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ORIGINAL PAPER

Chagas disease and housing improvement in northeastern Brazil: a cross-sectional survey

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Abstract Chagas disease was investigated in two new settlements of landless rural workers inhabiting prefabricated, triatomine-proof houses and in four neighboring older communities where mud huts were still well suitable for vectors. Through a cross-sectional survey and entomological assessment, we surveyed 148 houses/families in the two new settlements and in 47 houses/families in the four older localities. We determined seroprevalence of Chagas disease through IFI and Elisa (eluates) assays and searched for vectors in the domestic and peridomestic environments. Seroprevalence reached 0.6 % (3/466) in the new settlements and 0.8 % (1/115) in the older communities. Triatomines were not found in the new settlements, while 7 *Triatoma brasiliensis*, 4 *T. pseudomaculata*, 1 *Panstrongylus lutzi,* and 145 *Rhodnius nasutus* were collected in the older localities. In addition, a colony of *T. brasiliensis* (n=55) was encountered inside a school attended by children of the region. Parasite strains isolated from the insects were characterized as *T. cruzi* I. Despite the low prevalence of Chagas disease in both scenarios, entomological surveillance must be strengthened and housing improvement reinforced in order to control vector transmission. The risk of infection by the vectors was lower in the settlements of improved homes, where conditions for colonization of the peridomestic environment by transmitting insects were not observed.

Keywords Triatomines · *Trypanosoma cruzi* · Housing improvement · Chagas disease · Northeastern Brazil

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Introduction

Chagas disease (American Trypanosomiasis) is a tissue and hematologic parasitosis inflicted by the flagellate protozoa *Trypanosoma cruzi* Chagas, 1909, transmitted by hematophagous insects, the triatomines (Hemiptera, Reduviidae), as well as by ingestion of contaminated food, blood transfusion, and congenitally from mother to child (Alkmim-Oliveira et al. 2013; Sales-Campos et al. 2014). Vector and blood-borne transmission of Chagas disease have been successfully targeted by control programs based on insecticide treatment of houses and screening of blood banks in Brazil and other Latin American countries (Dias 2009), reducing significantly the disease incidence, as assessed in recent seroprevalence surveys which have shown that positivity is very uncommon in children and young adults in extra-Amazonian traditionally endemic areas (Coutinho et al. 2014; Lima et al. 2012). Nevertheless, in rural regions where insect vectors have sylvatic natural habitats and are able to reinvade and recolonize previously treated dwellings, insecticide-based control strategies are challenged by the existence of a very slack epidemiological and entomological surveillance (Ramos and Maul de Carvalho 2001; Santana et al. 2011; Sarquis et al. 2012). This is the current scenario of northeastern Brazil, where *Triatoma brasiliensis* Neiva, 2011; *Triatoma pseudomaculata* Corrêa and Espinola, 1964; and *Rhodnius nasutus* Stal, 1859 may reach the domestic and peridomestic structures from their natural stocks in the wild environment, in search of food supply in domestic animals (Sarquis et al. 2004; Almeida et al. 2009; Carvalho-Costa et al. 2010; Sarquis et al. 2010; Lima et al. 2012; Almeida et al. 2012; Bezerra et al. 2014).

Stable transmission of *T. cruzi* to man, able to produce endemic Chagas disease and the consequent generation of symptomatic chronic cases, requires more than the presence and circulation of the parasite in the peridomestic environment. The same habitats must be shared by insect vectors and man, i.e., colonization of the dwellings by triatomines, where they feed regularly on man and reproduce (Dias and Dias 1968). From a demographic and economic point-of-view, the colonization of vast South American areas, including the outback of southern, central, and northeastern Brazil, was characterized by landlordism, unequal income distribution, and extreme poverty (Ribeiro 1979). These factors generated the appropriate habitat where man and *T. cruzi* vectors could coexist, i.e., the traditional dwellings in which a wood frame is packed with mud, whose bare walls and crevices provide shelter for triatomines during the day, so they can suck the blood of the residents by night on a regular basis. However, rural exodus has changed the rural landscape, where the original mud huts have become increasingly rare, being substituted by new houses, built with bricks and plaster, inhibiting the colonization by insect vectors.

The objective of this study is to describe the prevalence of Chagas disease in two distinct scenarios, within close proximity, where native vectors present a high pressure of house infestation. One situation is two new villages of resettled landless workers living in prefabricated houses, while the other is composed of four old established communities with the traditional mud hut.

Materials and methods

Study area

A total of 6 localities were investigated (Table 1): two new rural settlements, Bela Vista and Serra Dantas; and four older rural localities, Pacatanha, Arapuá, Sítio Flores, and Gurgel, all situated in Jaguaruana municipality, Ceará state, Brazil. In the older communities, inhabitants practice subsistence agriculture and cattle rising. All localities were situated in the same proximity in a poor, hilly region, 23 km from the Jaguaruana urban center, access by car only possible during the dry season. Deforestation has been widespread and the Caatinga original vegetation was greatly altered due to intense human interference, resulting in highly reduced biodiversity, with the native vegetation devastated in consequence of tillage and herbage.

Bela Vista and Serra Dantas were established around 2000 by the Brazilian government for agrarian reform, a program directed toward the resettlement of rural landless workers. In these settlements, 154 families and 644 people are currently living in standardized and modern houses. Until installation, the landless peasants were encamped in canvas or plastic tents, having lived in this precarious manner for at least ten years.

Assessment of housing characteristics

We approached all dwellings; however, the research was conducted only in those compatible with owner agreement. Thus, 148 of the 174 dwellings in the 2 settlements and all 47 in the four rural communities were evaluated regarding the type of walls, roofs, and presence or absence of bathrooms. The peridomiciles, in which chickens, goats, sheep, cows, pigs, dogs, and cats circulated freely, were also assessed. Artificial triatomine habitats, consisting of animal shelters and piles of roofing tiles, bricks, and wood were investigated. Concerning the Bela Vista and Serra Dantas settlements, all houses have the same pattern. Houses are standardized and completely different from those preexisting in the rural surroundings.

Table 1 Characteristics of six surveyed localities in the municipality of Jaguaruana, state of Ceará, northeastern Brazil, 2007–2008

	New settlements		Old communities			
	Bela Vista	Serra Dantas	Arapuá	Sítio Flor	Gurgel	Pacatanha
Characteristic						
Houses/families assessed	118	30	17	13	10	7
Mean number of subjects per house	4.3	4.5	4.4	2.3	3.2	2
Blood samples collected	387	79	63	19	23	10
Females (%)	45.4	51.1	51.3	0	50	42.9
Subjects under 18 years old (%)	43.7	38.2	39.7	14	43.8	21.4
Mean age±standard deviation	25.4±21.8	27.4±20	27.2±21.8	21.2±19.4	30.3±21.12	43±31.6

Seroprevalence survey

A finger prick blood sample was collected on filter paper (Whatman No. 1). The filter papers were dried at room temperature and stored at 4 °C until processing. The eluates were tested for anti- *T. cruzi* antibodies by both indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assays (ELISA). Subjects were considered seropositive if both tests reacted positive (World Health Organization 1991).

Triatomine captures in artificial and natural ecotopes

In the four old communities, active intradomiciliar and peridomiciliar triatomine searches were exhaustively performed by two entomological technicians, only with tweezers and no dislodgment substances. In the triatomine sylvatic habitats, we searched only in trees, with aid of traps (Noireau et al. 2002), because there are no rocky outcrops in the study region, the natural *T. brasiliensis* ecotope. Besides, investigations were restricted just to the Pacatanha and Sítio Flor localities, since the palm trees as well as other native vegetation were severely reduced in the other study localities. The searches consisted of an average of 80 traps per locality, installed in the canopies of carnauba palm trees (*Copernicia prunifera*), spread randomly over different locations up to 1500 m^2, ~200 m from the dwellings. All the captured triatomines were stored in labeled plastic containers and forwarded to the laboratory in Rio de Janeiro for quantification and identification by species and evolutionary stage. Insect stools were removed by abdominal compression, diluted in saline solution and examined fresh through an optical microscope in search of the flagellates.

Characterization of isolated strains of *T. cruzi*

The parasite DNA was extracted with phenol–chloroform 1:1 and precipitated with sodium acetate and ethanol. A miniexon multiplex PCR assay was carried out for *T. cruzi* typing (*TcI*, *TcII*, Z3, or *T. rangeli*) (Fernandes et al. 2001). The amplified products were analyzed in ethidium bromide-stained agarose gels (2.5 %) and visualized under ultraviolet light. Four reference strains were adopted as controls: F (*TcI*), Y (*TcII*), Rb III (Z3), and R1625 (*T. rangeli*) (Zingales et al. 1998; Fernandes et al. 2001).

Ethical and legal aspects

The study was approved by the Human Research Ethic Committee of Fiocruz (protocol number 0139/01). The use of animal as bait in the traps was licensed by Committee of Scientific Use of Animals, Fiocruz (protocol LW-24/13). The Brazilian Institute of Environment and Renewable Natural Resources (Sisbio) approved the capture and transport of the triatomines from Caatinga to Rio de Janeiro.

Results

Housing characteristics

All houses of the four old communities, as well as those of Bela Vista and Serra Dantas, possessed tile roofs. Considering the houses of Pacatanha, Sítio Flores, Gurgel, and Arapuá, 37.7 % (n=16) had plastered mud walls; 34.9 % (n=15) had bare mud walls (Fig. 1.1); 23.3 % (n=10) had a mixture, part brick part mud walls, and only 4.7 % (n=2) had finished plastered brick walls. In all four communities, bathrooms were absent in 79.1 % of the houses. Permanent annexes, such as chicken coops, pigsties and corrals, were present in the peridomiciles of all houses, on average 1.54±1.48 annex/house, most in Arapuá (average 2.24±1.52 annexes/house) and least in Sítio Flores (average 0.75±0.87 annex/house). Chickens, followed by dogs, represented the main species of domestic animals circulating in the peridomestic environment. Regarding Bela Vista and Serra Dantas, all houses had finished painted plastered brick walls and ceramic tiled cement floors (Fig. 1.2). All consisted of one bedroom, living room, kitchen, indoor bathroom, a small porch, and a small utility area in the rear. The backyards were very small, with few domestic animals, most of them, chickens, dogs, and cats. The houses were uniformly arranged in rows, on both sides of the street.

Seroprevalence of Chagas disease

We tested 387 people in Bela Vista and Serra Dantas, 19 in Sítio Flor, 63 in Arapuá, 10 in Pacatanha, and 23 in Gurgel. Only four residents were *T. cruzi* positive in both IIF and ELIS A. Therefore, seroprevalence was considered 0.6 % (3/466) in the new settlements and 0.8 % (1/115) in the old communities. Regarding the four residents *T. cruzi* positive in both tests, the age/sex distribution was as follows: two men, one 60 years old and the other 61 and two adult women, one 25 and the other of unknown age.

Triatomine captures in artificial and natural ecotopes

In the new settlements Bela Vista and Serra Dantas, we did not find triatomines. Regarding the old communities, in Arapuá three *T. brasiliensis*, one *T. pseudomaculata,* and one *P. lutzi* were captured inside a house, only *P. lutzi* being infected. In Sítio Flor, one *T. brasiliensis* and one *T. pseudomaculata*, both adult males, were captured inside a dwelling; three *T. brasiliensis* and three *T. pseudomaculata* were captured in peridomiciles, none of them infected (Table 2). In Gurgel, no triatomines were found. In Pacatanha, 55 specimens of

Fig. 1 *1* Traditional rudimentary mud huts in which a wood frame is mud packed, with bare walls whose crevices provide shelter for triatomines during the day, so they can suck the blood of the residents at night on a regular basis. *2* Standardized housing in new settlements. The brick walls plastered and painted hinder the establishment of triatomine colonies

T. brasiliensis in all developmental stages were caught inside a small elementary school (Fig. 2), most of them hidden behind wall posters (Fig. 2.1) and in cardboard boxes containing books and other school supplies. Others were circulating freely, crawling on the walls of one classroom where ~20 children were attending class (Fig. 2.2). Most of the walls bore a great amount of triatomine vestiges such as feces and exuvia (Fig. 2.3). Thirty-three of the 55 *T. brasiliensis* were examined, however none was infected. Along the entire length at the inside top of the roof, there were many bird nests directly above the classroom, as there was no ceiling (Fig. 2.4). Perhaps these birds were the sole food source for the triatomines, which would explain the absence of infection since fowl are refractory to *T. cruzi*.

Regarding triatomine presence in the *C. prunifera* palm trees, 132 *R. nasutus* specimens were captured in Pacatanha. Sixty-one were examined, of which 18 (46.2 %) were infected with *T. cruzi*. In Sítio Flor, 13 *R. nasutus* were captured. Nine were examined, but only one (11.1 %) was infected. The miniexon multiplex PCR characterized the isolated strains of *T. cruzi* from bug feces as *T. cruzi* I.

Discussion

This study describes some epidemiological features of Chagas disease in a scenario under sociodemographic transition, in which rural communities present a reduction in population and are, to some extent, substituted by planned resettlements, arranged in the form of streets, with standardized, prefabricated improved housing. Data confirm low prevalence of Chagas disease, both in the old communities and in the new settlements, suggesting interruption of permanent transmission of the parasite. As almost all dwellers in the new settlements also spent most of their lives in rudimentary and infestation vulnerable housing, there is a great intersection in Chagas disease exposition histories between the two sets, which may explain similar prevalence rates.

Table 2 Chagas disease vectors collected in six localities in the municipality of Jaguaruana, state of Ceará, northeastern Brazil, 2007–2008

	New settlements		Old communities			
	Bela Vista	Serra Dantas	Arapuá	Sítio Flor	Gurgel	Pacatanha[a]
Species/ecotope						
Triatoma brasiliensis						
Intradomicile	–	–	3 M	1 M	–	8 N_1, 3 N_2, 2 N_3, 2 N_4, 5 N_5, 10 F, 25 M
Peridomicile	–	–	–	3 M	–	–
Triatoma pseudomaculata						
Intradomicile	–	–	1 M	1 M	–	–
Peridomicile	–	–	–	3 M	–	–
Panstrongylus lutzi						
Intradomicile	–	–	1 M	–	–	–
Peridomicile	–	–	–	–	–	–

[a] In Pacatanha, all triatomines were collected inside a school

M male, *F* female, $N_{1–5}$ first–fifth instar nymphs

Fig. 2 Chagas disease vectors inside a small elementary school. *1* Bugs hidden behind wall posters, *2* nymph crawling on wall, *3* vestiges of feces on wall, *4* bird nests in the roofing

Standardized prefabricated housing in the new settlements, even though located amidst a triatomine-infested area, is significantly more secure when compared to that of the older communities. Few houses in the older communities could be considered safe concerning the colonization by Chagas disease vectors. In addition, many structures in the house peridomestic environments, such as goat and sheep corrals, chicken coops, and pigsties, are highly vulnerable to triatomine colonization. In these structures, Chagas disease vectors can easily feed on domestic animals (Sarquis et al. 2006; Coutinho et al. 2012). Such environments act as a link between natural insect habitats and the houses (Freitas et al. 2004; Lima et al. 2008; Carvalho-Costa et al. 2010). Thus, despite regular chemical treatment with pyrethroids, we found Chagas disease vectors in some older dwellings in the communities, including inside houses. Perhaps the best example of coexistence between triatomines and man, in a region where these insects are abundant in nature, is the primary school attended by children living in all study sites. The presence of bird nests in the roofing of the school enabled an intense colonization by triatomines inside the classrooms. The intestinal contents of these insects were examined without evidence of the presence of trypanosomes, suggesting that birds, refractory to *T. cruzi* infection, are their main food source.

On the other hand, some insects captured in the houses of the older communities were positive for trypanosomes. *T. cruzi* genotyping demonstrated the presence of *T. cruzi* I, usually existent in a sylvatic cycle (Guhl et al. 2009). This coexistence of vectors and man, also leads to the risk of food-borne outbreaks of Chagas disease if, for instance, the utensils used for preparing school lunches are contaminated with triatomine feces, which were plentiful on the school walls. Although more common in the Amazon, food-borne Chagas disease outbreaks also occurs sometimes in northeastern Brazil (Valente et al. 2009; Bastos et al. 2010).

Previous studies highlighted the presence of native triatomine species in Northeastern Brazil (Alencar 1987). Thus, *T. brasiliensis*, a species that inhabits rocky shelters, *T. pseudomaculata,* eminently arboreal and *R. nasutus*, primarily harboring in palm trees, are the principal transmitters of Chagas disease in Ceará (Silveira et al. 2001; Lima et al. 2008). Chemical treatment with pyrethroid insecticides has been the cornerstone of Chagas disease control in Brazil, although in certain regions, it is impossible to eradicate the triatomine species, which potentially invade the domestic and peridomestic spaces from the wild environment. With the significant reduction in Chagas disease incidence rates, we progressed to the monitoring phase, in which the entomological surveillance actions are essential.

In the studied region, vulnerability to Chagas disease arises from interplay between historical factors related to the type of colonization and the consequent sociodemographic landscape, together with ecological factors characterized by the natural circulation of the parasite and its vectors. Transmission occurs at very low intensity, and the vulnerability is associated mainly with casual interaction with vectors that can potentially invade homes or contaminate food. The interaction with the vectors will be much less frequent in the settlements of improved homes, where conditions for colonization of the peridomestic environment by transmitting insects were not observed.

This survey describes, at a micro-regional level, how dynamic the determinants of Chagas disease can be, once they are a result of extreme rural poverty. Associated to specific socioeconomic conditions, these determinants can undergo sudden changes when alternatives to local development arise. Since it is impossible to eliminate the native insects, significant improvements in housing, income distribution and poverty elimination should provide the conditions to break the cycle of Chagas disease transmission in Northeastern Brazil.

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