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Investigation of Chagas disease in four periurban areas in northeastern Brazil: epidemiologic survey in man, vectors, non-human hosts and reservoirs

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ABSTRACT

Chagas disease was investigated in four periurban areas of Ceará state, northeastern Brazil, through serological, parasitological and molecular methods in humans, reservoirs and vectors. A cross-sectional survey revealed a seroprevalence rate of 1.2% (13/1076 residents, six also proving positive through PCR). *Trypanosoma cruzi* infection was not detected in children under 10 years old. *Triatoma pseudomaculata* prevailed in the peridomiciles: 63 specimens, 69% (34/49) infected with trypanosomatids. *Rhodnius nasutus* was captured in *Copernicia prunifera* palm trees (n = 280; 25.0% infected with trypanosomatids) and inside dwellings (n = 8, all uninfected). *Trypanosoma cruzi* seropositive reservoirs, represented by *Didelphis albiventris* (n = 27), *Rattus rattus* (n = 24), *Thrichomys laurentius* (n = 2), *Mus musculus* (n = 1) and *Monodelphis domestica* (n = 1), were identified. Among domestic dogs (n = 96) seroprevalence reached 21.9%. Miniexon multiplex PCR assays characterized TcI in triatomines. Both TcI and TcII were detected in wild mammal hosts. We conclude that *Trypanosoma cruzi* circulates within a domestic zoonotic cycle, requiring continuous surveillance. Insecticide application to domiciles does not appear to prevent continuous reintroduction of wild triatomine specimens, presenting a challenge to authorities involved in Chagas disease control.

1. Introduction

Chagas disease, or American trypanosomiasis, is a systemic parasitosis caused by the flagellate protozoa *Trypanosoma cruzi* which is mainly transmitted by hematophagous insects, the triatomines, via the insect's feces after a bite.[1] The protozoan can also be transmitted from mother to child congenitally and during birth,

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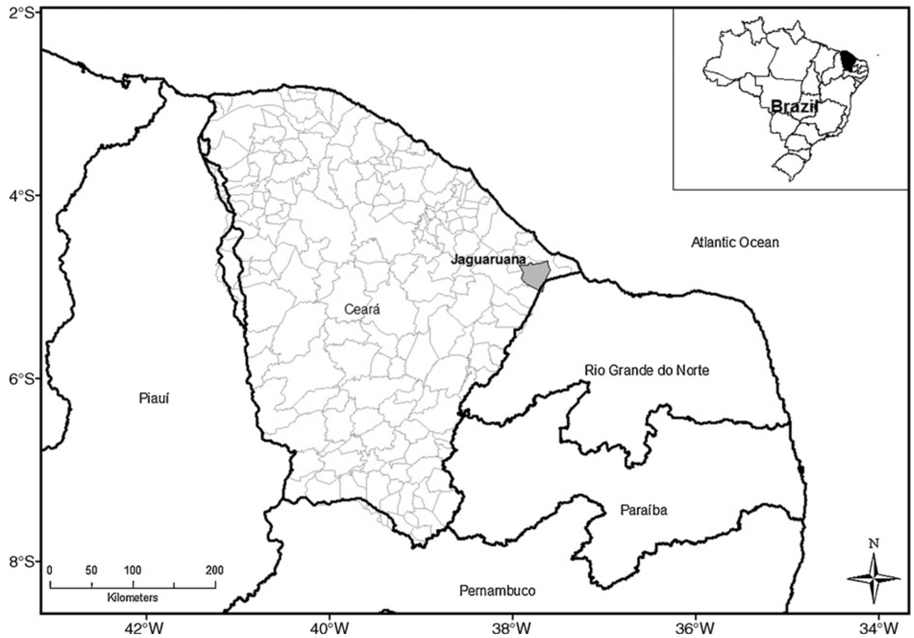

Figure 1. Location of the Jaguaruana municipality in the state of Ceará, region Northeastern, Brazil.

parenterally through blood transfusion, or in laboratory accidents and through the ingestion of foods contaminated with *T. cruzi*, as has been reported in Brazil in localized outbreaks.[2,3]

It is estimated that about 18 million people are infected by *T. cruzi*, primarily in Latin America, with nearly 21 000 registered deaths each year.[4] Chagas disease is broadly diverse with respect to prevalence, mode of transmission, protozoan genetics, pathogenicity, vector species and hosts. The disease transmission to man is essentially related to two socio-demographic factors, low quality of dwellings in the endemic regions promoting the establishment of domiciliary vector colonies and human ecological intervention.[5,6] Transmission of *T. cruzi* by blood derivatives and organ transplantation has become an ever increasing concern in many non-endemic countries as a result of ongoing immigration from Central and South America.[2,7–10]

Chronic and acute Chagas disease can be a life threatening disease. However, infection is frequently asymptomatic and evolves to an indeterminate phase, from which approximately 30% of cases develop a chronic stage characterized by myocarditis or dilation of hollow viscera.[11]

The infection is associated with two main lineages of *T. cruzi*: *T. cruzi* I (TcI) and *T. cruzi* II (TcII). *T. cruzi* I is linked to the enzootic sylvatic cycle, infecting man to a less severe degree of the disease in the Central American countries and in the Amazon basin; TcII is related to the domestic cycle in the Southern cone, producing more severe disease.[6] However, a more discriminating classification has recently been proposed, clustering *T. cruzi* strains into six discrete typing units, TcI to TcVI.[12]

After the Southern Cone Initiative, a multinational program for Chagas disease eradication,[13] Brazil was certified as free from *Triatoma infestans*-associated transmission of *T. cruzi* by the Pan-American Health Organization.[14] Nevertheless, vector borne transmission of *T. cruzi* associated with indoor invasion and potential domiciliation of wild triatomine species[15] still seems to persist in some Central and South American regions.[16] The interaction between man and sylvatic triatomines naturally infected with *T. cruzi* has been generating a great number of acute cases, mostly from food-borne outbreaks.[3,17,18] This study aims to characterize the circulation of *T. cruzi* in man, triatomines and mammal reservoirs in periurban areas in the State of Ceará, northeastern Brazil, providing an ecological and epidemiological scenario of Chagas disease in the studied area.

2. Materials and methods

2.1. Study area

The study was developed in the Jaguaruana municipality, situated in the Jaguaribe River Valley, state of Ceará (4°50′90″S, 37°46′48″ W; Figure 1). The altitude is 20 m, annual temperatures range from 23–33 °C and rainfall is approximately 850 mm/year. The rainy season extends from January to June, the dry season from July to December, and the population is approximately 31 600, half of which reside in rural areas. The survey was carried out in four localities situated in the periurban region, during five consecutive days in the dry season and five in the wet season. The periurban regions were defined as transitional zones or surroundings where urban and rural activities were juxtaposed and landscape features were subject to rapid modifications induced by anthropogenic activity. These localities, Dió, Caatinguinha, Córrego das Melancias and Perímetro Irrigado, consist of loamy and sandy soil in the domain of the Caatinga (scrublands), a characteristic vegetation of the semi-arid Brazilian northeast region. It is common to find the *Copernicia prunifera* palm tree growing in close proximity to the dwellings, most of which have been standing for over 40 years, and although the plastered brick walls and roof tiles prevail, several homes were in poor condition, with cracks and crevices as well as dilapidated plaster. Bare clay walls were scarce.

All dwellings in the four communities were visited, and a census was performed in order to assess the population of the studied areas. Córrego das Melancias, the largest community, was the poorest and had the greatest number of residents with 545 people and Caatinguinha the smallest with 111 people; Dió had 503 residents and Perímetro Irrigado 253. There was good cooperation from the inhabitants; 77.3% agreed to participate for Chagas disease diagnostic testing. Most of the non-participation was attributed to absence due to daily activities at the moment of our visit, which was held during the daytime.

2.2. Prevalence of *Trypanosoma cruzi* infection in man

In two cross-sectional surveys, all residents who agreed to participate (n = 1076), including children, were investigated after signing a written consent form. To evaluate the seroprevalence of Chagas disease, finger prick blood samples were collected on Whatman 50 filter paper for an initial trial. Two samples from each individual were wrapped in transparent plastic film. In order to detect anti-*T cruzi* IgG, an indirect immunofluorescence assay (IIFA) (Biomanguinhos®, Rio de Janeiro, Brazil) was performed. Additional intravenous blood samples were collected in glass tubes from positive or inconclusive individuals in accordance with the IIFA, in order to conduct confirmatory serology (IIFA plus ELISA [Biomanguinhos®]) as well as hemoculture and PCR tests.

For hemoculture, approximately 3 mL of whole blood was placed in medium Novyl, McNeal and Nicolle (NNN) medium, covered with an overlay of liver infusion tryptose (LIT), mixed with 10% fetal calf serum and 140 mg/mL of gentamicin sulfate. For PCR, 10 mL of blood was collected and added to the same volume of guanidine-HCl 6 M/ EDTA 0.2 M solution, kept at room temperature and submitted to *T. cruzi* k-DNA detection with primers 5′- AAATAATGTACGGG(T/G)GAGATGCATGA-3′, and 5′-GGTTGCATTGGGTTGGTGTAATATA-3′, in order to amplify the 330 bp fragment containing the hyper variable regions of *T. cruzi* minicircle k-DNA. This study was approved by the Research Ethics Committee of Fiocruz (*protocol number 039/04*).

2.3. Entomological survey, detection and characterization of *Trypanosoma cruzi* infection in triatomines

Triatomines were searched for within each dwelling and in the peridomestic environments (chicken coops, pigpens, corrals, perches and piles of bricks, tiles, wood and straw). *Copernicia prunifera* palm trees within 200 m proximity of the houses were also investigated for bugs, with the aid of efficient live-bait adhesive traps appropriate for triatomine capture.[19] The caught bugs were stored in labeled plastic containers and forwarded to the laboratory for identification of species and developmental stages according to Lent and Wygodzinsky's keys.[20]

All specimens that arrived alive in the laboratory were inspected in order to assess the natural *T. cruzi* infection rate. Feces, removed by abdominal compression, were diluted in saline and examined under an optical microscope (400X), the numbers of epimastigote and trypomastigote forms per μL quantified using a Neubauer chamber.

Trypanosoma cruzi strains isolated from triatomines were characterized through PCR. 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*), as previously described.[21,22] 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*).[21,22]

2.4. Reservoir survey, detection and characterization of *Trypanosoma cruzi* in small mammals

Small mammals were captured with live traps (Tomahawk® and Sherman® models) baited with a mixture of peanut butter, banana, oat and bacon. The traps were placed along linear transects at 10m intervals between capture stations in all study sites. The trapping period was five nights per site in each survey. Three surveys per site were conducted during three consecutive years (2004, 2005 and 2006). The total sampling efforts took 1200 traps-nights. All small mammals were captured and handled in compliance with recommended safety procedures.[23] Voucher specimens were deposited in the collection of the National Museum at Rio de Janeiro Federal University, Rio de Janeiro, RJ, Brazil. The wild animal captures were licensed by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) license numbers 0202/2004 and 13373.

For detection and characterization of *T. cruzi*, rodents and marsupials were anesthetized (ketamine, 100 mg/kg) and had their blood collected by cardiac puncture. Two tubes containing NNN medium, covered with an overlay of LIT, were inoculated with 0.2 ml of blood from each specimen. The tubes were examined every other week for two months in cases where serological results were negative and for longer periods in positive cases with subsequent amplification in the same medium for posterior characterization. An immunofluorescence antibody test (IFAT) was applied to detect antibodies against *T. cruzi* according to standard procedures,[24] with a commercial anti rat conjugate (Sigma-Aldrich®, Saint Louis, MO, USA). For the opossum sera assay, an anti-opossum Ig fraction produced in rabbits was used followed by a commercial anti rabbit conjugate as described elsewhere.[25] The antigen adopted in *T. cruzi* serology was obtained from parasites harvested from axenic culture in the exponential phase.

2.5. Assessment of *Trypanosoma cruzi* infection in domestic dogs

In order to investigate the role of household pets in the circulation of the parasite, the presence of anti *T. cruzi* antibodies was evaluated in dogs living in the four localities, upon owner consent. Ten ml of peripheral blood obtained from the dorsal-tibial or radial veins by venipuncture was collected from 96 adult mongrel dogs (80 males and 16 females) for serological survey and examined through ELISA and IIFA. In order to assess the possible existence of cross-reaction with anti *Leishmania chagasi* antibodies, specific IIFA was also performed.

3. Results

3.1. Seroprevalence in man

Seroreactivity by age groups is presented in Figure 2. Of the 1076 blood samples collected on filter paper, 48 (4.5%) were positive for antibodies against *T. cruzi* in the IIFA tests performed on finger prick blood samples, of which 13 remained positive by confirmatory serology in both IIFA and ELISA, yielding 1.2% seroprevalence. No child under 10 years old proved positive, and hemoculture was negative in all individuals submitted to confirmatory assays, while the PCR confirmed positivity for six residents, all over 30 years old.

3.2. Entomological survey, detection and characterization of Trypanosoma cruzi in vectors

Considering the four studied localities, *Triatoma pseudomaculata* prevailed in the peridomiciles: 64 specimens were collected, 69% (34/49) infected with flagellate trypanosomatids. Nevertheless, most of the *Triatoma pseudomaculata* captures (21 specimens) took place in the

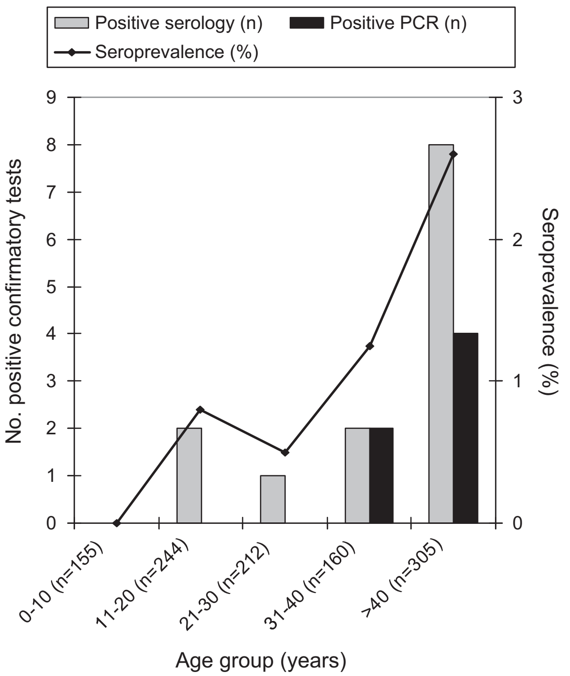

Figure 2. Prevalence of *Trypanosoma cruzi* infection in four periurban areas in Jaguaruana, State of Ceará, Northeastern Brazil (p = 0.009, test for linear trend).

Table 1
Number of triatomines captured in four periurban localities of Jaguaruana, State of Ceará, Brazil, in both dry and rainy seasons

Locality and Season	Ecotopes		
	Inside dwellings	Peridomestic environment	Palm trees (*Copernicia prunifera*)
Dió			
Rainy	0	1	4
Dry	0	9	48
Córrego das Melancias			
Rainy	1	0	6
Dry	0	21	103
Perímetro Irrigado			
Rainy	1	0	23
Dry	0	0	37
Caatinguinha			
Rainy	6	0	19
Dry	2	33	40
Total	10[a]	64[b]	280[c]

[a] 8 *Rhodnius nasutus*, 1 *Triatoma brasiliensis*, 1 *Panstrongylus lutzi*.
[b] 1 *Triatoma brasiliensis*, 63 *Triatoma pseudomaculata*.
[c] All specimens *R. nasutus*.

Córrego das Melancias and Caatinguinha peridomiciles. *Rhodnius nasutus* was captured not only in *C. prunifera* palm trees (n = 280; 24.7% [38/154] infected with trypanosomatids), but also inside dwellings (n = 8, all uninfected winged specimens). *Triatoma brasiliensis* was practically absent from all four localities, save only one unique infected adult specimen captured inside a house and another, uninfected, in the peridomestic environment. In addition, one adult infected specimen of *Panstrongylus lutzi* was captured inside a house. Flagellates in 28 samples collected from bug feces were characterized as TcI. Table 1 presents the results of triatomine captures in the four studied localities by season (dry or rainy); insects were more abundant during the dry season in the peridomestic environment and in palm trees.

3.3. Circulation of Trypanosoma cruzi in reservoirs

Six small mammal species were captured, *Didelphis albiventris* and *Monodelphis domestica* (Didelphimorphia: Didelphidae), *Thrichomys laurentius* (Rodentia: Echimyidae), *Galea spixii* (Rodentia: Caviidae) and *Rattus rattus* and *Mus musculus* (Rodentia: Muridae). The *T. cruzi* infection rates in small mammals are presented in Figure 3. Four *R. rattus* and five *Didelphis albiventris T. cruzi* strains were genotyped as TcI. In addition, two *D. albiventris* strains were characterized as TcII.

3.4. Seroprevalence survey in domestic dogs

Of the 96 blood samples collected from domestic dogs, 43 (45%) were seropositive for *T. cruzi* by IIFA, 45 (47%) by ELISA and 21 (22%) simultaneously positive by both tests. In the 21 seroreactive dogs, the titers ranged from 80 to 320 in the IIFA reaction and the optical density (OD) varied between 0.404 and 2.383, with a 0.354 cut-off in ELISA. Of the 43 cases reactive by IIFA, four (9%) also displayed

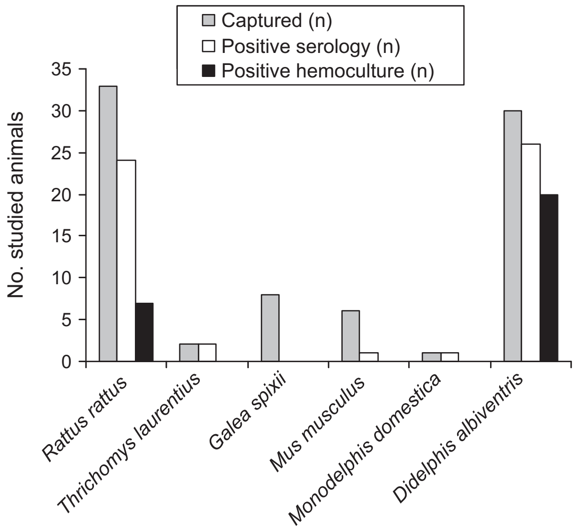

Figure 3. *Trypanosoma cruzi* infection assessment through serology and hemoculture in small mammals captured in the periurban areas of Jaguaruana Municipality, Ceará, Brazil.

reactivity to *Leishmania chagasi* although no dog exhibited any clinical signs whatsoever of leishmaniasis.

4. Discussion

According to our survey, the prevalence of Chagas disease in the studied periurban areas of Jaguaruana municipality is low, *T. cruzi*-infection not even detected in children less than 10 years old, suggesting that Chagas disease transmission is virtually interrupted in the assessed areas. This certainly reflects the success of measures based on periodical insecticide treatment of the dwellings in controlling vector-borne transmission, intensified in the early 1990s, which drastically reduced Chagas disease in vast Brazilian regions. In northeastern Brazil, Chagas disease transmission occurs inside houses, so the higher prevalence in adults can not be attributed to work activities as in some Amazonian regions. A previous study performed in 2001 and 2002 in rural areas in the same region revealed that the seroprevalence of Chagas disease was somewhat higher, reaching 3.1%.[26] Nonetheless, positive examinations were also non existent in children under 10 years old.[26] In these previously studied rural areas, inadequate dwellings that were subject to triatomine colonization were more frequent than in the periurban localities assessed herein. In this context, another factor contributing to the low seroprevalence of Chagas disease is progressive dwelling improvement, since the traditional mud huts, which allowed the establishment of intradomiciliary triatomine colonies, have become increasingly rare.

In this study, we demonstrated that *T. cruzi* circulates intensely in the domestic and peridomestic environments, where triatomines often colonize structures like corrals, pig pens and chicken coops, the triatomine species most frequently found being *Triatoma pseudomaculata*. In a previous study in four rural localities of Jaguaruana, *Triatoma brasiliensis* was significant, as well as its infection rate, when compared to *Triatoma pseudomaculata*,[27] while in the periurban regions *Triatoma brasiliensis* was very scarce. Besides, the authors disclosed that the rate of *T. cruzi* infection in *Triatoma pseudomaculata* captured in rural areas (18%)[27] was much lower than that in the same species obtained in periurban areas (69.1%).

The natural habitats of *Triatoma pseudomaculata* are bird nests and many species of trees, as this potential Chagas disease vector does not prefer a specific tree species, although an association with *Mimosa tenuiflora* (the black acacia bush) was established in regions under anthropic modifications.[28] Our data reinforce the evidence that *Triatoma pseudomaculata* is well adapted to artificial environments.[29] Previous studies in this region and other areas in northeastern Brazil demonstrated that this species is often infected with *T. cruzi* (infection rates ranging from 15–18%),[27] and migration of sylvan *Triatoma pseudomaculata* specimens towards the domestic environment has been reported. This process is facilitated by the presence of this species in the *M. tenuiflora* bush, because the wood is usually stored in the peridomiciles for both firewood and construction of pens for domestic animals, as structures apparently have been built with wood already naturally infested.[28] In addition, the attraction of *Triatoma pseudomaculata* to light was recorded, which demonstrates the importance of the active dispersal of this species.[30] It has been proposed that intervention in the natural environment of *Triatoma pseudomaculata* is the main determinant in the colonization of peridomestic ecotopes.[31] Nevertheless, *Triatoma pseudomaculata* is not frequently encountered inside houses, corresponding to 2.5% of intradomicile specimens collected in a previous survey carried out in the studied area.[27] In the present survey, we did not capture any specimens inside houses, practically all being captured in chicken coops and store rooms. Genetic studies concerning the population dynamics of *Triatoma pseudomaculata* in the Brazilian northeast suggest that peridomestic populations are continuously replaced by wild specimens, as demonstrated through the assessment of gene flow between these ecotopes.[32]

Copernicia prunifera palm trees are abundant in the study region. They are an important characteristic of the semi-arid landscape of the Caatinga biome, in the Jaguaribe River Valley, furnishing an important natural resource to the local population as the source of palm wax (Brazilian wax). These palm trees are frequently situated near the dwellings and represent the preferential habitat of *R. nasutus*.[33,34] In these palm trees, *R. nasutus* feeds primarily on birds, which are refractory to *T. cruzi*. In the present study, we surveyed palm trees situated in the peridomestic environment within 200 m of the houses. In addition, adult *R. nasutus* specimens were found inside dwellings, demonstrating the potential of this species to fly into the houses, probably attracted by light. Established *R. nasutus* colonies in domestic animal enclosures has previously been reported in the studied region, principally in straw roofing,[31] demonstrating that this species, like *Triatoma pseudomaculata*, is also an important link between the sylvatic and domestic cycle of Chagas disease. Feeding behavior studies performed with *R. nasutus* indicate that it may be viewed as a potential *T. cruzi* vector as it avidly searches for a food source, has a lengthy feeding time and frequently defecates during feeding.[35] Palm tree micro habitats contain a complete trophic network comprising different species, including distinct families of insects (including triatomines), amphibians, birds and marsupials.[36] The epidemiological importance of palm trees in the transmission of *T. cruzi* has been displayed in the Brazilian Amazon.[15,37,38] We also demonstrate that the abundance of triatomines in the peridomestic space (such as piles of wood, corrals, and chicken huts) is higher during the dry seasons, suggesting that the scarcity of food sources during the drought stimulates the insects to search for blood meals in domestic animals.

In the present study, the *T. cruzi* strains from triatomines were characterized as TcI, the lineage associated with the zoonotic sylvatic cycle of the parasite.[6] *Trypanosoma cruzi* infection in mammal reservoirs captured in the peridomestic environment was also investigated. *Didelphis albiventris* and *R. rattus* were the specimens most frequently trapped, presenting high *T. cruzi* infection rates. As reviewed by Noireau et al.,[39] marsupials of the genus *Didelphis* are important *T. cruzi* reservoirs and may act as 'vectors' of the parasite, as they are able to maintain long-lasting infections and high parasitemias, concomitantly producing amastigotes in tissues and epimastigotes in their scent glands. In addition, *D. albiventris* exhibits a synanthropic behavior adapting to human environments, mainly as an environmental disturbance or pest. Opossums, therefore, may be considered excellent links between the sylvatic and domestic cycles of *T. cruzi* transmission. In our study, five *D. albiventris T. cruzi* strains were genotyped as TcI and two as TcII. Despite the proposal that TcI is more well adapted to opossums than TcII, as demonstrated by the frequent detection of TcI but not TcII in *Didelphis*,[40] the isolation of TcII in *D. albiventris* has been evidenced.[39] *Trypanosoma cruzi* II is the lineage associated with severe disease in man in former endemic areas in Brazil, and its presence in the peridomestic environment could point to an increased risk of resurgence of symptomatic human disease if control measures were discontinued or weakened.

Among the rodents, *R. rattus* was the species most frequently captured, presenting a high *T. cruzi* infection rate, also illustrating the presence of the parasite in the domestic environment. All *R. rattus T. cruzi* strains were characterized as TcI, the association between *R. rattus* and TcI also being recently demonstrated in Chile.[41] The synanthropic characteristics of these mammals could be considered as the possible link between the sylvatic and the peridomestic cycles of *T. cruzi*, since these small mammals, particularly *D. albiventris* and *R. rattus*, are potential reservoirs and, in tune with the increase of deforestation in these regions, have become closer to the residents, possibly transforming the sylvatic cycle into a synanthropic position.

In our study, the seroprevalence survey performed in dogs revealed a very high *T. cruzi* infection rate, as dogs frequently are infected with *T. cruzi*.[39] In some Latin-American regions dogs may represent important sentinels for the detection of *T. cruzi* circulation in human habitats.[42] The high *T. cruzi* infection prevalence observed in dogs reinforces the close relationship between the zoonotic cycle of *T. cruzi* and the human population in the studied area. Additionally, rudimentary cattle, goats, pigs and sheep raising is an important characteristic of the human settlements in semi arid northeastern Brazil (the Caatinga biome), within a very close physical proximity to the animal shelters and human dwellings.

The eco-epidemiology of Chagas disease in northeastern Brazil displays peculiar characteristics when compared to the vast former endemic areas where transmission was chemically interrupted in central, southeastern and southern Brazil. Therefore, restraining the continuous introduction of *T. cruzi* into the domestic and peridomestic environment seems to be extremely difficult due to the characteristics of the potential Chagas disease vectors in the region. In this scenario, the presence of the parasite in the peridomiciles poses a real risk of Chagas disease transmission if control measures are interrupted. Chagas disease control in such an ecological background must be strictly constant, including continuous entomological surveillance of the man's environment as well as structural improvement of the houses, in order to prevent triatomines' colonization.

Authors' contributions: MML conceived the study; MML and OS designed the study; all authors analyzed data; MML, FAC-C and TGO drafted the manuscript; all authors critically revised the manuscript for intellectual content, read and approved the final version. MML is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: Procedures involving humans and animals were approved by the Ethical Research Committee and the Animal Use and Care Committee, respectively, of the Oswaldo Cruz Foundation, Rio de Janeiro Brazil.

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