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Multiple Approaches to Address Potential Risk Factors of Chagas Disease Transmission in Northeastern Brazil

Natalia Faria Daflon-Teixeira,[1] Carolina Coutinho,[2] Taís Ferreira Gomes,[1] Helena Keiko Toma,[3] Rosemere Duarte,[4] Márcio Neves Bóia,[5] Filipe Anibal Carvalho-Costa,[6,7] Carlos Eduardo Almeida,[8]* and Marli Maria Lima[1]*

[1]*Laboratório de Ecoepidemiologia da Doença de Chagas, Instituto Oswaldo Cruz, IOC/Fiocruz, Rio de Janeiro, Brazil;* [2]*Instituto de Comunicação e Informação Científica e Tecnológica em Saúde/Fiocruz, Rio de Janeiro, Brazil;* [3]*Laboratório de Diagnóstico Molecular e Hematologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil;* [4]*Departamento de Ciências Biológicas, Escola Nacional de Saúde Pública (ENSP)/Fiocruz, Rio de Janeiro, Brazil;* [5]*Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, IOC/Fiocruz, Rio de Janeiro, Brazil;* [6]*Laboratório de Epidemiologia e Sistemática Molecular, IOC/Fiocruz, Rio de Janeiro, Brazil;* [7]*Escritório Regional Fiocruz Piauí, Fundação Oswaldo Cruz, Teresina, Brazil;* [8]*Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, Campinas, Brazil*

Abstract. Chagas disease is one of the most significant systemic parasitosis in Latin America, caused by *Trypanosoma cruzi*, which is mainly transmitted by hematophagous insects, the triatomines. This research was carried out in both domestic and wild environments throughout a Northeastern rural locality. Triatomines were captured in both peridomicile and wild environments, obtaining 508 specimens of triatomines, of which 99.6% were *Triatoma brasiliensis*. Insects were captured in 10 (18.5%) peridomiciles with an average of 8.3 triatomines per residence. *Triatoma brasiliensis* nymphs and adults were found in six peridomiciles, generating a 11.1% colonization. No *T. cruzi* infection was detected in the 447 peridomestic insects analyzed. On the other hand, of the 55 sylvatic *T. brasiliensis* molecularly examined for *T. cruzi*, 12 (21%) were positive, all harboring *T. cruzi* I. The blood meal analysis by enzyme-linked immunosorbent assay from gut content revealed that both peridomestic and wild triatomine populations fed mainly on birds, refractory to the parasite, which may explain the null rate of natural infection prevalence in the domestic environment. However, infected triatomines for potential home infestation within the radius of insect dispersion capacity were registered in rock outcrops around the dwellings. Anthropogenic environmental influences are able to rapidly alter these scenarios. Therefore, to avoid disease transmission to humans, we recommend constant vector control combined with periodic serological surveillance. The associated methodology presented herein may serve as a model for early detections of risk factors for Chagas disease transmission in the Brazilian Northeast.

INTRODUCTION

The bloodsucking insects of the subfamily Triatominae (Hemiptera: Reduviidae) are the vectors for the protozoon *Trypanosoma cruzi*, the etiological agent of Chagas disease. In endemic regions, the bug distribution map is intimately related to poverty because domestic transmission occurs in low-income populations living in precarious housing conditions.[1–3]

Currently in Brazil, the Chagas disease epidemiological scenario is divided into two situations regarding the eco-epidemiological transmission features. In the Amazon region, where there are no vectors colonizing households, there has been an increase in *T. cruzi* transmission to humans because of food contaminated with triatomine feces.[4] The second is characterized by endemic areas where *Triatoma infestans* was found in the past. There were 13 states and 726 municipalities infested by this vector that currently has only residual foci that eventually emerge in the states of Bahia and Rio Grande do Sul.[5] This triatomine species was probably originating from Bolivia and widespread in Brazil until the late 1990's, being commonly found in dense colonies harboring high *T. cruzi* rates inside houses.[3,6]

An intensive Chagas Disease Control Program (ChDCP) was adopted to combat *T. infestans* and to avoid contamination via blood banks. Brazil was certified as free of Chagas disease transmission by this species and by blood transfusion.[5–7] Although the ChDCP did not directly target native vectors, domiciliary captures for these insects has also decreased dramatically because of house spraying directed toward *T. infestans*. However, even though this species has never been encountered in Ceara state, performed serological surveys , even before ChDCP, frequently evidenced autochthonous cases of Chagas disease in the state, leaving no doubt about the role of native vectors in Chagas disease epidemiology. In addition, several authors have reported that in regions formerly infested by *T. infestans*, the control is opening a niche for dispersion and infestation of native triatomines.[8–10]

Because the residence features may strongly influence triatomine infestation, it has been suggested that transmission can be greatly affected by the manner in which the endemic area inhabitants treat their domiciles and surroundings.[11] Deforestation can reduce the natural food supply, forcing both bugs and potential *T. cruzi* reservoirs to change their behavior to search for food and shelter in artificial environments, such as peridomiciles and even intradomiciles. In a scenario where both vectors and potential mammal reservoirs are in a synanthropic process, there is an evident risk of (re)emerging Chagas disease transmission for the residents.[12] The Brazilian Northeast has been struggling to control *T. cruzi* transmission by autochthone triatomine species, mainly *Triatoma brasiliensis* and *Triatoma pseudomaculata* among others, frequently found colonizing domestic structures.[13,14] As sylvatic triatomine populations are uncontrollable, *T. brasiliensis*, currently considered the most notorious species in Brazil, represents a perennial source for house infestations and, consequently, an unceasing threat.

This study aims to evaluate the triatomine fauna, investigate the feeding sources and determine the *T. cruzi* genotype

* Address correspondence to Carlos Eduardo Almeida, Rua Monteiro Lobato, 255, Barão Geraldo, Campinas, São Paulo 13083-862, Brazil, E-mail: almeida_ce@hotmail.com or Marli Maria Lima, Laboratório de Ecoepidemiologia da Doença de Chagas, Instituto Oswaldo Cruz/ Fiocruz, Av. Brazil 4365, Rio de Janeiro 21045-900, Brazil, E-mails: mmlima@ioc.fiocruz.br and marlilima@uol.com.br.

harbored by the insects captured in a rural locality of Ceará state where these bugs frequently colonize domiciles. Moreover, we investigate the sylvatic areas around the houses as the probable infestation sources. The socioeconomic conditions of residents and the use of dwelling surroundings, such as peridomestic annexes and domestic animals are also considered. By combining all this information, we are able to detect risk situations and, hence, provide a model for other sites.

MATERIALS AND METHODS

Study area. Russas city lies in the lower Jaguaribe river micro-region (lat. 4°56′25″S, long. 37°58′33″W), and the rural locality, called Cipó, is situated approximately 20 km from the city center. The area is inhabited by people living in precarious economic conditions as a consequence of the periodic droughts. Notwithstanding, several families (approximately 230 people) live in this locality in 80 dwellings. We managed to obtain information about dwelling characteristics and peridomiciliary environments in 48 (60%) houses because 32 (40%) were uninhabited or locked during our visits. There is no municipal water supply, sewerage, nor regular garbage collection. Therefore, trash is usually accumulated in the household surrounding areas. The vegetation is dense and open shrub. Rock outcrops are present in considerable amounts throughout the region extending from the wild environments up to some peridomiciles.

Domiciliary unit (DU) profiles. To ascertain the local population profile and triatomine infestation, all dwellings were visited except those closed or abandoned, and a census was taken to evaluate the residents' habits. In intradomiciles and peridomestic environments, the triatomine search took place during the day with the aid of flashlights and tweezers, not using insect dislodging substances. All fissures inside and outside dwelling walls were carefully investigated, as well as picture frames, photographs, furniture, and beds. In the peridomicile, environments consisted of the entire area around the houses including temporary or permanent constructions for resident use or animal shelter, such as chicken coops, corrals, perches, and brick-tile-wood/piles and straw, all also investigated.

Investigation in the wild environments. Rocky outcrops were present throughout the locality in the backyards of the dwellings. As *T. brasiliensis* is the most important Chagas disease vector in the northeast[15] and awareness that their natural habitat is rocky outcrops,[16] searchers were inclined toward this vector. Fifty-five rocky outcrops, located in a range of 70–1,300 meters from houses, were investigated at night because of the insect's nocturnal activities, incorporating the "capture by exhaustion" method, that is, collectors continued the captures until no more bugs were visible. For both natural and artificial environments, we obtained permission from dwelling owners/residents for the investigation.

All captured triatomines were stored in labeled plastic containers and forwarded to the laboratory in Rio de Janeiro for quantification, specific identification, and evolutionary stage determination.[13,15,16] Some samples of reference of each triatomine species collected are deposited in the *Laboratório de Ecoepidemiologia da doença de Chagas, Instituto Oswaldo Cruz, IOC/Fiocruz*. To investigate the natural *T. cruzi* infection (NTcI), insect feces were removed by abdominal compression, diluted in saline solution and examined under optical microscopy. A Neubauer chamber was enlisted to quantify epimastigote and tripomastigota forms per microliter.

Feeding sources. The blood meal type in the gut contents of nymph and adult triatomines was identified by indirect enzyme-linked immunosorbent assay (ELISA), following Burkot et al.[17] Triatomine feces obtained by abdominal compression were diluted in carbonate bicarbonate buffer and applied to polystyrene microplates. After washing, the conjugate (anti-rabbit IgG, horseradish peroxidase conjugated) was added for a new incubation and washing. This procedure was conducted with the buffer application, and the plates were analyzed in a microplate reader with a 492-nm operational filter and a 600-nm reference filter. The cutoff point was defined as the mean value of the negative controls plus two standard deviations. Positive samples were those reading 10% over the cutoff. The following antisera were tested: birds, chicken (*Gallus gallus*), cat (*Felis domestica*), cockroach (*Periplaneta americana*), dog (*Canis familiaris*), opossum (*Didelphis marsupialis*), horse (*Eqqus caballus*), human (*Homo sapiens*), lizard (*Tupinambis meriane*), rodent (*Rattus norvegicus*), goat (*Capra hyrcus*), armadillo (*Dasypus novencinctus*), and sheep (*Ovis aries*).[18]

***Trypanosoma cruzi*.** The parasite isolation of infected bugs[19] was adapted to the technique by Bronfen et al.[20] In a biological safety cabinet, class II, each infected insect was placed in Falcon tubes containing 5 mL of white sterilizing solution (HCL, 0.25 g; NaCl, 6.5 g; concentrated HCL, 1.25 mL; ethanol, 250 mL; and distillated water, 750 mL) for 1 hour 30 minutes. With the aid of forceps, we cut the posterior portion of the triatomine abdomen to remove the intestinal tract. The intestine was diluted in phosphate buffered saline (PBS); macerated with a glass stick and dispensed in a glass tube containing 4 mL of LIT medium (Liver Infusion Tryptose) supplemented with 10% fetal bovine serum, plus 6.6 mg/mL ampicillin; incubated at 28°C; and examined 7 days later. From the protozoa that successfully grew, 0.5 mL of the parasite culture was transferred to another tube with LIT medium including an antibiotic to enhance parasite replication. DNA extraction from the epimastigote obtained culture medium was achieved with the reagent DNAzol® (Cincinnati, OH) as recommended by the manufacturer.

For genotyping, we used the protocol of Fernandes et al.[21] Amplifications via polymerase chain reaction consisted of 35 cycles of denaturing at 94°C, annealing at 50°C, and extension at 72°C. Polymerase chain reaction products were subjected to electrophoresis on 2.5% agarose gel and visualized under ultraviolet (UV) light. With this methodology, amplifications that produce a fragment of 200 bp are characterized as *T. cruzi* I (TcI), those with 250 bp are *T. cruzi* II, and fragments with 150 bp are zymodeme 3. According to Fernandes et al.,[21] the mini-exon is a tandemly repeated gene that has proved to be a useful marker for typing trypanosomatids by using the following primers (TCI: 5′ACAC TTTCTGTGGCGCTGATCG; TC2: 5′TTGCTCGCACACTCGGCTGCAT; TC3: 5′CCGCGWA CAACCCCTMATAAAAATG; TR: 5′CCTATTGTGATCCCC ATCTTCG; and ME: 5′TACCAATATAGTACA GAAACTG). For details on the entire methodology, please see Fernandes et al.[21] DNA of a *T. cruzi* culture (Clone Brener strain) was adopted as a positive control and water was the negative.

Entomological indicators. To determine the entomological indicators, we analyzed the household infestation (HI), triatomine density, household colonization (HC), and the triatomine NTcI in accordance with the equations standardized by Pan American Health Organization (PAHO).[22]

Household infestation. Number of infested houses × 100/Number of inspected houses.

Household triatomine density. Proportion of insects captured by inspected dwellings in a location. Household triatomine density = Number of captured triatomines × 100/Number of inspected dwellings.

Household colonization. Percentage of infested houses with triatomine nymphs in a location. Household colonization = Number of houses with triatomine nymphs × 100/Number of inspected houses.

Triatomine NTcI. Prevalence of *T. cruzi* natural infection in a given triatomine population. Natural *T. cruzi* infection = Number of infected triatomines × 100/Number of examined triatomines.

Geographic distribution and infestation foci. All triatomine infested domiciles and rocky outcrops in the Cipó locality were georeferenced with a global position system, and the spatial analysis was displayed in a geographic information system with the TerraView version 3.3 software (Boston, MA). Because rock outcrops are considered the main natural *T. brasiliensis* ecotope, we generated a buffer with a radius of 200 m around the infested rocks to assign higher risk areas within the triatomine dispersion capacity. This method is based on the assumptions that rocky outcrops in sylvatic environments are the main natural *foci* for *T. brasiliensis* reinfestation[23] and DUs insecticide spraying to eliminate vectors is being successfully conducted. The radius for the buffer was based on the flight capacity of *T. infestans*[24] because of the absence of the same information for *T. brasiliensis*.

RESULTS

Household data. One hundred ninety-seven people were living in the investigated houses with an average of four people per structure. Although the dwellings were relatively large, averaging five rooms, the houses only had one bedroom because most residents (75%) had the habit of sleeping in hammocks slung in one unique room. All dwellings had tile roofs, 46% had incomplete plaster walls, and 83% had cement floors. Few dwellings possessed indoor plumbing with piped water, internal bathroom absent in 40%. Some residents (14.6%) improvised some kind of shower as a bath recourse whereas the majority (85.4%) used a bucket in rudimental constructions, usually outside.

Dogs, cats, and chickens freely circulated inside 63% of the dwellings. Most residents also reported the presence of synanthropic rodents inside the houses. Peridomiciliary annexes were observed in 85% of the dwellings, consisting of barns, corrals, henhouses, and pigpens. Also, there were wood/tile/brick piles besides rock outcrops situated in close proximity to the houses. Of the 48 surveyed dwellings, 46 (96%) had at least one type of animal breeding indoors such as dogs, cats, and/or chickens. We registered 1,090 animals living in the households, representing an average of 22.7 animals per inspected house. Table 1 shows the main types of livestock in Cipó.

Triatomine captures took place in 54 homesteads (67.5%), producing bugs in 10 peridomiciles with a 18.5% infestation. Four hundred forty-seven bugs were collected, rendering an average density of 8.3 insects per homestead. No intradomicile presented bug infestation or vestiges, such as eggs, feces, or exuvia. In peridomiciliary areas, we surveyed 90 annexes, finding only two species, a 4th stage nymph and an adult female of *T. pseudomaculata* captured in a woodpile and a chicken coop, respectively. Nevertheless, both were discarded from the analysis in view of the irrelevant number. All other captured specimens were *T. brasiliensis*, mainly in woodpiles. In a sole woodpile located less than 10 m from the dwelling, we collected 279 specimens (Table 2). However, most of the captures were in the rock outcrops as four of the seven investigated rocks were infested.

In all annexes and infested rocks, the amount of captured nymphs was higher than that of adults (Table 3), but in four peridomiciles, only adults were collected, generating a colonization rate of 11.1%.

***Trypanosoma cruzi* genotyping.** No triatomine from the peridomestic environment was *T. cruzi* infected via optic microscopy methods. In the wild environment, we investigated 10 rocks and all were infested, obtaining 61 *T. brasiliensis* specimens. The intestinal contents of 55 (90.2%) specimens indicated that 12 (21.8%) were infected (Table 4). The protozoan strains from these bugs were isolated and characterized via amplification of a fragment of the non-transcribed mini-exon gene. As all exhibited a 200 pb fragment (Supplemental Figure 1), they were identified as *T. cruzi* I (= TcI and Discrete Typing Unit-DTU I). Therefore, there was no need to apply other steps for the DTU genotyping protocol for intraspecific nomenclature.[25,26] In addition, we confirmed that the remaining protozoans, by optical microscopy, were really *T. cruzi*.

Feeding sources. The ELISA analysis from both peridomestic and wild environments had 66 reacting antisera. Fifty-four insects (82%) fed on a single source, 12 (18%) reacting to two or three antisera, confirming the *T. brasiliensis* eclecticism to the food source, being able to feed on different vertebrate hosts (Table 5). Considering unique feedings, in the peridomestic and the wild environments, triatomines fed mainly on chicken (44%), armadillo (19%), and rodent (9%) blood. No sample was positive for human blood. Most food sources belonged to both environments. The opossum antigen was detected in one *T. cruzi*–infected triatomine captured in the wild environment. Considering multiple feedings, the combination of chicken/armadillo had the highest prevalence (42%), being noticed in both peridomestic and wild habitats.

Geographical distribution of infestation foci. All 10 inspected rock piles in the wild environment were infested.

TABLE 1

Breeding type, number of animals, number of surveyed households, and average number of animals per household surveyed in the Cipó locality, Russas municipality, Ceará, Brazil

Breeding type	Total *N*	Number of surveyed households	Animal average number per surveyed household
Chicken/Duck/Turkey	552	43 (89%)	12.8
Dog	28	28 (58%)	1.0
Cat	31	30 (62%)	1.0
Sheep	42	5 (10%)	8.4
Goat	187	20 (42%)	9.4
Pig	82	27(56%)	3.0
Cattle	151	15 (31%)	10.1
Horse/Donkey	11	10 (21%)	1.1
Bird	6	1 (2%)	6.0

Table 2

Investigated and infested annexes and captured triatomines in the peridomestic environments in the Cipó locality, Russas municipality, Ceará, Brazil

Annexes	Infested annexes	Surveyed domiciles	Captured triatomines
Corrals/Cattle	1	–	–
Corrals/sheep-goat	1	–	–
Pigsty	12	–	–
Henhouse	12	2	2
Woodpiles	16	3	373
Barns	11	1	8
Rocks	8	4	64
Perches	15	–	–
Tile piles	9	–	–
Brick piles	5	–	–
Total	90	10	447

In this work, we considered some rocky formations in peridomiciles together with annexes due to the house proximity.

The radius of a 200-m buffer around these rock piles showed that most of the surveyed households (72%) were in the influence area generated by the buffer.

Human inquiry. The census applied to the residents living in the inspected dwellings revealed some local characteristics: a young population, mostly children, adolescents, and young adults (Table 6), 96% born in the Cipó locality. Only 59% of the residents reported literacy, and most of the population worked in agriculture. However, some inhabitants were small traders, civil servants and retirees, children, and young people frequenting school.

DISCUSSION

American trypanosomiasis transmission has dropped in Brazil since the success of the ChDCP, an announced achievement in 2006.[27] This program targeted *T. infestans*, a species introduced and widely adapted to intradomiciles in Brazil, which has made possible the chemical insecticide control. To date, *T. infestans* has been reported solely in isolated cases.[28] However, after the ChDCP success, Brazilian health authorities realized that some autochthonous triatomines, such as *T. brasiliensis* and *T. pseudomaculata*, were also responsible for hyper-endemic foci of Chagas disease transmission in the Northeast region because *T. infestans* has never been found in Ceará, Paraíba, and Rio Grande do Norte states—states with high rates of human infection. Besides, in a recent Chagas disease outbreak in Rio Grande do Norte state, the involvement of *T. brasiliensis* has been suggested.[29] Home investigation and spraying are highly laborious and costly because native triatomines keep high pressure of domiciliary reinfestation after home spraying with chemical insecticides. Therefore, dwellings can be reinfested few months after treatment.[30,31] What is worse, many endemic municipalities have no available staff nor financial resources for Chagas disease control because of other epidemic priorities (e.g. Zica, Dengue, Leishmaniasis, and, more recently, yellow fever) and the severe Brazilian economic crises.

If on one hand *T. infestans* could be eliminated because it was restricted to domestic environments in Brazil, then on the other hand native triatomines represent a challenge because they keep foci in the sylvatic environment. Several authors[1,29,30] have stated that not only *T. brasiliensis* and *T. pseudomaculata* are able to infest homes in the region. Other local species such as *Panstrongylus megistus*, *Rhodnius nasutus* and *P. lutzi* are also frequently found in domiciles. Nevertheless in our study, *T. brasiliensis* was almost the only species captured in the Cipó locality. This triatomine is geographically disseminated in all states in the north of the São Francisco river in the Brazilian Northeast where it has been encountered in high densities and prevalence with regard to *T. cruzi* natural infection.[15]

Prior studies in the northeast region confirmed the presence of *T. brasiliensis* in rock outcrops.[16,23,32] It is well known that this species is predominant in the Russas municipality, Ceará,

Table 3

Number of specimens according to the developmental stage caught in annexes in peridomestic environments of the Cipó locality, Russas municipality, Ceará, Brazil

	Local of capture			
Evolutive stage	Henhouses	Woodpiles	Barns	Rocks
Nymphs				
1°	–	10	–	5
2°	–	39	2	18
3°	–	82	2	17
4°	1	116	1	15
5°	–	96	1	5
Adults				
Male	–	20	1	–
Female	1	10	1	4
Total	2	373	8	64

Table 4

Number of specimens by evolutive stage and *Trypanosoma cruzi* infection on rocks in the wild environment of Cipó locality, Russas municipality, Ceará, Brazil

Triatomines	Caught, *N*	Examined, *N* (%)	Infected, *N* (%)
Developmental stage			
1°	–	–	–
2°	2	2 (100)	–
3°	9	7 (77.8)	2 (15.8)
4°	13	11 (84.6)	4 (16.7)
5°	21	19 (90.5)	4 (18.2)
Adult			
Male	8	8 (100)	1 (8.3)
Female	8	8 (100)	1 (12.5)
Total	61	55 (90.2)	12 (21.8)

Table 5

Triatomine blood meal sources detected by indirect enzyme-linked immunosorbent assay

	Peridomestic	Wild	Overall
Unique feeding source			
Chicken	16	8	24(44%)
Opossum	1	1	2(4%)
Sheep	1	1	2(4%)
Reptile	1	1	2(4%)
Rodent	5	0	5(9%)
Armadillo	8	2	10(19%)
Hemolymph	8	1	9(17%)
Subtotal for unique feedings	40	14	54
Multiple feeding sources			
Chicken/rodent	1	0	1(8%)
Chicken/armadillo	3	2	5(42%)
Opossum/hemolymph	0	1	1(8%)
Bird/chicken/opossum/rodent	1	0	1(8%)
Bird/chicken/rodent/armadillo	3	0	3(25%)
Bird/chicken/hemolymph/armadillo	1	0	1(8%)
Subtotal for multiple feedings	9	3	12
Total	49	17	66

TABLE 6
Inhabitants of Cipó, Russas municipality, Ceará, Brazil, 2010–2011 stratified by gender and age

Age group (years old)	Female	Male	Total
Child/teenager (0–19)	34	33	67 (34.0%)
Young adult (20–40)	33	31	64 (32.5%)
Adult (41–65)	22	25	47 (23.9%)
Seniors (≥ 66)	10	9	19 (9.6%)
Total	98	99	197

widely distributed in the wild environment, and inhabiting domestic and peridomestic ecotopes.[33–36] In the Cipó locality, *T. brasiliensis* remains the prevalent triatomine species representing 99.6% of the captured specimens and present in both sylvatic and domestic environments and frequently associated with other triatomines, such as *T. pseudomaculata*, *P. megistus* and *R. nasutus*.[1,13,16,37,33] However, it is postulated that *T. brasiliensis* is favored in a niche competition with other species, which may explain the almost exclusive presence of this species in the areas surrounding the studied locality.[35]

In Bahia state, Brazil, the possible factors involved in HI by *T. juazeirensis* (a member of *T. brasiliensis* species complex) and *T. pseudomaculata* have been investigated.[38] The authors observed that the chance of *T. pseudomaculata* to infest peridomestic areas is 17 times higher in preserved environments, whereas *T. juazeirensis* seems to prefer households situated in areas where the biodiversity has been altered and subject to human activities.[38] These data corroborate the prevalence of *T. brasiliensis* compared with *T. pseudomaculata* in Cipó, where homesteads associated to livestock and agricultural activities have led to severe degradation of the Caatinga biome. One must be aware that *T. brasiliensis* s.l. passed through a taxonomic revision, and all "species" south of the São Francisco River were afterward recognized as independent evolutionary units of a species complex.[16,39]

Wood and rocks have cracks that can provide shelter for *T. brasiliensis* breeding, greatly favoring infestation.[40,41] Studies carried out in rural areas of nearby sites in Jaguaruana[33] and Russas[12,40,42] municipalities have demonstrated that in the man-made environments, the triatomine captures are most productive in goat/sheep corrals, henhouses, and tile/brick/wood piles. In Cipó, most of the homesteads possess peridomestic annexes, and most residents raise at least one type of domestic animal. In addition, all infested triatomine annexes were of wood. In fact, the greatest triatomine density appeared in a woodpile, which was also inhabited by frogs, lizards, and chickens. It has been demonstrated that small synanthropic animals, such as rodents (potential *T. cruzi* reservoirs),[23,36] use these structures as shelters, providing a feeding source for these insects. Nonetheless in our study, the insects were most frequently captured in rocks, the natural breeding sites of *T. brasiliensis*, in the peridomestic environments. These rocks are in close proximity to the dwellings, where domestic and sylvatic animals move freely, contributing blood as a food source for triatomine breeding in this environment. Indeed, the high adaptation capacity of *T. brasiliensis* to human environments favors the infestation and maintenance of colonies both in rocks and in peridomestic annexes.

This scenario represents a challenge for vector control in Cipó because the insect populations in peridomestic environments are the most difficult to eliminate because insecticides have less residual effect outside mainly because of weather exposure.[42,43] The absence of triatomines and vestiges, such as feces or exuvia, inside the dwellings does not rule out the possibility of triatomine specimen existence within domestic environments (which could not be found), as emphasized by Abad-Franch et al.[12]

Triatoma brasiliensis is considered an important vector of Chagas disease because of the high natural *T. cruzi* prevalence together with the natural harboring of different *T. cruzi* strains.[14,23,35] In our research, only specimens captured in the Cipó wild habitat were infected, and the molecular typing only revealed the presence of *T. cruzi* I, indicating limited *T. cruzi* diversity in the bugs in the study area. This parasite lineage has a particular distribution throughout the Americas with some strains prevalent in certain geographical areas and transmission cycles present in both domestic and sylvatic environments.[44,45]

The socioeconomic survey demonstrated precarious household conditions which does not seem to influence triatomine infestation as no bug was caught in intradomiciles. However, we did not discard the possibility of home infestation with low triatomine densities, as detailed by Valença-Barbosa et al.[46] On the other hand, we cannot rule out the possibility of transmission to humans because the capture of infected triatomines in the wild environment indicates that the protozoon cycle is active in this habitat with infected bugs perhaps eventually invading domestic and peridomestic habitats. According to Almeida et al.,[23] the sylvatic bug populations with high natural *T. cruzi* prevalence maintains an active gene flow with domestic populations. Thus, the interconnection of wild and domestic protozoon cycles in the presence of *T. brasiliensis*, both in natural and artificial environments, makes possible the appearance of infected insects in the peridomiciliary environment.[38] In the studied locality, in both peridomiciliar and wild environments, birds and chickens were the main blood sources which may explain the absence of human population infection. Based on the environments where *T. brasiliensis* were found, Alencar[35] assumed that this triatomine has feeding preference for chicken, followed by cat, goat, and dog blood. Fowls were the most prevalent animal raised, followed by goats and cattle. However, cattle are the only domesticated animals that do not live in close proximity to the domiciles. *Triatoma brasiliensis* exhibited low anthropophily in our study when compared with other domestic species, probably because of the absence of intradomiciliary bugs.

We recognize some restraint in the ELISA technique to detect feeding sources as the hosts for standardization of the test are limited to a set of domestic or synanthropic animals. However, the method has some advantages over some newer approaches based on direct DNA sequencing via the Sanger method, which poses a limitation in the capacity to detect multiple feeding sources. Therefore, we consider the ELISA technique still useful, particularly to detect feeding on domestic animals and multiple blood feeding sources because *T. brasiliensis* is known to present dietary eclecticism.[23,47] To obtain more precise results, for further studies, we recommend the combination of both techniques as one may resolve limitations of the other or the use of metabarcoding technique.[48]

This study suggests that in addition to regular and systematic measures of chemical control in conjunction with effective entomological surveillance, the location of Cipó requires housing improvement together with better health conditions and education to provide knowledge for the prevention and control of Chagas disease. Despite the absence of any *T. cruzi* human infection detection, the epidemiological scenario is dynamic and geographically diverse. It is known that anthropogenic environmental influences may rapidly change these circumstances.[8] Therefore, we strongly recommend the combined monitoring approach herein presented for this and other rural localities across the semi-arid region of northeastern Brazil where *T. brasiliensis* and other native species frequently infest dwellings.

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Authors' addresses: Natalia Faria Daflon-Teixeira, Taís Ferreira Gomes, and Marli Maria Lima, Laboratório de Ecoepidemiologia da Doença de Chagas, Instituto Oswaldo Cruz/Fiocruz, Rio de Janeiro, Brazil, E-mails: nataliadaflon@gmail.com, taisferreiragomes@gmail.com, and mmlima@ioc.fiocruz.br. Carolina Coutinho, Instituto de Comunicação e Informação Científica e Tecnológica em Saúde/Fiocruz, Rio de Janeiro, Brazil, E-mail: carolina.coutinho@icict.fiocruz.br. Helena Keiko Toma, Laboratório de Diagnóstico Molecular e Hematologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, E-mail: hktoma@globo.com. Rosemere Duarte, Departamento de Ciências Biológicas, Escola Nacional de Saúde Pública/Fiocruz, Escola Nacional de Saúde Pública, Rio de Janeiro, Brazil, E-mail: rduarte@ensp.fiocruz.br. Márcio Neves Bóia, Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, IOC/Fiocruz, Rio de Janeiro, Brazil, E-mail: mboia@ioc.fiocruz.br. Filipe Anibal Carvalho-Costa, Laboratório de Epidemiologia e Sistemática Molecular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, and Escritório Regional Fiocruz Piauí, Fundação Oswaldo Cruz, Teresina, Brazil, E-mail: carvalhocosta70@hotmail.com. Carlos Eduardo Almeida, Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, Barão Geraldo, Campinas, Brazil, E-mail: almeida_ce@hotmail.com.

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