66	IES	Up to 2 MW	Yes	Yes	Yes
15	RS	RS	---	M	54	IES	Up to 2 MW	No	Yes	Yes
16	RS	RS	---	M	59	IES	Up to 2 MW	No	Yes	Yes
17	RS	---	RS	M	58	I	Up to 2 MW	No	Yes	Yes
18	RS	RS	---	M	68	I	Up to 2 MW	Yes	Yes	Yes
19	RS	RS	---	F	60	I	Up to 2 MW	Yes	Yes	Yes
20	RS	RS	---	F	60	IES	Up to 2 MW	No	Yes	No
21	RS	RS	---	F	50	IES	Up to 2 MW	No	Yes	Yes
22	RS	RS	---	F	69	IES	Up to 2 MW	No	Yes	Yes
23	RS	---	RS	F	58	IES	Up to 2 MW	No	Yes	No
24	RS	---	RS	F	52	IES	Up to 2 MW	Yes	Yes	No
25	RS	---	RS	F	61	IES	Up to 2 MW	No	Yes	Yes
26	RS	RS	RS	F	68	IES	Up to 2 MW	No	Yes	No
27	RS	RS	---	F	61	IES	Up to 2 MW	No	No	No
28	RS	RS	---	M	53	CES	Up to 2 MW	No	Yes	Yes
29	RS	RS	---	F	64	IES	Up to 2 MW	Yes	Yes	Yes
30	RS	RS	---	M	60	IES	Up to 2 MW	No	Yes	No
31	IND	IND	IND	F	61	CHS	Up to 2 MW	Yes	Yes	Yes
32	RS	RS	---	F	62	IES	Up to 2 MW	No	No	Yes
33	RS	RS	---	F	61	IES	Up to 2 MW	No	Yes	Yes
34	RS	RS	---	F	47	IHS	Up to 2 MW	No	Yes	Yes

Id: identification; **ELISA**: enzyme-linked immunosorbent assay; **IIF**: indirect immunofluorescence; **HAI:** hemagglutination inhibition assay; **Fam hist CD**: family history of Chagas disease; **Resided REH:** resided in rammed earth house; **RS**: reagent serum; **NRS**: non-reagent serum; **CHS**: complete high school; **MW**: minimum wage;**- No: not; NI**: non-informed; **F**: female; **M:** male; **I**: illiterate; **IES:** some elementary school; **---:** test not performed; School – education level; Insect presence – reports the presence of insects; **IND:** indeterminate; **CES**: complete elementary school; **IHS:** incomplete high school.

TABLE 3

Sociodemographic characteristics of participants.

	Positive		Negative				
Variable	**n**	**%**	**n**	**%**	**PR**	**P-value**	**95% CI**
Sex							
female	25	4.5	532	95.5	1.27	0.53	0.60−2.68
male	9	3.5	246	96.5			
Age (years)							
≥ 50	32	10.7	266	89.3	27.6	< 0.01	6.66−114.40
< 50	2	0.4	512	99.6			
Income							
< 1 minimum wage	1	0.9	104	99.1	0.20	0.11	0.03−1.46
≥ 1 minimum wage	33	4.7	45	95.3			
Schooling							
until IES	30	6.2	451	93.8	5.15	< 0.01	1.83−14.47
from CES	4	1.2	326	98.8			
Place of birth							
Limoeiro do Norte	23	4.4	504	95.6	1.13	0.73	0.56−2.29
other cities	11	3.9	274	96.1			
Occupation							
farmer	7	3.4	197	96.6	0.74	0.46	0.33−1.67
other	27	4.6	554	95.4			
retired	21	14.7	122	85.3	7.25	< 0.01	3.72−14.14
other	13	2.0	629	98.0			
housewife	3	5.4	539	94.6	0.13	< 0.01	0.04−0.42
other	31	4.3	698	95.7			

IES: some elementary school; **CES:** complete elementary school; **PR:** prevalence ratio; **95% CI:** 95% confidence interval.

TABLE 4

Analysis of biological risk factors for CD in participants.

	Positive		Negative				
Variable	**n**	**%**	**n**	**%**	**PR**	**P-value**	**95% CI**
Family history of CD							
yes	12	4.9	231	95.1	1.33	0.42	0.67−2.67
no	21	3.7	546	96.3			
Resided in rammed earth houses previously							
yes	29	7.2	373	92.8	6.17	< 0.01	2.19 −17.37
no	4	1.2	338	98.8			
Knowledge of the triatomine							
yes	28	5.1	519	94.9	2.23	0.06	0.93−5.31
no	6	2.3	255	97.7			
Presence of insects and/or rodents at home							
yes	25	3.9	616	96.1	1.13	0.10	0.53−2.40
no	9	3.4	252	96.6			
Donated blood previously							
yes	4	3.3	119	96.7	0.75	0.81	0.27−2.08
no	30	4.4	659	95.6			
Received a blood donation previously							
yes	2	3.4	57	96.6	0.80	1.00	0.19−3.24
no	32	4.3	720	95.7			
Has pets in intradomicile and/or peridomicile habitats							
yes	23	3.8	576	96.2	0.74	0.41	0.37−1.50
no	11	5.2	202	94.8			
Nurtures dogs							
yes	13	3.7	343	96.3	0.79	0.50	0.40−1.56
no	21	4.6	435	95.4			

CD: Chagas disease; **PR**: prevalence ratio; **95% CI:** 95% confidence interval.

TABLE 5

Statistical analysis of the use of healthcare services and comorbidities in participants.

	Positive		Negative				
Variable	**n**	**%**	**n**	**%**	**PR**	**P-value**	**95% CI**
Visits to the physician per year							
> 2 times	26	6.9	352	93.1	4.61	< 0.01	1.79−11.87
≤ 2 times	5	1.5	330	98.5			
Undergoes medical examinations frequently							
yes	21	6.5	302	93.5	2.16	0.03	1.08−4.33
no	12	3.0	387	97.0			
Reports a health problem							
yes	27	7.4	339	92.6	4.70	< 0.01	2.07−10.67
no	7	1.6	439	98.4			
Reports hypertension							
yes	18 9.9		163	90.1	3.92	< 0.01	2.04−7.53
no	16	2.5	615	97.5			
Reports gastrointestinal problems							
yes	2	3.4	57	6.6	0.80	1.00	0.19−3.25
no	32	4.2	721	5.8			
Reports diabetes							
yes	1	1.9	52	98.1	0.43	0.72	0.06−3.11
no	33	4.3	726	95.7			

PR: prevalence ratio; **95% CI**: 95% confidence interval.

Limoeiro do Norte has reported CD cases for many decades, with an estimated prevalence of 16.7% in the year of 1967, as well as the presence of several species of triatomine (predominance of *T. brasiliensis* and *T. pseudomacula)*[13,18,21]. The decline in the prevalence of infection by *T. cruzi* in Limoeiro do Norte from 16.7% in 1959 to 4.6% in 1970-1977 and to 4.2% in 2013 can be explained, among other factors, by vector control measures and greater awareness among the local population[13]. Even with this significant reduction, the high (4.2%) CD prevalence in this study compared with other Brazilian studies can be explained by the higher sensitivity and specificity of recently used methods[14,23,24,25,26].

In this study, the infection rate was higher (27 times) in the population aged ≥50 years. This result is consistent with those found in other regions, suggesting that CD is residual with these individuals who likely acquired the disease decades previously. Presently, more effective control of vector-borne transmission has been implemented in these regions[14,17,24,25,26].

CD positivity was 5 times higher among those who had only partially attended elementary school compared with those who completed elementary school. This result was also observed in previous studies, regardless of the region of the country[23,27,28,29], indicating that a higher education level is associated with a lower CD prevalence.

The CD prevalence was 2.63 times higher in the group of women aged >15 years who had a history of abortion compared with those who did not. Care must be taken in interpreting these data, since we did not ask whether the abortion was spontaneous or induced and, although CD can lead to abortion, the etiology can be multifactorial[30]. However, it is important to include this variable in future prospective studies.

An important association was observed between those who had a history of residing in a rammed-earth house and positivity for CD; the CD prevalence was 6.17 times higher in those who had lived in rammed-earth housing compared with those who had not. This higher seroprevalence was not affected by currently dwelling in a house of masonry, but was related to having previously lived in rammed-earth houses. It is likely that the residents of these houses had been infected in previous homes with conditions suited for vector transmission. There is strong evidence suggesting that masonry houses offer greater protection against vector-borne transmission[31,32].

In this study, older participants reported better knowledge of triatomines than younger participants, and this knowledge was significantly related to age. This same finding was verified in the City of Botucatu, State of São Paulo in the past decade[29]. This finding was expected, as the current generation typically considers CD as a major public health problem of the past as a result of the government programs for vector control.

The high prevalence of hypertension among infected patients is likely not associated with CD, although hypertension was the most common cardiovascular disease among the chagasic population in other regions[16,33,34,35]. There is a consensus that CD and its association with other chronic diseases can increase morbidity and mortality and worsen the quality of life of those with hypertension[34]. This fact should cause concern and reorient

the prevention and monitoring activities for those aged >50 years of age[36]. Information about comorbidities were collected via interviews, which is an important limitation of this study. Issues related to providing information to a population with a limited education and the lack of clinical and laboratory exams to confirm the reported comorbidities were also limitations of this study.

The magnitude and dispersal of vector-borne diseases, including dengue fever and, more recently, Zika virus, which result in the allocation of the majority of investments to vector-borne disease control programs, further weakens the few remaining triatomine control programs. Efforts to educate local populations in the identification of triatomines and to notify health authorities must be expanded and strengthened in order to maintain previous achievements in controlling this disease. Currently, in most regions of Brazil, chronic cases of CD caused by infections acquired in the past prevail, with an increase in the occurrence of acute CD observed in the states of the Legal Amazon. Changing this epidemiological framework of CD in Brazil should promote important discussions about future actions and strategies concerning the surveillance, prevention, and control of vectors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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