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ORIGINAL ARTICLE

Assessing the entomo-epidemiological situation of Chagas disease in rural communities in the state of Piauí, Brazilian semi-arid region

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Background: In northeastern Brazil, the wild nature of *Trypanosoma cruzi* vectors has challenged control actions. This study aims to describe the entomological and epidemiological scenario of Chagas disease in rural communities in the state of Piauí.

Methods: A cross-sectional study (n=683 individuals/244 dwellings) was carried out to obtain serum samples, sociodemographic data and intra- and peridomestic triatomines.

Results: The overall seroprevalence rate was 8.1%, with no positive tests among subjects <30 y of age. Prevalence rates reached 34.3% and 39.1% among subjects 61–75 and >75 y of age, respectively; 1474 triatomines were collected, of which 90.3% were found in peridomiciliary structures and 9.7% inside houses; 87.2% were classified as *Triatoma brasiliensis*. *T. cruzi* infection rates in insects were 0.5% by light microscopy and 0.9% by culture in NNN/LIT medium. Five cultivated isolates were submitted to molecular genotyping, three of which were identified as *T. cruzi* I and two as *T. cruzi* II.

Conclusions: Although no vector transmission currently occurs, prevalence rates are high in adults and the elderly. This disease should be targeted by primary healthcare providers. Insect surveillance and control activities should not be discontinued in an environment favourable to the perpetuation of house colonization by triatomines.

Keywords: Brazil, Chagas disease, *Triatoma*, *Trypanosoma cruzi*

Introduction

Chagas disease is caused by the flagellated protozoan *Trypanosoma cruzi*, which is transmitted in enzootic cycles involving mammals and haematophagous insects called triatomines (kissing bugs [order Hemiptera, family Reduviidae]).[1] When brought into contact with natural *T. cruzi* transmission cycles in a process that involves the domiciliation of triatomines, humans can be infected when they are used as a food source by the insect vector.[2]

The disease has an often unrecognized—but potentially severe—acute febrile initial phase, progressing chronically over decades to cardiac and/or digestive clinical forms characterized by cardiomyopathy and oesophageal or colonic motility dysfunction.[3] In Brazil and other endemic Latin American countries, Chagas disease is a frequent cause of heart failure and cardiac conduction disorders, requiring complex care.[4] In addition, stroke is a frequent complication of Chagas disease in asymptomatic and oligosymptomatic patients.[5]

However, most cases (almost 70%) have a benign evolution and are clinically classified as indeterminate, without apparent impairment of myocardial function, but requiring periodic electrocardiographic and echocardiographic follow-ups.[6] These individuals can transmit *T. cruzi* infection through blood donation if routine serology testing of blood banks is not performed.[7] Women with Chagas disease in the indeterminate and chronic phases can transmit the infection congenitally to their children, occurring in 5–10% of births from seropositive mothers.[8]

Stable and continuous transmission of *T. cruzi* to humans generates an endemic epidemiological pattern in Latin America, occurring in specific socio-environmental and demographic scenarios that permit the colonization of human domiciles by triatomines.[9,10] For centuries, human settlements characterized by economic poverty and inadequate housing in vast rural areas in Brazil have made Chagas disease hyperendemic in many regions. The first country-based serological survey, carried out in the 1970s and 1980s, revealed that Piauí, Sergipe, Bahia, Minas Gerais, Rio Grande do Sul and Goiás were the five states with the highest prevalence rates, often >5%.[11]

In Brazil and other South American countries, Chagas disease has been targeted by effective control campaigns based on the spraying of pyrethroid insecticides on the walls of houses in rural communities. These actions, which in Brazil were coordinated by the former federal agency Superintendência de Campanhas de Saúde Pública (SUCAM), succeeded in virtually stopping vector transmission in vast areas,[12] and associated with the growing urbanization of the Brazilian population, improvements in the quality of rural houses and the mitigation of rural poverty, have substantially reduced the incidence of Chagas disease.[13] In addition, obligatory serological screening of blood donated from the late 1980s onwards has interrupted blood-borne transmission of *T. cruzi*.[14]

Given its chronic nature, developing insidiously over decades, the morbidity and mortality associated with Chagas disease is still high in Brazil. However, this likely reflects past transmission conditions, where contemporary cases of Chagas disease in adults and the elderly were contracted before the effective control and virtual eradication of vector transmission cycles in Brazil.[15]

Despite the control of transmission in many endemic countries, two new epidemiological scenarios have emerged in the last 2 decades. The first is the growing recognition of the acute form of the disease in outbreaks caused by food contamination with infected sylvatic insects and/or their faeces, especially in the Amazon, but also in Brazilian states outside the Amazonian region.[16,17] The second is the emergence of the disease in developed countries, associated with the immigration of Latin Americans, occurring by donation of blood and solid organs.[18,19]

In northeastern Brazil, the wild nature of *T. cruzi* vectors has challenged control actions. In this region, the species *Triatoma brasiliensis* complex, *Triatoma pseudomaculata* and *Rhodnius nasutus* are the main vectors of *T. cruzi*. They naturally inhabit crevices of rocky outcrops, the space under the peeling bark of Caatinga trees and the crown of palms, respectively. These species are able to recolonize the domestic environment after insecticide spraying, bringing back the risk of *T. cruzi* transmission to domestic animals and eventually humans.[20–22] This requires continuous entomological surveillance.

In 2016 we verified frequent colonization of the interior of houses by *T. brasiliensis macromelasoma* in rural communities in the state of Piauí, with evidence that they were using humans as a blood source.[23] This finding pointed to the possibility of vector transmission of Chagas disease re-emerging in these locations, motivating the present investigation. Therefore the aim of this study was to characterize the epidemiological situation of Chagas disease in known endemic regions in which both triatomine entomological surveillance had been discontinued in the state of Piauí and no serological surveys had been performed in the last 15 y. We intended to integrate seroprevalence data with entomological indicators and to characterize domestic transmission cycles through isolation of *T. cruzi* in insects collected in homes.

Population and methods

Description of the study area

The study was conducted in the municipality of São João do Piauí (latitude: 8°21′39″ South; longitude: 42°15′4″ West; altitude 228 m) in the southeastern region of Piauí state (northeastern Brazil). São João do Piauí is located in the Brazilian semi-arid region, which contains 1262 municipalities in the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and Minas Gerais, with a total population of 28 million. São João do Piauí has 19 548 inhabitants, with 13 470 in the urban area and 6078 distributed around 85 rural communities (see map in Figure 1). The climate is tropical, semi-arid and hot, with 656 mm of precipitation per year, concentrated in the months December–March, and temperatures ranging between 25.6 and 28.9°C. The municipality is in the Caatinga biome, characterized by shrubby xerophytic vegetation. The topographical relief is marked by elevated plains with large plateaus and slopes ranging from 150 to 300 m in altitude. There are also steep valley slopes and elevations with plenty of rocky outcrops.

Study design

This study aimed to assess whether there are active cycles of vector transmission of Chagas disease in the included communities. For this, serological and entomological data were generated through three approaches: a cross-sectional serological survey, the characterization of insect vectors and the calculation of entomological indicators, including the rates of natural *T. cruzi* infection of triatomines and the molecular characterization of parasites.

Sampling, recruitment and statistical analysis of the seroprevalence survey

A cross-sectional study was performed in order to obtain blood samples from residents (n=683) of 12 rural communities. The communities included were Bonsucesso, Chiqueirinho, Eugênio, Grajau, Jacaré, Lagoa da Serra, Poço do Rego and São José in the so-called upper part and Curtume, Junco, Saco and Riacho in the lower part of the municipality (Figure 1). The recruitment strategy involved visiting all houses and aimed to include all residents of the communities studied, which were randomly selected in the area of the municipality. Residents who were not at home (n=150) or those who refused blood collection (n=240) were lost. In the end, the sample included 11.2% of the rural population of São João do Piauí. During the visits, sociodemographic data were

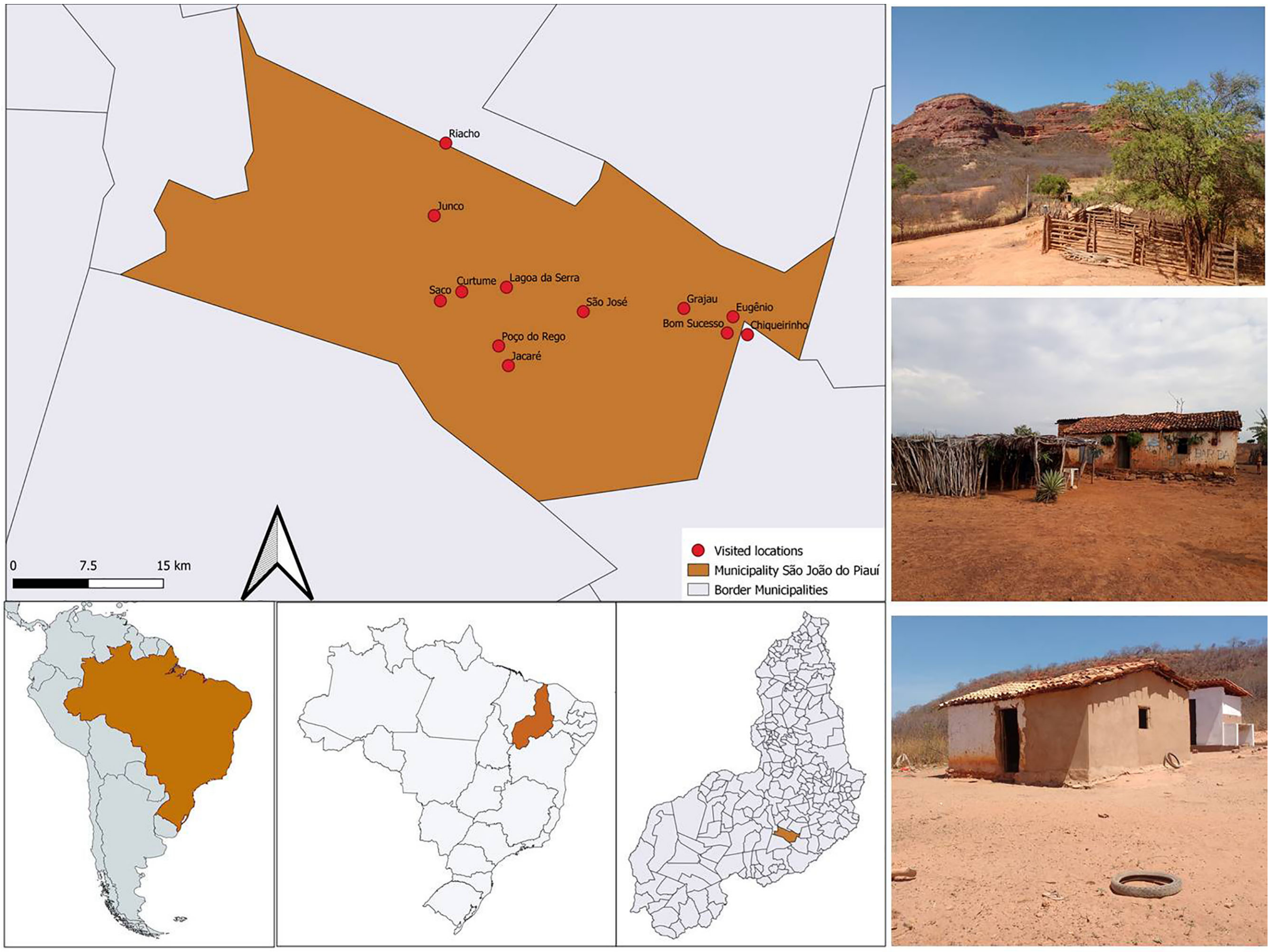

Figure 1. Map illustrating the location of the rural communities studied in the years 2018 and 2019 in the municipality of São João do Piauí, Piauí state and photographs illustrating the local landscape.

obtained and triatomines captured in the home and peridomestic environment (see below). Prevalence ratios and 95% confidence intervals (CIs) were calculated. Associations were considered statistically significant for $p<0.05$ using the Fisher's exact test. In this analysis, the dependent variable was seropositivity for Chagas disease and the independent variables were age group, gender, locality and occupation. Analysis was performed with Epi Info 2000 (Centers for Disease Control, Atlanta, GA, USA).

Serological procedures

Blood samples (5 mL) were collected from residents by vacuum venipuncture. The serum obtained was transported to the Central Public Health Laboratory of Piauí (LACEN-PI) and submitted to two different serological techniques for detection of anti-*T. cruzi* antibodies. For the enzyme-linked immunosorbent assay (ELISA) technique, the Chagatest ELISA recombinante v.3.0 kit (Wiener Lab, Rosario, SF, Argentina) was used and for the indirect immunofluorescence (IIF) technique the IFI Chagas BioManguinhos kit (BioManguinhos, Rio de Janeiro, RJ, Brazil) was used. The subjects who were reactive in both techniques were considered positive for Chagas disease. All residents received test results and were clinically evaluated and received treatment guidelines.

Entomological survey

The domestic environment was searched for triatomines. Searches were carried out with the aid of lanterns inside and around the houses, which included chicken coops, piles of tiles and bricks and corrals of animals, including pigs, goats and sheep. Insects were transported alive to the Diptera and Hemiptera Entomological Surveillance Laboratory at Fiocruz, in Rio de Janeiro, to be classified by species, sex and developmental stage. The following entomological indices were calculated: intradomicile infestation rate (houses with triatomines inside/total households assessed $\times$ 100), peridomestic infestation rate (houses with triatomines in the peridomestic environment/total households assessed $\times$ 100) and rate of natural infection by *T. cruzi* (number of positive triatomines/total triatomines examined $\times$ 100).

Table 1. Prevalence and distribution of *T. cruzi* seropositivity in rural communities in São João do Piauí, northeastern Brazil, 2018–2019, as assessed through ELISA and IIF

	Positivity rate[a], n/N (%)	Prevalence ratio (95% CI)	p-Value
Age group (years)			
0–15	0/114	–	
16–30	0/193	–	
31–45	9/165 (5.5)	0.49 (0.22 to 1.12)	0.114
46–60	13/118 (11)	1	
60–75	24/70 (34.3)	3.11 (1.69 to 5.70)	<0.001
>75	9/23 (39.1)	5.19 (1.87 to 14.34)	0.002
Profession			
Farmer	30/287 (10.5)	2.88 (1.35 to 6.17)	0.003
Other	8/213 (3.8)	1	
Retired	17/40 (30.9)	1131 (524 to 2442)	<0.001
Community			
Bom Sucesso	1/30 (3.3)	0.42 (0.05 to 3.49)	0.660
Chiqueirinho	5/39 (12.8)	1.64 (0.50 to 5.30)	0.497
Curtume	2/65 (3.1)	0.39 (0.07 to 1.95)	0.273
Eugênio	13/61 (21.3)	2.72 (1.03 to 7.19)	0.041
Grajau	2/67 (3)	0.38 (0.07 to 1.89)	0.266
Jacaré	1/22 (4.5)	0.56 (0.06 to 5.09)	1.000
Junco	4/126 (3.2)	0.40 (0.11 to 1.46)	0.167
Lagoa da Serra	3/50 (6)	0.76 (0.19 to 3.06)	1.000
Poço do Rego	4/18 (22.2)	2.84 (0.85 to 9.50)	0.101
Riacho	11/68 (16.2)	2.07 (0.76 to 5.62)	0.184
Saco	5/64 (7.8)	1	
São José	4/73 (5.5)	0.70 (0.19 to 2.50)	0.733
Gender			
Male	25/325 (7.7)	1	
Female	30/358 (8.4)	0.99 (0.94 to 1.03)	0.425

[a]Subjects were considered positive when they were reactive in both ELISA and IIF techniques.

Identification and molecular characterization of *T. cruzi* in insects

The captured insects were dissected to obtain their intestinal contents, which was used to prepare faecal suspensions diluted in saline and examined by light microscopy. Intestinal contents were also seeded in NNN/LIT culture medium for trypanosomal growth. Cultures were followed for 30–40 d to assess the presence of parasites. DNA from isolated parasites was purified using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) with modifications. Briefly, at the elution step, 100 μL AE buffer was added to the silica membrane column and maintained at room temperature for 10 min before DNA elution, as described by Moreira et al.[24] DNA was stored at −20°C until use and the purity and concentration were determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260/280 nm and 260/230 nm. The isolated parasites were submitted to a genotyping protocol to determine the discrete typing unit with multilocus conventional polymerase chain reaction, following a combination of methodologies previously described.[25–27]

Results

Prevalence, distribution and factors associated with Chagas disease in the studied communities

The overall positivity rate of the 12 rural communities surveyed was 55/683 (8.1%), as presented in Table 1. Three results were inconclusive (positive in ELISA and negative in IIF) and were classified as negative. Among the research subjects, 74 (10.8%) had already undergone serological tests for Chagas disease. Among these, 19 had received positive results in the past and 36 did not know their seropositive status for Chagas disease. The communities of Eugênio (13/61 [21.3%]) and Poço do Rego (4/18 [22.2%]) presented the highest positivity rates. There were no positive examinations among the 307 subjects <30 y of age. Chagas disease prevalence rates were significantly higher in older groups, reaching 24/70 (34.3%) and 9/23 (39.1%) among subjects ages 61–75 and >75 y, respectively. The rates were similar between males and females (7.7% and 8.4%, respectively). Positivity rates in males and females in each age group were not significantly different, as presented in Figure 2. Considering women of

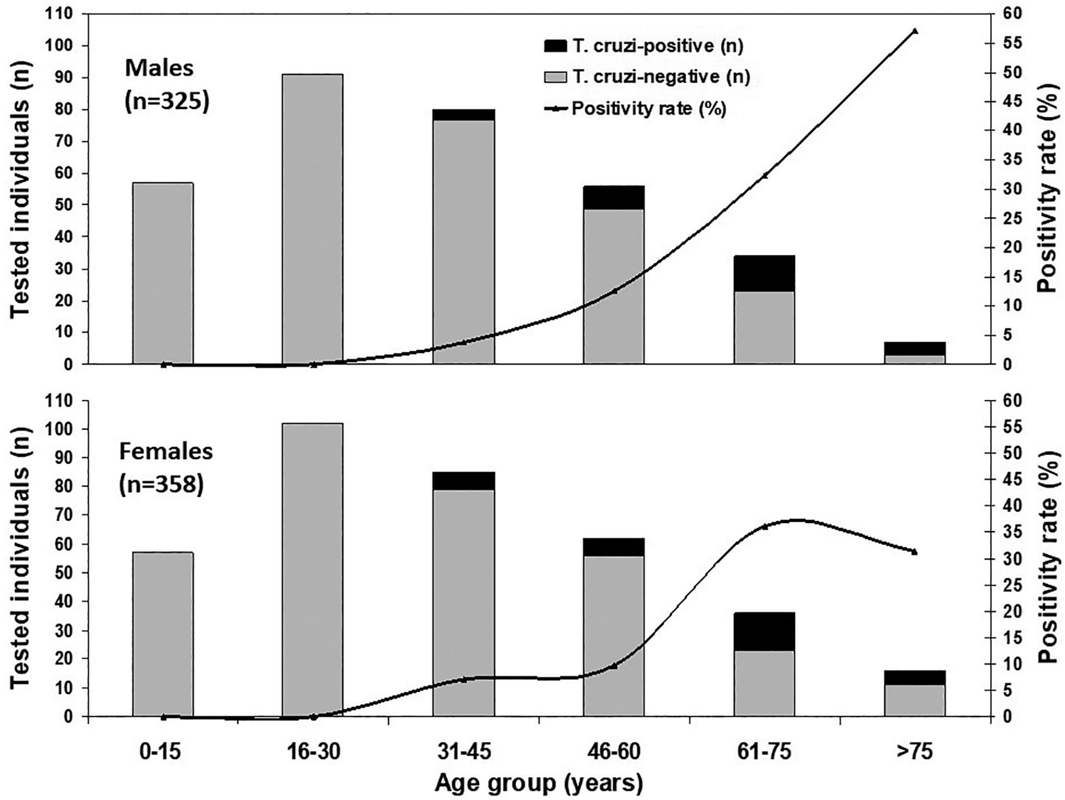

Figure 2. Chagas disease seroprevalence rates by age and gender in São João do Piauí, Piauí state, 2018–2019, as assessed through ELISA and IIF.

childbearing age, seroprevalence in the 15–30 and 31–45 y age groups was 0 and 7.1%, respectively.

Among people >18 y of age, seropositivity rates were significantly higher among farmers (30/287 [10.5%]) than among people with other vocations (8/213 [3.8%]). Among retirees, 17/40 (30.9%) were positive. Among the 244 households investigated, 50 (20.5%) had at least one positive resident (results not shown). Regarding the presence of insects that transmit Chagas disease, the houses where there was colonization by triatomines more often had at least one positive resident (24/73 [32.9%] vs 26/171 [15.2%]; p=0.002). In addition, homes with the presence of triatomine faeces on the walls also had a significantly higher proportion of seropositivity (at least one resident) (26/99 [26.3%] vs 24/145 [16.6%]; p=0.046).

Species diversity of triatomines and entomological indices

During visits to the 244 houses, blood samples were collected and 1474 triatomines were captured, 1331 (90.3%) of which were found in peridomestic structures such as chicken coops, pens, heaps of tiles and firewood, while the remaining 143 (9.7%) were found inside the houses (Table 2). The proportion of immature instars (nymphs) among the insects captured in the peridomestic structures and inside the houses was 71.4% and 73.4%, respectively, demonstrating the process of colonization of human dwellings by Chagas disease vectors. Among the insects collected, 1286 (87.2%) were classified as belonging to the *T. brasiliensis* complex, 158 (10.7%) as *Triatoma sordida*, 27 (1.8%) as *T. pseudomaculata* and 3 (0.2%) as *Triatoma melanica*. Among the insects classified as belonging to the *T. brasiliensis* complex, 353 could be classified as subspecies: 334 were *T. brasiliensis brasiliensis* and 19 were *T. brasiliensis macromelasoma*.

Collected insects were distributed in 95 sites, 32 inside the houses and 63 in the peridomestic environment. The main collection sites inside homes were the bedrooms (177 adult insects [mean 7.4 insects per site] and 234 nymphs [mean 9.8 insects per site]). Regarding the peridomestic capture sites, chicken coops stood out, with 107 adults (mean 4.5 insects per site) and 446 nymphs (mean 18.6 per site), followed by tiles (54 adults [mean 2.3 per site] and 135 nymphs [mean 5.8 per site]) and goat and sheep pens (27 adults [mean 4.5 per site] and 102 nymphs [mean 17 per site]) (not shown). Considering the set of houses studied, the overall infestation rate (inside houses or in peridomestic environments) was 73/244 (29.9%). All houses were bricks structures, so there were no mud (*pau-a-pique*) houses in the included communities. Among these houses, 119 (48.8%) did not have complete plastering. The intrahome colonization rate was 11.5% (n=28). This rate was not different when comparing houses with complete and incomplete plastering (11/106 [10.4%] vs 16/119 [13.4%]; p=0.309). The rate of peridomestic infestation was

Table 2. Distribution by species, stage, place of capture and infection rates by *T. cruzi* of specimens of triatomines collected in the homes of 12 rural communities in São João do Piauí, Piauí, Brazil, 2018–2019

	Inside houses			Peridomestic structures		
Species/instar	N (collected)	Positive in light microscopy, n/N	Positive in cultivation of gut content, n/N	N (collected)	Positive in light microscopy, n/N (%)	Positive in cultivation of gut content, n/N (%)
T. brasiliensis complex						
Females	14	0/13	0/13	151	2/135 (1.5)	2/135 (1.5)
Males	20	0/19	0/19	163	0/140	4/140 (2.8)
N1	30	0/8	0/8	56	–	
N2	4	–	–	107	0/51	0/51
N3	16	0/2	0/2	204	0/98	1/98 (1)
N4	20	0/9	0/9	190	1/97 (1)	1/97 (1)
N5	20	1/18 (5.6)	0/18	291	4/235 (1.7)	1/235 (0.4)
Total	124	1/69 (1.4)	0/69	1162	7/756 (0.9)	9/756 (1.2)
T. sordida						
Females	–	–	–	31	0/28	0/28
Males	–	–	–	25	0/23	0/23
N1	–	–	–	22	–	–
N2	–	–	–	15	–	–
N3	1	–	–	15	0/1	0/1
N4	–	–	–	12	–	–
N5	–	–	–	37	0/15	0/15
Total	–	–	–	157	0/65	0/65
T. pseudomaculata						
Females	1	–	–	4	0/3	0/3
Males	3	0/3	0/3	4	0/4	0/4
N1	1	–	–	0	–	–
N2	4	–	–	0	–	–
N3	9	–	–	1	–	–
N4	18	–	–	9	–	–
N5	1	–	–	4	0/1	0/1
Total	3	0/3	0/3	4	0/8	0/8
T. melanica						
Females	–	–	–	2	0/2	–
Males	–	–	–	1	0/1	–
N1	–	–	–	–	–	–
N2	–	–	–	–	–	–
N3	–	–	–	–	–	–
N4	–	–	–	–	–	–
N5	–	–	–	–	–	–
Total	–	–	–	–	–	–

N1–5: first to fifth instar nymphs.

23.1% (n=52). Inside 90 houses (40.6%) there were traces of triatomines (insect faeces on the walls). Intradomiciliary infestation rates were higher in the communities of Lagoa da Serra (31.3%), Riacho (37.5%) and Chiqueirinho (26.3%). The mean number of insects captured inside homes per positive household was higher in Junco (31.8±35.6 insects), Lagoa da Serra (30.2±33.1 insects) and Poço do Rego (33.3±50 insects). These results are presented in Table 3.

Characterization of *T. cruzi* infection in insects

A total of 904 insects had their intestinal content examined by light microscopy, with trypanosomatids observed in 8 (0.5%) of these. The intestinal contents of 996 insects were cultured on NNN/LIT medium and a growth pattern morphologically compatible with *T. cruzi* was observed in 9 (0.9%). These isolates were submitted to genotyping with *T. cruzi* I identified in three isolates and *T. cruzi* II identified in two isolates (Figure 3).

Table 3. Entomological indices calculated from specimens of triatomines collected in rural communities of São João do Piauí, Piauí, Brazil, 2018–2019

	Infestation rate inside homes, n/N (%)	Infestation rate in the peridomestic environment, n/N (%)	Infestation rate in any place, n/N (%)	Insects collected (n)	Insects per positive domicile, mean±standard deviation
Community					
Bom Sucesso	2/13 (15.4)	1/13 (7.7)	2/13 (15.4)	16	5.3±4.5
Chiqueirinho	5/19 (26.3)	3/19 (15.8)	7/19 (36.8)	135	19.2±28.6
Curtume	1/24 (4.2)	7/24 (29.2)	8/24 (33.3)	87	10.8±13.6
Eugênio	2/25 (8)	8/25 (32)	10/25 (40)	50	5.0±4.8
Grajau	2/26 (7.7)	6/26 (23.1)	6/26 (23.1)	87	14.5±24.8
Jacaré	0/10 (0)	2/10 (20)	2/10 (20)	20	10.0±1.4
Junco	0/28 (0)	5/28 (17.9)	5/28 (17.9)	223	31.8±35.6
Lagoa da Serra	5/16 (31.3)	6/16 (37.5)	8/16 (50)	242	30.2±33.1
Poço do Rego	1/11 (9.1)	2/11 (18.2)	3/11 (27.3)	100	33.3±50.0
Riacho	6/16 (37.5)	7/16 (43.8)	11/16 (68.8)	306	27.8±34.8
Saco	1/25 (4)	6/25 (24)	7/25 (28)	163	23.2±32.8
São José	3/31 (9.7)	2/31 (6.5)	4/31 (12.9)	45	11.2±11.2

Discussion

The present study sought to describe the current epidemiological scenario of Chagas disease in an area where vector-borne transmission occurred over decades, possibly since the beginning of colonization of the Brazilian northeastern hinterlands in the Caatinga biome by ranchers and farmers.[28] The main finding was the absence of infection in people <30 y of age. This is consistent with the virtual eradication of transmission achieved in the 1990s following actions for vector control with pyrethroid insecticides.[29] In contrast, high seroprevalence rates were observed in the older age groups, so that Chagas disease affects near one quarter of the population >60 y of age. Data suggest that in the studied communities, despite there being no more active transmission of *T. cruzi*, the prevalence is very high in the elderly, who often do not know their positivity status. Many of these patients have never had an initial clinical assessment to classify Chagas disease in different forms/stages or sought supportive treatment.

Although highly prevalent in the rural population studied, Chagas disease is not addressed by the primary healthcare system in the region.[30] The healthcare system is unable to diagnose and treat extant cases. In this sense, Chagas disease remains, in the 21st century, a hidden and neglected disease. Although transmission has been interrupted, diagnosis and treatment of Chagas disease should not be discontinued in the region. Primary healthcare teams in these locations—and in other settings with similar socio-environmental characteristics in the Brazilian semi-arid region—should be trained to provide diagnostic testing and treatment for the population.

It can be inferred that there is a large contingent of adults with Chagas disease in rural communities in the semi-arid region of the state of Piauí who could be receiving clinical, electrocardiographic and echocardiographic follow-up and treatment. In addition, the current consensus is that there are benefits to antiparasitic treatment of young adults with benznidazole. This study showed that in rural communities, up to 10% of the population 30–45 y of age is positive for *T. cruzi* infection and could benefit from parasiticidal treatment with benznidazole.[31] The seroprevalence rates described in this study are compatible with those estimated for other hyperendemic regions in South America. In Argentina, serological positivity ranging from 27 to 47% was described in Chaco.[32,33] However, the Brazilian semi-arid region seems to differ from Bolivia in that the latter is still reporting infection in children.[34,35]

Another important finding of the study is the presence of women >30 y of age, still of childbearing age, who are seropositive for Chagas disease. Considering the fact that testing of pregnant women is not carried out in the region, there is a risk of congenital transmission of *T. cruzi* in the population. This transmission route has been considered a challenge for the control of Chagas disease in areas where vector transmission has been controlled.[36,37]

Regarding entomological data, an unfavourable scenario was described, pointing to the risk of recrudescence of vector transmission. In a physiogeographic landscape very favourable to the presence of triatomines, these insects still colonize a large proportion of the studied houses, mostly *T. brasiliensis* complex species. *T. brasiliensis* naturally inhabits cracks in rocky outcrops present in the landscape of Caatinga.[22] From their wild habitats, these triatomines tend to colonize anthropic environments in search of food among domestic animals. From the colonization of peridomestic structures such as chicken coops, insects can invade and colonize houses, feeding on humans and bringing the risk of *T. cruzi* transmission.[21]

In addition to *T. brasiliensis*, the entomological survey demonstrated the presence of *T. sordida* and to a lesser extent *T. pseudomaculata*. *T. sordida* is a typical species of the Cerrado, its centre of dispersion. It must be taken into account that the

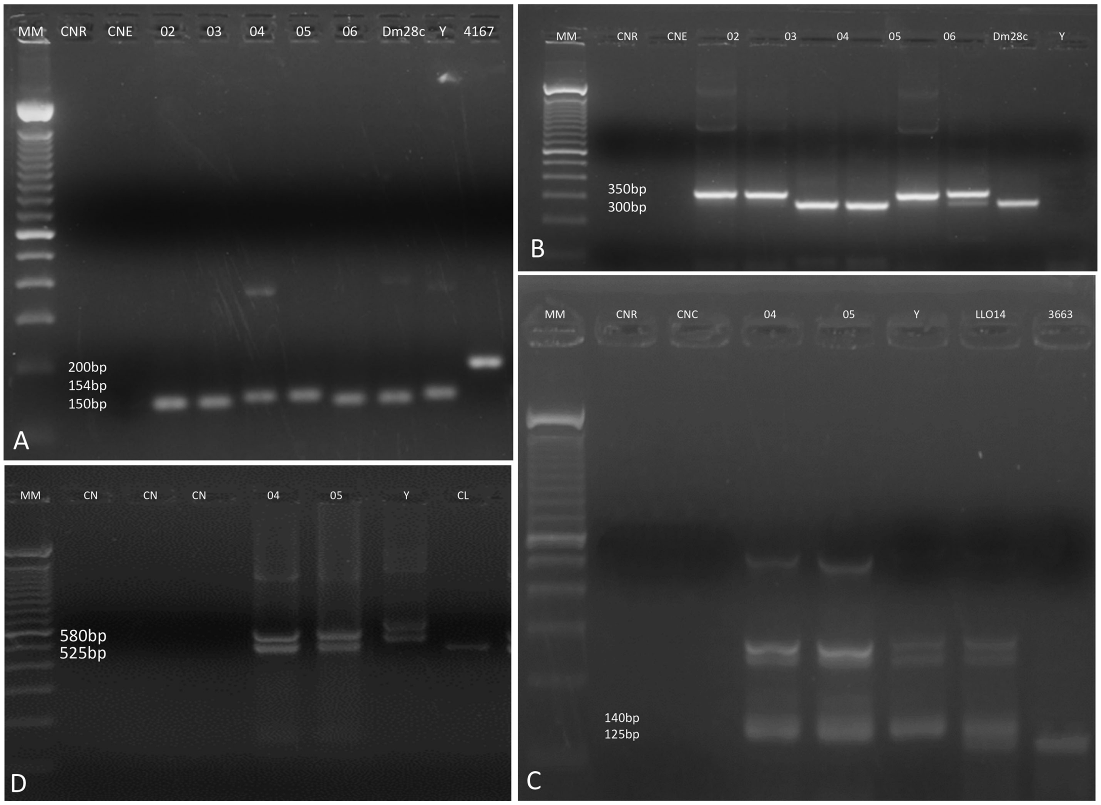

Figure 3. Molecular typing of *T. cruzi* isolates obtained from cultivation of faecal suspensions of triatomines collected in rural communities of São João do Piauí, 2018–2019. (A) Agarose gel electrophoresis demonstrating amplification products of *T. cruzi*, target 1 (intergenic spacer of spliced leader [SL-Irac]) with UTCC/Tcac primers. The reference strains are Dm28c for *T. cruzi* I (150 bp), Y for *T. cruzi* II (154 bp) and 4167 for *T. cruzi* III and IV (200 bp). Samples 02, 03 and 06 are compatible with *T. cruzi* I and samples 04 and 05 are compatible with *T. cruzi* II. (B) Amplification of *T. cruzi* target 2 (intergenic region of mini-exon genes [SL IR I and II]) with TCC/TC2 TC1 primers. The reference strains are Dm28c for *T. cruzi* I (350 bp) and Y for *T. cruzi* II (300 bp). Primers do not amplify the reference strain 4167 for *T. cruzi* III and IV. The products demonstrate that samples 02, 03 and 06 are compatible with *T. cruzi* I and samples 04 and 05 are compatible with *T. cruzi* II. (C) Amplification of *T. cruzi* target 3 (D7 domain of the 24Sα ribosomal RNA gene) with D75, D76 and D71 primers. The reference strains are Y for *T. cruzi* II (140 bp), 3663 for *T. cruzi* III (125 bp) and LL014 for *T. cruzi* V (125+140 bp). The products demonstrate that samples 04 and 05 are compatible with *T. cruzi* II. (D) Amplification of *T. cruzi* target 4 (A10 nuclear fragment) with Pr1, Pr6 and Pr3 primers. The reference strains are Y for *T. cruzi* II (580 bp) and CL for *T. cruzi* VI (525 bp). The products demonstrate that samples 04 and 05 are compatible with *T. cruzi* II.

studied area, although located in the Caatinga biome, can be considered an ecotonal zone in the south of the state, close to the Cerrado biome, and that *T. sordida* has already been identified in this area in Piauí state, as reviewed by Forattini et al.[38] The influence of the ecotonal physiogeographic landscape in the state of Piauí upon the triatomine fauna, which includes typical species from different biomes, has also been recorded in the state capital, Teresina.[39]

In the present study, it was shown that a large proportion of the triatomines captured inside houses were immature stages (nymphs). In addition, there was also the collection of insect eggs inside houses, suggesting the colonization of households and blood-feeding on humans. Although the rate of natural infection of insects by *T. cruzi* was low, the circulation of this parasite in the domestic environment was demonstrated by parasitological and molecular techniques.

The results indicate the danger of interrupting entomological surveillance and insecticide spraying of households in rural locations in the northeastern semi-arid region of Brazil. They also point to the need for housing improvements and education of residents so that they can recognize the insects and report their presence to the authorities. In recent years, almost all insect control resources have been directed towards combating the *Aedes aegypti* mosquito and the emergence of arboviruses that have become public health priorities.[40]

Conclusions

The present study reveals the current epidemiological scenario of Chagas disease in rural locations in the Caatinga biome in northeastern Brazil. The results suggest that although no vector transmission currently occurs, prevalence rates are high among adults and the elderly. The disease should be targeted by primary healthcare actions in the region. Insect surveillance and control activities should not be discontinued in an environment favourable to the perpetuation of house colonization by triatomines.

Authors' contributions: JSP, KJLM, FACC, JSM conceived the study and were responsible for the experimental design, data analysis, manuscript preparation and supervision of field and laboratory activities. TV and CB conducted and supervised *T. cruzi* genotyping assays using molecular methodologies. BBCE, DFMS, RAO and EP were responsible for field collections and initial processing of insects and biological samples. RSJ, AHRS and LRS were responsible for entomological analysis, identification of the natural infection of insects with *T. cruzi*, taxonomic classification, organization and analysis of entomological data.

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Competing interests: None declared.

Ethical approval: The study was approved by the Research Ethics Committee of the Oswaldo Cruz Institute of the Oswaldo Cruz Foundation (protocol no. CAAE: 89970718.7.0000.5248). Informed consent was obtained from all individuals. For children and adolescents <18 y of age, the consent form was completed by a guardian. Children and adolescents also provided an assent form.

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