The risk of oral transmission in an area of a Chagas disease outbreak in the Brazilian northeast evaluated through entomological, socioeconomic and schooling indicators

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ABSTRACT

Chagas disease is a neglected tropical disease strongly associated with low socioeconomic status, affecting nearly 8 million people – mainly Latin Americans. The current infection risk is based on acute case reports, most of which are typically associated with oral transmissions. In the semi-arid region of Northeastern Brazil, serious outbreaks of this transmission type have surged in the last years. One of those occurred in 2016 in the state of Rio Grande do Norte. Rural residents of four municipalities surrounding Marcelino Vieira ingested sugar cane juice – which was probably ground with *Trypanosoma cruzi*-infected insects. Eighteen cases of Chagas disease were confirmed serologically, with two deaths reported. Socioeconomic information, schooling of residents and the structure of peridomestic and domestic environments in the rural area of Marcelino Vieira, along with entomological indicators, were investigated to understand better the factors related to the outbreaks in this region. We found triatomines (mainly *Triatoma brasiliensis*) in 35% (24/67) of domiciliary units and all rocky outcrops inspected (*n* = 7). Overall, 25% (91/357) of examined *T. brasiliensis* were infected by *T. cruzi* in artificial ecotopes, with almost the same prevalence in the sylvatic environment (22%; 35/154). Among all ecotopes investigated, wood/tile/brick piles were the ones linked to high insect infestations and triatomine *T. cruzi* infection prevalence. Ninety-five percent of people interviewed recognized the triatomines and knew the classic route of transmission of disease – triatomine bite-dependent. However, only 7.5% admitted knowledge that Chagas disease can also be acquired orally – which poses a risk this transmission route currently recognized. Here, we highlight the physical proximity between humans and triatomine populations with high *T. cruzi* infection prevalence as an additional risk factor to oral/vector contaminations. In sum, residents have low income, low level of education, and/or a willful disregard for the routes of Chagas disease transmission (specifically oral transmission), a combination of factors that may have favored the Chagas disease outbreak. We here provide recommendations to avoid further outbreaks.

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1. Introduction

The protozoan parasite, *Trypanosoma cruzi*, is the etiologic agent of Chagas disease – one of the most relevant neglected tropical diseases in Latin America (WHO, 2020). Classic transmission occurs via excreta from blood-sucking bugs that are eliminated during or shortly after the blood meal of infected insects. These blood-sucking bugs belong to the Triatominae subfamily (Hemiptera: Reduviidae) (Lent and Wygodzinsky, 1979). *Triatoma infestans*, the primary vector, was likely introduced from the Bolivian Andes and adapted to households across Brazil – probably during a period known as "the sugar age" (1530–1700), during the exploration of sugar cane, coffee, and ores in Colonial Brazil (Gurgel et al., 2009). The Southern Cone countries (Argentina, Brazil, Chile, Uruguay, Paraguay, Bolivia and Peru) joined efforts in 1991 to combat transmission by *T. infestans* and to control the transmission via blood-/organ donations (Schmunis and Yadon, 2010). As a result, Brazil was effectively free of *T. infestans* by 1996, and has eliminated transfusional Chagas disease transmissions nearly completely (Dias, 2007). However, native vectors, such as *T. brasiliensis* and *T. pseudomaculata*, currently challenge control measures by invading and colonizing human domiciles in endemic regions in northeastern Brazil (Dias et al., 2000; Lima et al., 2015). Additionally, outbreaks by oral contamination have been reported in several Brazilian regions (Dias et al., 2016). The oral route is recognized currently as the main source of infection, especially in the Brazilian Amazon, and most likely the result of the consumption of fresh açaí berries and juice in this region (Dias et al., 2001; WHO, 2020).

Chagas disease outbreaks in the Amazon region have been intensively investigated from epidemiological, ecological, and molecular perspectives. Such work has led to the genotyping of the parasite from vectors, *T. cruzi* reservoirs, and humans (e.g. Coura et al., 2002; Monteiro et al., 2012; Jansen et al., 2018) as well as studies that detailed additional eco-epidemiologic features of the outbreaks (Briceño-León, 2007; Coura et al., 2013). Outside Amazônia, as in the state of Santa Catarina (southern Brazil), an oral Chagas disease outbreak in 2005 lead to research that tracked the parasite through molecular techniques in its reservoirs (*Didelphis aurita* and *D. albiventris*). The results of this study suggest that *T. tibiamaculata* was the responsible vector (Steindel et al., 2008). In the municipality of Guarapari, in the state of Espírito Santo (southeastern Brazil), an oral case of Chagas disease transmission resulted in the death of a two-year-old child (Dario et al., 2016). From this incident, the sylvatic (rodents) and domestic (dogs) reservoirs of *T. cruzi* were examined. Through genotyping the parasite (DTUs I, II, III and IV) in the human, fatal case they found the same genotypic combination harbored by the vector (*T. vitticeps*) (Dario et al., 2016).

In the Brazilian Northeast, research on Chagas disease outbreaks is not prioritized compared with other areas of Brazil, even though outbreak events are also serious. For example, in 1986 in the municipality of Catolé do Rocha in Paraíba, an outbreak occurred from oral infection via sugarcane juice ingestion (Shikanai-Yasuda et al., 1991). Dias et al. (2008) reported an outbreak in Bahia possibly associated *T. sordida* with fatal cases; however direct evidence for the oral infection was not found. Another outbreak with similar characteristics occurred in the state of Ceará, in the municipalities of Aratuba and Redenção in 2006. This event was thought to be caused by the consumption of contaminated vegetable soup (Pamplona De Góes Cavalcanti et al., 2009). Finally, a recent (2019) Chagas disease outbreak occurred in the state of Pernambuco, (Góes, 2019). Authorities could not obtain detailed eco-epidemiologic information, although it is believed that this outbreak was a result of contaminated food or beverage associated with a religious event. Each of these events exemplifies the connection with outbreaks and oral infection as a result of food or beverage contamination in semiarid region of Brazilian Northeast.

This study examines factors surrounding another recent outbreak in the state of Rio Grande do Norte in 2016 documented by Vargas et al. (2018). In total, 18 cases were confirmed serologically in the municipalities of Tenente Ananias, Marcelino Vieira, Alexandria and Pilões (10

in Marcelino Vieira alone), and two deaths were reported. Based on the patient reports, Vargas et al. (2018) suggested that infections occurred from a single event involving the consumption of sugarcane juice – probably obtained by the grinding of sugar cane with *T. cruzi* infected insects. *Triatoma brasiliensis* were found infected by the parasite in the same location of the sugar cane mill in Marcelino Vieira (Vargas et al., 2018). Considering the high *T. cruzi* prevalence in domestic and peridomestic *T. brasiliensis* populations in Rio Grande do Norte, Lilioso et al. (2017) proposed that this species is the likely vector involved in the outbreak. Our objective was to understand the factors related to Chagas disease outbreaks in northeastern Brazil, and more specifically on the case of Marcelino Vieira. Because this municipality was the center of this outbreak, we concentrated our efforts on this location. We described the eco-epidemiological conditions, the socioeconomic status of the residents, and the housing structures in the rural area. Also, we evaluated the knowledge of people regarding the risk factors related to Chagas disease, including the potential for oral infections. To determine additional factors possibly linked to the outbreak, we mapped the distribution of infected/non-infected insect populations and their foci – in both sylvatic and peridomestic ecotopes.

2. Materials and methods

2.1. Ethics statement

This research was approved by the UNICAMP Research Ethics Committee (protocol No. 2631,532). The collection and transportation of triatomines were conducted with the assistance of technicians from the municipal and state health departments and had the Sisbio IBAMA License Nº 58,373–1 approval. We obtained permission from homeowners/residents to collect insects from all dwellings and properties, and all interviewed residents signed (or printed digitally) a Free and Informed Consent Form (FICF). The SISGEN register is A5C8D0D.

2.2. Study area and environments

We conducted the study in the municipality of Marcelino Vieira (6° 17′ 38″ S, 38° 10′ 01″W), located within the Caatinga biome in Northeastern Brazil. The region is characterized by seasonally dry forests with a mosaic of thorny shrub vegetation (IBGE, 2017). We chose this site because (i) it had the most recorded infections of the outbreak, and (ii) it is the location of the sugar cane mill – the potential source of the outbreak. Inspections for triatomine infestations were conducted in the following environments: the sylvatic (rocky outcrops), the peri-domestic, and the intra-domestic. Although peri-domestic and intra-domestic environments are markedly different (see topic "2.4"), we employed the term domiciliary unit (DU) for the combination of peri-domestic and intra-domestic environments from each house to apply the entomological indications (see topic "2.4").

2.3. Surveys with rural property residents

We conducted a cross-sectional study through interviews with residents of 67 DUs from our rural localities of Marcelino Vieira by using a questionnaire. These DUs included patients affected by the Chagas disease outbreak. One resident (over the age of 18) from each dwelling was interviewed to obtain socioeconomic and educational information. Specifically, we collected data on age, gender and years of schooling of residents. We grouped them into the following age groups: 18–30, 31–59 and over 60 years. To establish the socioeconomic characteristics, we followed the Brazilian Association of Research Companies (ABEP), which is based on a proprietorship score and level of education. We classified the total income per household based on Kamakura and Mazzon (2016), but it was adapted to the reality of the local residents from the rural and poor site studied, as follows: monthly family income (in USD) for 5 classes (A-E) of more than $270.7 (A), $203.2 (B1),

$152.4 (B2), $114.3 (C1), $85.7 (C2), $226.7 (D), and $64.4 (E).

2.3.1. The resident's knowledge regarding Chagas disease-related risk transmissions

We evaluated the Chagas disease and vector knowledge of each resident. Specifically, the questions focused on the (i) recognition of triatomine by morphology; (ii) identification of ecotopes associated with DU infestation; (iii) recognition of the disease name; (iv) knowledge of classic disease transmission; (v) knowledge of alternative forms of disease transmission (as oral); (vi) potential contact with triatomines; and (vii) source of knowledge regarding Chagas disease (e.g. schools, TV, technicians from the health departments, etc.). To test vector recognition, we presented each respondent with a selection of immature and adult triatomines and non-hematophagous insects (e.g., beetles).

2.3.2. Characteristics of dwellings construction

We evaluated the construction of each dwelling via home inspection and classified it by its probability of triatomine colonization. For example, we recorded whether walls were coated and whether the roof had a ceiling to prevent the insect colonization. The survey conducted here was adapted from a survey produced by FUNASA (Brazilian Health Foundation) to guide house improvement programs (FUNASA, 2017).

2.4. Insect captures and entomological indicators

We searched for triatomines inside homes (intra-domestic), which includes all areas closed by doors where humans live or work (Almeida et al., 2008). In these environments, we examined cracks in the walls, the back of paintings, mattresses, cabinets, curtains, and under/behind furniture. We also searched peridomestic environments, represented by spaces surrounding homes. In these environments, several man-made structures are found – mainly shelters for domesticated animals (e.g., chicken, goats, cows, and dogs). Besides livestock shelters, it is common to find woodpiles and brick/tile piles (i.e., artificial ecotopes) to store material for rudimentary ovens and to build houses, walls, roads, fences and other purposes. We took note of all ecotopes found in each house visited.

We also examined the primary and native sylvatic ecotopes of *T. brasiliensis* (e.g., rocky outcrops; Lent and Wygodzinsky, 1979) as well as alternative natural ecotopes (e.g., xique-xique cacti, lower tree trunks, downed trees, and animal dwellings; Valença-Barbosa et al., 2014). We identified these locations using Google Earth Pro-software (Wuthrich, 2006) or by consulting the residents. We divided sampling into four groups of localities (A, B, C and D) – distributed according to their geographic proximity. Sylvatic points that were not around DUs

were also considered, named "O" (outside localities; Fig. 1). In this environment we performed searches in beginning at sunset (~17:00 local time) and continued collection for approximately five hours. All insects were collected using cap lamps, gloves, and tweezers.

2.5. Ecotypic indicators

The Ecotypic Prevalence in DUs (EPD) reflects the prevalence of a given ecotope in each locality. It is represented by the presence of ecotopes/houses investigated. The Prevalence of Ecotypic insect colonization (PEC) considers the EPD / ecotopes with triatomines.

2.6. Entomological indicators in anthropic habitats

All collected triatomines were kept alive after the capture. We assessed species, evolutionary stage, and gender (if adults) of each sample in the laboratory. Entomological indicators were calculated according to Pan American Health Organization (OPAS, 2003), as follows:

  i) Domiciliary infestation index (DI) = number of DU with adult triatomines x 100 / number of DU inspected;
 ii) Domiciliary triatomine density (DTD) = number of captured triatomines / number of DU inspected;
iii) Domiciliary colonization index (DCI) = number of DU with nymphs x 100 / number of DU inspected;
 iv) Natural Infection index by *T. cruzi* (NI) = the number of infected triatomines x 100 / number of examined triatomines.

2.7. Entomological indicators in sylvatic habitats

The natural infection index/prevalence by *T. cruzi* in the sylvatic environments (NIS) was calculated apart from NI. The triatomine density in the sylvatic environment (TDS) was estimated according to Almeida et al. (2009), using the following equation: TDS = total triatomines collected / total collectors/work hours in the sylvatic environment.

2.8. Trypanosoma cruzi natural infection

We developed our procedure using traditional optical microscopy techniques (OM). We obtained triatomine feces using abdominal compression and deposited the sample on a slide, adding 5μL of saline solution. We then covered the slide with a 22×22 mm glass coverslip. We examined the intestinal content using a binocular optical microscope with a 400x magnification by screening all fields for *T. cruzi*. We
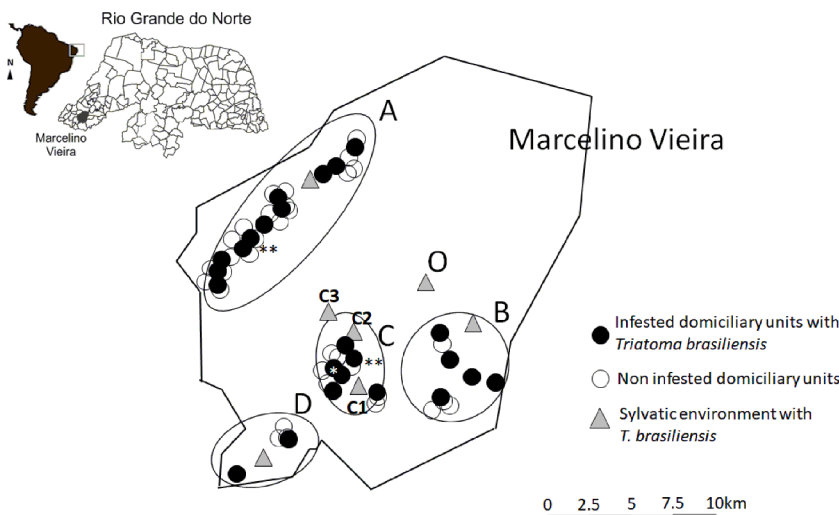

Fig. 1. Survived localities (A, B, C and D) in Marcelino Vieira, Rio Grande do Norte, Brazil. Black dots show domiciles positive for triatomine captures. Open circles show domiciles where triatomines were not found. Triangles are sylvatic locations surveyed for *Triatoma brasiliensis*. The point O was a sylvatic environment outside any locality. Because locality C had three sylvatic environments they are indicated as C1–3. Asterisks indicate points where we found concomitant infestation by *T. brasiliensis* and *Rhodnius nasutus* (*) or by *T. brasiliensis* and *T. pseudomaculata* (**).

conducted the molecular diagnosis simultaneously using Polymerase Chain Reaction (PCR), for comparative purposes, to assure that the trypanosomatids observed were *T. cruzi*. We macerated the gut of a portion of collected insects using a sterile crusher and liquid nitrogen. We then used The DNeasy Blood & Tissue Kit (Qiagen) to isolate the DNA, following the manufacturer's protocol. We used PCRs the primers 121 and 122 (*T. cruzi* kDNA), as previously described by Sturm et al. (1989) and Wincker et al. (1994), with termocycles described by Moreira et al. (2017). For all insects that tested positive for *T. cruzi*, we amplified the 330 bp fragment. If positive for *T. cruzi*, an amplicon could be seen in 2% (w/v) agarose gels by electrophoresis at 80 V for 40 min. We used samples with water as controls for comparison with the DNA Dm28 strain from *T. cruzi* culture (TcI). An additional PCR was conducted to verify intestinal DNA did not have inhibitors for amplification; therefore, for the same samples, we amplified the mitochondrial CytB gene as the target. For this purpose, we amplified the insect Cyt B gene as the target, by using the cybTprR /CYTBR (Oliveira et al., 2017) and 7432F primers (Monteiro et al., 2003), with PCR conditions previously described.

2.9. Statistics

We used chi-square analysis to evaluate the significance among entomological indicators obtained among localities and McNemar's chi-square to compare traditional and molecular techniques for *T. cruzi* detection. We evaluated statistical significance using 95% confidence intervals and an alpha of 0.05. All analyses were conducted using the R (R Development Core Team, version 3.6.3; https://www.r-project.org/).

2.10. Relationships among Trypanosoma cruzi infection, habitat and localities for Triatoma brasiliensis

To explore the interaction among habitat sources, *T. cruzi* prevalence and localities, we used the software Cytoscape 3.7.2 (Shannon et al., 2003) to build a network. For this purpose, we used only insects that tested for the *T. cruzi* natural infection via OM.

3. Results

3.1. Surveys with rural property residents

We interviewed 67 people – composed of one person per DU inspected. Respondents varied among age groups: (i) 17.9% were 18–30 years old, (ii) 43.3% were 31–59 years old and (iii) 38.8% were 60 years old or older. Most respondents were women (76.1%). The majority of people also had less than five years of schooling or were illiterate (Table 1).

For the ones who have a formal monthly salary, the main source of income derived from retirement related to age or disabilities (55.2%), governmental social programs (6.0%), from livestock and subsistence farming activities (31.3%) and other activities 7.5%. According to socioeconomic parameters, families should receive a minimum monthly of USD 197.91 (see: https://www.gov.br/; dollar quotation in September 16, 2020); but 25% lived with on less than USD 40.5. Therefore, family

income is mostly dependent on self-subsistence agricultural activities (Table 2).

Regarding the knowledge of the residents on Chagas disease, 95% of those interviewed recognized the insect vector, knew the ecotope it occupies in DUs, and most of the respondents (92.5%) understood that the triatomine transmits a disease. Only 7.5% reported that they knew the disease could be transmitted by contaminated food/drink (oral route). Thirty percent of the interviewees said they believe to have been bitten by the vector in the last 3 years. Most of them supposed to have been bitten by the finding of blood-engorged insects around where they were sleeping (e.g. under beds, behind furniture, curtains, etc.) (Table 3). The main sources of information on Chagas disease came from within the community (i.e., neighbors) according to 49.3% of respondents (Table 4).

3.2. Characteristics of dwellings construction

We found that 97% (65/67) of the houses surveyed did not have covered roofs; however, only 12% (8/67) did not have plastered walls (i. e., walls without a coating).

3.3. Insect captures

In the entomological survey, we found 35% (24/67) of houses had triatomines in the peridomiciles. Inside dwellings, we captured six specimens of *T. brasiliensis* in two houses (a single adult female in one DU and 5 nymphs in coated walls in a second DU). *Triatoma brasiliensis* was also the most captured species in all ecotopes (98–100%), except for a chicken coop – which exhibited an abundant ($N = 251$) infestation by *T. pseudomaculata*. *Rhodnius nasutus* ($N = 7$) were also found in a chicken crop. Because *T. brasiliensis* has greater epidemiological relevance (Costa et al., 2003) and was more abundant, in this study, we will only report on findings associated with this species.

3.3.1. Ecotopes

We measured the prevalence for the finding of key-ecotopes (Ecotypic Prevalence in DUs; EPD) and the proportion of those with triatomine infestations (Prevalence of Ecotypic Colonization; PEC). Overall, henhouses were the most common ecotope found in DUs (EPD=85%). However, we found greater insect colonization in woodpiles (PEC=47.1%). Both EPD and PEC varied among localities (Table 5). For both indicators, the distribution was significant among localities at $p < 0.05$ (see details in topic 3.4.1)

3.4. Entomological indicators for anthropogenic environments

Overall, the value of domiciliary infestation (DI) (35.8%) was slightly, yet not significantly, higher than the overall domiciliary colonization index (DCI) (32.8%). Domiciliary triatomine density (DTD) was 7.7, reflecting the number of triatomines collected relative to the number of houses inspected. Overall, natural *T. cruzi* infection prevalence in anthropogenic habitats (NI) was 25%. These indicators varied among localities; and the most remarkable variation was in the NI,

Table 1
Level education of 67 residents interviewed in the rural area from the municipality where the Chagas disease outbreak occurred: Marcelino Vieira. Rio Grande do Norte. Brazil. 2018.

Education level	N	Years of schooling	%
Illiterate / Incomplete elementary I / Complete Elementary I	37	< 5 years	55.2
Incomplete Elementary II / Complete Elementary II	22	6–8 years	32.8
Incomplete High School / Complete High school	8	9 years or more	11.9

Table 2
Distribution of monthly income, according to ABEP (2018), with adaptation according to the minimum salary for 2018. Data from people interviewed in Marcelino Vieira, RN, Brazil.

Class	Average family monthly income (USD*)	Number of families	%
A	270.7	1	1.5
B1	203.2	2	3.0
B2	152.4	8	12.2
C1	114.3	18	27.3
C2	85.7	20	30.3
D-E	64.2	18	25.7

* 1 BRL = 0.18 USD (average dollar for September 2020).

Table 3
Responses from residents form the study area (*n* = 67) on Chagas disease transmission and vector identification. Answers were recorded as yes or no. Marcelino Vieira. Rio Grande do Norte. Brazil. 2018.

Questions	*n* = 67	Percent yes
Do you know the vector? (barbeiro/procotó)	64/67	95.5
Do you know where it hides?	52/67	77.6
Do you know whether the insect transmits any disease?	62/67	92.5
Do you know the name of the disease?	44/67	71.0
Do you know how the disease is transmitted?*	62/67	92.5*
Do you know another form of infection? (as orally)	5/67	7.5
Do you think you have had contact with "barbeiros" or have been bitten by these insects in the last 3 years?	20/67	30
People who answered they knew the disease is transmitted only via the insect's bite		

Table 4
Sources where the residents obtained information about Chagas disease in Marcelino Vieira, Rio Grande do Norte, Brazil, 2018.

Source of information	*n* = 67*	%
Neighbor	33	49.3
Family	13	19.4
School	9	13.4
TV	9	13.4
Vector control surveillance agents	5	7.5
When it was bitten	1	1.5

* an resident may have had more than one answer.

ranging from 0% (A) to 100% (C). Localities B and C were slightly closer to rocky outcrops and exhibited higher DI, DTD, and DCI. DTD also had high variation, ranging from 3.1 (A) to 16.9 (C) (Table 6). For all indicators, the distribution was significant among localities at *p* <0.05 (see details in topic 3.4.1).

3.4.1. Entomological indicators for sylvatic environments

All localities had sylvatic foci (rocky outcrops) physically close to DUs (<250 m), with some locality having more than one focus (e.g., C; Fig. 1). Overall, we collected 1,096 insects in sylvatic environments, varying from 29 (C2) to 287 (D). We had only a few specimens for which to test the natural index of *T. cruzi* infection in the sylvatic environments (NIS) because most of the insects either were too emaciated to be analyzed or died shortly after collection. For indicators in anthropogenic environments, the natural infection also ranged among sylvatic environments, even within the same locality. For example, in the locality A, where no peridomestic insects were infected; however, we found 100% (10/10) infection in bugs from the sylvatic environment. In the sylvatic habitat, overall triatomine density (TDS) was 9.7. TDS ranged from 1.8

(C2) to 17.9 (D) (Table 7). For indicators, the distribution was significant among localities at *p* < 0.05 (see details in topic 3.4.1).

3.4.1. Statistics for the entomological indicators

Chi-squared tests evidenced that the composition of entomological indications among localities were all significant (all $\chi^2 > 100.6$, $df = 3–6$, $p < 0.0001$).

3.5. Comparison of Trypanosoma cruzi detection on insects via traditional and molecular methods

We randomly selected 159 samples of *T. brasiliensis* for PCR analysis to identify infection by *T. cruzi*. We based the criterion on ecotypic/

Table 6
Entomological indicators based on the search for *Triatoma brasiliensis* in the 67 domiciliary units inspected.

| Entomological indicators | Number of houses inspected | Capture points | | | | |
		A 39	B 9	C 14	D 5	Total 67
DI		28.2% (11/39)	55.6% (5/9)	42.9% (6/14)	40% (2/5)	35.8% (24/67)
DTD		3.1 (119/39)	14.1 (127/9)	16.9 (237/14)	7(35/5)	7.7 (518/67)
DCI		23.1% (9/39)	55.6% (5/9)	42.9% (6/14)	40% (2/5)	32.8% (22/67)
NI		0% (0/163)	82.5% (33/40)	23.8% (30/126)	100% (28/28)	25.4% (91/357)

DI = Domiciliary infestation index; DTD = Domiciliary triatomine density; DCI = Domiciliary colonization index; NI = Natural Infection index by *T. cruzi*.

Table 7
Entomological indicator based on the search for *Triatoma brasiliensis* in the rocky outcrops Marcelino Vieira, Rio Grande do Norte. Brazil. 2018.

Localities	*n* (collected)	NIS (Infected/ analyzed)	TDS	Dist. from the nearest residence
A	119	100% (10/10)	7.4	50 m
B	127	NA	7.9	100 m
C1*	237	0%(0/6)	17.9	100 m
C2*	29	9%(3/34)	1.8	50 m
C3*	104	0%(0/6)	6.5	100 m
D	287	24%(23/98)	17.9	250 m
O	193	NA	12.1	740 m
Total	1096	25.5%(35/154)	9.7	

NIS = natural index of *T. cruzi* infection in the sylvatic environments; *because the locality C had three sylvatic environments, we indicated them in the map. NA = not analyzed. TDS = triatomine density in the sylvatic environment.

Table 5
Ecotopes susceptible and infested with by *Triatoma brasiliensis* in Marcelino Vieira, RN, 2018. Values below the localities indicate number of DUs surveyed.

Ecotope (n)	Localities									
	A		B		C		D		Total	
	39		9		14		5		67	
	%EPD	%PEC	%EPD	%PEC	%EPD	%PEC	%EPD	%PEC	%EPD	**%PEC**
Piles of tiles/bricks	30.8(12/39)	41.7(5/12)	77.8(7/9)	42.9(3/7)	57.1(8/14)	12(1/8)	60(3/5)	33.3(1/3)	45(30/67)	43.3(13/30)
Woodpiles	30.8(12/39)	33.3(4/12)	33.3(3/9)	100(3/3)	7(7/14)	100(7/7)	20(1/5)	0(0/1)	25.4(17/67)	47.1(8/17)
Hen house	87.2(34/39)	26.1(9/34)	66.7(6/9)	0(0/6)	93(13/14)	38.5(5/13)	80(4/5)	100(4/4)	85.1(57/67)	31.6(18/57)
Pigsty	5.1(2/39)*	0	0	0	0	0	20(1/5)*	100(1/1)*	1.5(1/67)*	100(1/1)*
Food storage	2.6(1/39)	100(1/1)	–	–	–		–		1(1/67)	100(1/1)

% Ecotypic Prevalence in DUs (%EPD) = presence of ecotopes /houses investigated. Prevalence of Ecotypic insect's colonization (%PEC) = Ecotopes inspected/ ecotopes with triatomines. *insufficient number for consideration.

geographical balancing. The number of samples varied across sampling spots within localities, such that more samples were included from areas with greater epidemiological importance. For example, we analyzed 30 insects from the site of the sugar cane mill (and probable outbreak site; population "*vii*" in locality B), and 34 insects from a site with reportedly numerous infestations (*n* = 191 insects collected). However, in sites where there were fewer infestations (*n*<70), we analyzed fewer samples (5–20). Insects from populations "*vii*" and "*ix*" had the highest *T. cruzi* prevalence, considering their sample size, using both Optical Microscopy (OM) (93% and 100%, respectively) and PCR (100% for both populations) methods (Table 8). We found no statistical difference in prevalence identified by the OM and PCR methods ($\chi^2 = 3.03$, $df = 1$, $p = 0.081$).

3.6. Relationships among Trypanosoma cruzi infection, habitat and localities for Triatoma brasiliensis

We used a total of 612 specimens analyzed via OM method. From this analysis, we demonstrate the relationship among the *T. cruzi* prevalence, ecotopes, and localities. At locality A, 21.8% (60/275) of the analyzed sample was collected in henhouses; the remainder of the sample originated from woodpiles in the peridomestic environment (all of which were negative for *T. cruzi* natural infection) and from rocky outcrops (*n* = 10) – of which were all positive for *T. cruzi*. Except for henhouses, the remaining peridomestic habitats (woodpiles, tile piles and woodpiles combined with tile piles) exhibited high proportion of infected insects (>45%). The presence of tiles seems to be more likely to shelter *T. cruzi* infected bugs, as higher *T. cruzi* prevalence is observed for tile alone and for the combination of wood and tile piles. Infected insects were identified in all localities that had rocky outcrops, with an overall 22% (35/154) (Fig. 2).

4. Discussion

Although vector-borne Chagas disease transmission has declined dramatically in Brazil since 1980, acute cases of the disease continue to be reported (Coura et al., 2002). More importantly, outbreaks, like those in the northeastern region of Brazil, have appeared in the recent decades (Pamplona De Góes Cavalcanti et al., 2009; Vargas et al., 2018). In this study, we focused our analyses on the municipality of Marcelino Vieira in the Rio Grande do Norte State, in light of the recent Chagas disease outbreak (Vargas et al., 2018). We employed an integrative approach,

Table 8
Trypanosoma cruzi detection in *Triatoma brasiliensis* populations analyzed by Optical Microscopy (OM) and Polymerase Chain Reaction (PCR), captured in (sub) locations in Marcelino Vieira, Rio Grande do Norte, 2018.

| *Triatoma brasiliensis* sampling spot	Ecotope	Locality	OM%	PCR%
I	Sylvatic	O	100(1/1)	100(1/1)
Ii	Peridomicile	A	0(0/5)	40(2/5)
Iii	Peridomicile		0(0/7)	42.9(3/7)
Iv	Peridomicile		0(0/10)	0(0/10)
V	Peridomicile		0(0/8)	0(0/8)
Vi	Sylvatic		100(8/8)	100(8/8)
vii	Peridomicile	B	93.3(28/30)	100(30/30)
viii	Peridomicile		100(4/4)	100(4/4)
Ix	Peridomicile	C	100(34/34)	100(34/34)
x	Peridomicile		100(10/10)	90(9/10)
xi	Peridomicile		0(0/4)	75(3/4)
xii	Sylvatic		46.2(6/13)	57.1(8/13)
xiii	Peridomicile	C	100(5/5)	100(5/5)
xiv	Peridomicile		75(15/20)	75(15/20)
Total			69.8(111/159)	76.7(122/159)
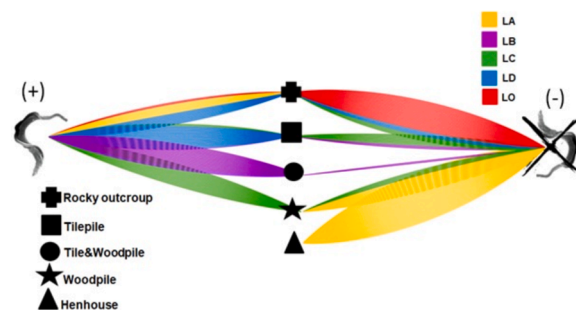
Fig. 2. Network showing the relationships among the habitats, localities, and *Trypanosoma cruzi* natural infection in *Triatoma brasiliensis* (*N* = 612). The thickness of the connections indicates sample size. The color of the connection indicates locality. The central nodes represent the ecotopes, whereas the right and left poles indicate the positive and negative infection of *T. cruzi* in the insects.

combining socioeconomic and entomological surveys, to identify factors potentially associated with the outbreaks (e.g., triatomine infestation and *T. cruzi* infection prevalence).

Most of the surveyed residents were 31 to 59 years old and had only minimal formal education (<5 years of schooling), as evident in the difficulties expressed by respondents when completing the informed consent form (FICF). Social conditions may explain this lack of formal education (e.g., transportation difficulty to schools in rural areas). Current research suggests that these factors (specifically literacy rates) are strongly associated with Chagas disease infection (Oliveira-Marques et al., 2005; Borges-Pereira et al., 2002; Aguilar et al., 2007). Economic conditions (especially those tied to level of education) also may be related to transmission rates. Marques et al. (2010) found that the highest prevalence of chronic Chagas disease was observed in people with low levels of education who also lived in poverty, as we have shown in our study. Retirement is an important source of income, which is consistent with the elderly population found and the ones with disabilities – probably caused by extreme poverty conditions. Moreover, only 4.5% of families have an income higher than the Brazil Minimum Monthly Wage (USD 197.91). In this study, the permanent income of surveyed residents was difficult to assess, since the income of rural workers is not permanent due to the productive cycles of crops and animals.

Awareness of transmission risk and mode may also affect infection rates among residents. Most of the respondents in our study reported that they understood the classic vector-borne transmission; however, only a small percentage of these respondents (7%) were aware of the risk of transmission through oral contamination. According to Colosio et al. (2007), in Paiçandu-PR, the greatest knowledge gap in the population was a contributing factor to disease transmission.

Previous research suggests that dwelling structures may create habitat for, and increase colonization risk of, triatomine infestations because walls without coating can work as shelters for triatomines in their cracks, and the roofs without ceilings present a direct pathways between the house and the external area – facilitating the circulation of the vector. We found that 12% of the houses are without coating or ceilings, which is slightly higher than houses in both urban and rural areas across northeast in Brazil (8%) (IBGE-PNADC/T, 2019). Only *T. brasiliensis* was found inside DUs (intradomestic populations). Our results support the findings of other surveys in the Rio Grande do Norte State (Barbosa-Silva et al., 2016), and in the state of Ceara (Vasconcelos et al., 2018; Daflon-Teixeira et al., 2019) regarding the prevalence of *T. brasiliensis* in anthropogenic habitats. What is more, we found a female *T. brasiliensis* inside a dwelling with coated walls. Although this individual could be an accidental invasion, we also found five nymphs inside a different dwelling with coated walls. The Pan American Health Organization (OPAS 2003) defined the finding of nymphs as indicators

of colonization because insects in this stage are unable to fly, and thus were likely residents rather than invaders.

The localities with the highest values of wild triatomine density were also those with the highest dwelling infestation, suggesting that triatomine density may act positively on infestations. Regarding the distances between rocky outcrops and DUs, all localities had sylvatic environments (<250 m) within the estimated range (550 m) of flight capacity (Cecere et al., 2004) for triatomines.

Chicken coops were present in 85.1% of the houses inspected, but of this, only 31.6% was infested. Although tile, brick and woodpiles were less prevalent in DUs (25.4–45%), 43–47% of these ecotypes were infested. Additionally, insect populations collected in these piles exhibited much higher *T. cruzi* prevalence, which was evidenced in the network. It is common to find piles of building materials and wood in proximity to structures and dwellings. These piles are readily used as artificial ecotopes by triatomines, as also observed by Coutinho et al. (2012) in Ceará. According to Lilioso et al. (2020), these ecotopes can shelter potential *T. cruzi* reservoirs, which are rodents of Caviid family (*Galea spixii* and *Kerodon rupestris*).

There are many factors that influence triatomine infestation in DUs (e.g., proximity of houses to rocky outcrops, debris piles near houses, and proximity of domesticated animals to living spaces). Although most people cannot relocate their houses further from the natural habitat for triatomines, educating residents about other factors in their control may reduce local infestation (Dias et al., 2000). That is, understanding the relationship between infestation and ecotopes may reduce transmission disease risk. We found that, with the exception of henhouses, the prevalence of infected insects in peridomestic environments was even larger than that for the sylvatic environment. Thus, educational programs could be used to inform residents about the risks associated with creating artificial ecotopes in the peridomestic environment.

We found no difference between the technique used to detect *T. cruzi*-infected triatomines. There as only an 8% difference between PCR and OM. Our results provide negligible support for the findings of Vinhaes et al. (2014), Moreira et al. (2017), and Lima-Oliveira et al. (2019) that *T. cruzi* infection can be underestimated by OM. Furthermore, Vinhaes et al. (2014) reported that prevalence of *T. cruzi*, as identified by OM by those involved in vector control measures, may be biased by the nutritional status of the insect at the time of capture and by exanimating dead insects (a result of low intestinal content). These last authors also reported difficulty of differentiating *T. cruzi* from other morphologically similar protozoa (*Blastocrithidia triatomae* and *T. rangeli*). It is important to note that *T. rangeli* has already been found in *T. brasiliensis* in the Rio Grande do Norte (Lima-Oliveira et al., 2019). However, given the lack of statistical evidence, we suggest the OM is still valid to estimate *T. cruzi* prevalence if insects are kept healthy and alive until analysis.

The state of Rio Grande do Norte appears to be an area of intense circulation of *T. cruzi* (Barbosa-Silva et al., 2016, 2019; Lima-Oliveira et al., 2019) if compared to the states of Ceará and Paraíba (Coutinho et al., 2012; Daflon-Teixeira et al., 2019), where the prevalence may be more variable (2.9–20.3%). These high *T. cruzi* circulation in Rio Grande do Norte State was also evidenced by Vicente and Camara (2019), who identified 40% (16/40) of dogs that were analyzed tested positive for *T. cruzi*. These studies underscore the risk of *T. cruzi* transmission to humans and the need to monitor *T. brasiliensis* domiciliary infestations to prevent outbreaks – particularly in the Rio do Norte state.

The outbreak extended to three municipalities beyond Marcelino Vieira: Tenente Ananias, Alexandria and Pilões (Vargas et al., 2018). These municipalities are located in the southwestern region of the state and are within a distance of 17 to 30 km from each other. We believe that the extent of the outbreak was underestimated because it was based on symptomatic patients that sought health assistance. Futhermore, the number of respondents that had contact with triatomines raises concern and should be a focus of future monitoring efforts if health officials seek to interupt transmission. The results of our study highlight the need for a human serological survey in the affected area, where peridomestic triatomine infestation by *T. cruzi* infected insects is high. According to WHO (2020), widespread diagnosis is required to treat patients infected by *T. cruzi* in the specific phase of the disease (acute and chronic), and in a timely fasion.

Concluding remarks

Thirteen percent of people reported they may have been bitten by triatomines in the last three years. Twelve percent of dwellings were found to be suitable for triatomine colonization (e.g. those living in a dwelling without coating or covered roof). Educational programs may change the way residents deal with spaces surrounding homes to avoid creating sites that attract *T. cruzi* infected insects. The proximity of infected bugs and humans is worrisome for both vector-borne and oral transmissions. We demonstrated that the population sampled in our study has all the characteristics of those affected by Chagas disease: low income, low level of education, and a willful disregard for the routes of Chagas disease transmission (specifically oral transmission). We reported that information about the disease usually comes from word of mouth in the community and still covers only the classic transmission route (via insect bites). Together, these factors present a situation that leads to increased risk of infections or new outbreaks because this scenario is probably similar for other points non investigated. Oral route transmission will be a challenge to public health programs working to combat Chagas disease; however, such programs that specifically target oral transmission and include community education and participation must be funded and administered in Northeastern Brazil.

CRediT authorship contribution statement

Jackeline Monsalve-Lara: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Maurício Lilioso:** Formal analysis, Investigation. **Carolina Valença-Barbosa:** Formal analysis. **Patricia J Thyssen:** Writing - original draft. **Danilo C Miguel:** Investigation, Methodology, Writing - original draft. **Cleanne Limeira:** Methodology, Writing - original draft. **Fernanda R Gadelha:** Investigation, Writing - original draft. **Fernanda V H M Fontes:** Investigation. **Dayane Pires-Silva:** Formal analysis, Writing - original draft. **L Lynnette Dornak:** Writing - original draft, Writing - review & editing. **Marli M Lima:** Writing - original draft. **Maria R Donalisio:** Conceptualization, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing. **Carlos E Almeida:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None.

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