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Entomological triatomine indicators in the State of Rio Grande do Norte, Brazil

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Abstract *This study aimed to describe the main entomological triatomine-related indicators in the western mesoregion of Rio Grande do Norte. This is a descriptive cross-sectional retrospective study developed on a historical analysis of information on the triatomine capture carried out by the Chagas Disease Control Program, from 2008 to 2013. Five species were captured, of which the* Triatoma brasiliensis *and* Triatoma pseudomaculata*, by occupying the domestic and peridomestic environment, sequentially, and* Panstrongylus lutzi *by the highest rate of natural infection. A prevalence of nymphs among the captured specimens, a higher triatominal density in the peridomicile, infestation, colonization and natural infection rates of 5.6%, 49.6% and 0.8%, respectively, a significant difference in the distribution of specimens between the municipalities investigated and lack of declining infestation and colonization rates between 2009 and 2012 was observed. Findings suggest the need for continuous surveillance, facilitated by the association between field teams and communities, with emphasis on the proposal of health education for the recognition and notification of triatomines by the population.*

Key words *Chagas disease, Entomology, Vector control*

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Introduction

Chagas disease still represents an important public health problem, given the current prevalence of *Trypanosoma cruzi* infection of approximately 5.7 million individuals in Latin America, with the highest number of infected in Argentina, Mexico and Brazil. In the latter, specifically, it is estimated that 1.2 million people are affected by the disease[1].

Vector transmission, which occurs through the penetration of parasites into the bloodstream through the gateway created in the skin by the bite of the hematophagous insect, still is the most important route of human infection by *T. cruzi*[1], and is facilitated by the great variety of triatomines in Brazil, which houses sixty-two species, of which thirty are in the domestic environment and ten are epidemiologically important[2].

In relation to the Brazilian Northeast, the same stands out for the dispersion rates, domestic infestation, colonization and natural infection, anthropophilia and number of captures of species *Triatoma brasiliensis*, *Triatoma pseudomaculata* and *Panstrongylus megistus*. In addition, species *Triatoma brasiliensis* and *Triatoma pseudomaculata* are found in all northeastern states and are a concern due to their great dispersion and difficult control[3].

In the state of Rio Grande do Norte, the first scientific description of *T. cruzi* infection was carried out in a seroprevalence study, which showed a positivity of 15.5% of the population[4] and, in spite of vector control programs adopted since that time, the presence of nine species of triatomines are recorded, among which are *Triatoma brasiliensis*, *Triatoma pseudomaculata*, *Panstrongylus megistus*, *Panstrongylus lutzi*, *Panstrongylus diasi*, *Rhodnius nasutus*, *Triatoma melanocephala*, *Triatoma petrochiae* and *Triatoma rubrofasciata*[3]. Recent data have shown the estimated *T. cruzi* seroprevalence at 6.5% in the rural population of the Western Potiguar mesoregion, without, however, describing natural triatomine infection, infestation and domestic colonization rates[5].

This study aimed to describe the species captured in the peridomestic and intradomestic environment, the occurrence of infestation and domestic colonization, and the natural infection rates of the different triatomine species to understand the challenges in controlling vector transmission control in an endemic rural area for Chagas' disease.

Methodology

Study design

This is a descriptive, cross-sectional and retrospective study developed through an analysis of the historical series of information on triatomine capture provided by the Chagas Disease Control Program (PCDCh), corresponding to the period 2008-2013.

Study location

Rio Grande do Norte is located in the northeastern region of Brazil, bordering to the north and east with the Atlantic Ocean, to the south with Paraíba and to the west with Ceará (Figure 1) and has a population of 3,168,027 inhabitants[6]. It has one of the lowest HDIs in the Brazilian Northeast, with a large ruralized area and high index of low quality human dwellings, which are characterized as favorable for the triatomine shelter.

The area of this study is located to the west of the state and has 448,904 inhabitants, characterizing it as the second most populous region[6]. It has a predominance of semiarid climate, low rainfall index and the Caatinga as main vegetation. Despite housing programs, it still has houses of mud and masonry without plaster, besides peridomestic environment with the presence of animal breeding, heaps of straw, bricks, tiles and wood, conditions conducive to the emergence of the vector.For the study, we considered nine municipalities in the region with epidemiological relevance to Chagas disease, which include Apodi, Campo Grande, Caraúbas, Felipe Guerra, Governor Dix-Sept Rosado, Janduís, Messias Targino, Mossoró and Upanema (Figure 1).

Entomological indicators

The following entomological indicators were considered for analysis: number of triatomines captured (males, females, nymphs and infected), number of triatomines found in the domestic and peridomestic environment, intradomestic triatominal density (number of triatomines captured in intradomestic environment/number of intradomestic environments investigated); peridomestic triatominal density (number of triatomines captured in peridomestic environment/ number of peridomestic environments investigated); colonization index (number of households with nymphs/total number of households

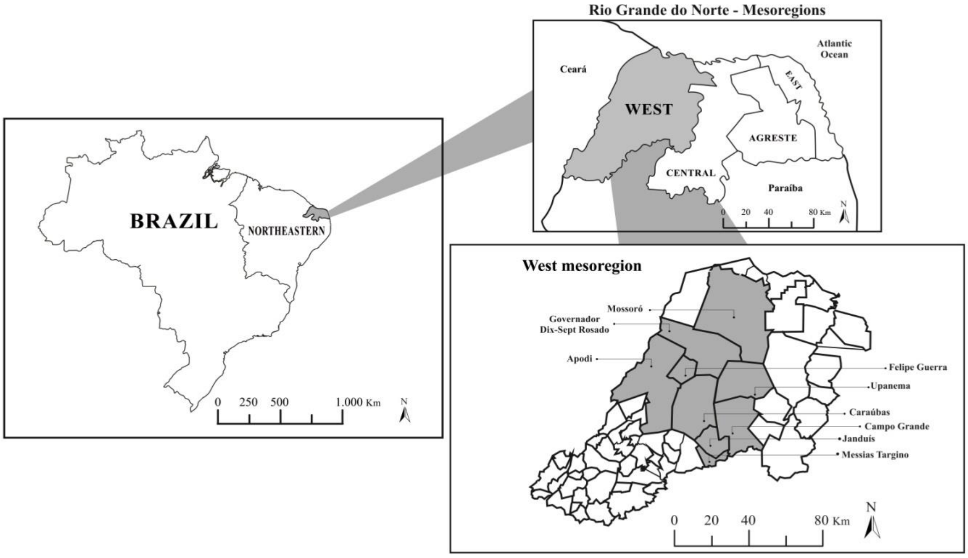

Figure 1. Map of Brazil demarcating the State of Rio Grande do Norte, the Western Mesoregion and the municipalities evaluated.

with triatomines x 100), infestation index (number of infested households/number of households investigated x 100) and natural infection index (number of infected triatomines/number of triatomines examined x 100).

Triatomines captured

The capture of triatomines in the peridomestic and intradomestic environment occurred actively (manual capture) and was carried out by endemic agents of the municipalities in household visits programmed or directly by residents of the homes studied.

In the intradomestic environment, all household dependencies were verified, besides any possibilities of shelter for triatomines, such as cracks, holes, crevices in the floor, internal and external walls, furniture and objects. In the peridomestic environment, the search was performed in animal husbandry annexes (poultry, sheep, goats, pigs, cattle and horses), wooden piles, bricks, tiles, straws and warehouses or storehouses.

Metal tongs of different sizes and lanterns were used for the inspection of cracks and places lacking luminosity that could serve as shelter for triatomines, without any use of traps. All the captured triatomines were placed in polyethylene bottles with shredded or folded paper and sealed with a perforated lid for better preservation. Vials were duly labeled, identified and recorded on the Chagas Disease Control Program form.

Taxonomy

The records of triatomine species cases in the investigated municipalities were provided by the Regional Chagas Disease Control Coordination of the Second Regional Health Administration of Rio Grande do Norte.

Entomological indices: natural infection, home infestation and colonization

The study on natural triatomine infection by *T. cruzi* was carried out in the Laboratory of the II Regional Health Administration located in the municipality of Mossoró (RN).

The direct parasitological technique was adopted by abdominal compression of the insect to collect the fecal material in saline solution (NaCl 0.9%), which was deposited on a slide and examined in an optical microscope with a magnification of 400x. In the positive cases, slides were stained by the Giemsa technique and sent for confirmation at the Central Laboratory in Natal (RN). The natural infection index was obtained from the ratio between the number of infected triatomines and the number of triatomines examined.

Data review

Descriptive statistics were used to obtain the relative and absolute frequencies of triatomine data. The Kruskal Wallis test was used to verify the association between the distribution of the number of captured triatomines and the study sites. The ANOVA and Tukey tests (multiple comparisons) were used to identify significant differences in entomological indexes between study sites. The Student's t test was selected to observe the difference of means between the intradomestic infestation index and the peridomestic infestation index in the study period. The simple linear regression test was performed to verify correlation between the entomological indices and the years of the study.

The analyses were performed through the Statistical Package for the Social Sciences (SPSS), version 20 (Chicago, IL, USA) and a significance level of 5% was established.

Results

According to a report provided by the Chagas Disease Control Program, 5,370 triatomines were captured, of which 50.6% (2,662/5,370) were in the form of nymphs and 75.5% (4,053/5,370) occupied the peridomestic environment (Table 1), so that statistical significance was detected between the intradomestic and peridomestic densities, with the latter being highlighted.

The general household triatominal density was 0.11. A statistical difference ($p < 0.05$) was observed between the number of triatomines captured and the locations included in the study, with a higher number of specimens in the rural area of Apodi.

The species captured were *Triatoma brasiliensis*, representing 56.9% (3,053/5,370), *Triatoma pseudomaculata*, with a percentage of 41.8% (2,247/ 5,370), *Panstrongylus lutzi*, corresponding to 0.7% (35/5,370), *Rhodnius nasutus*, with 0.5% of the total (25/5,370) and *Panstrongylus megistus*, indicated by 0.2% (10/5,370) (Table 1).

The species with the highest triatominal densities corresponded to *T. brasiliensis* and *T. pseudomaculata* (Table 2).

The overall natural infection rate among triatomines was 0.8%. Among the infected species found in intradomestic environment, *T. brasiliensis* and *T. pseudomaculata*, with 9 and 7 contaminated specimens, respectively, were highlighted (Table 1). While *P. lutzi* represented a lower triatomine concentration, it was responsible for the higher rate of natural infection among the captured triatomines (Table 2).

Of the 47,095 households investigated, 5.6% (2,630/47,095) evidenced triatomines, reflecting the overall household infestation index. The distribution of infested households among the studied sites showed statistical significance ($p < 0.05$), especially the municipality of Campo Grande, with the highest infestation rate seen in the analysis period, of 47.7% in 2012 (Table 3).

The overall colonization index corresponded to 49.6%, so that 17.3% (472/2,727) of the nymphs were located in the intradomestic space. Considering the species captured in the intradomestic environment, 71.3% (938/1,317) were *T. brasiliensis*. Of the species *T. pseudomaculata*, 85.4% (1,919/2,247) was found in the peridomestic environment (Table 1). Comparing the colonization coefficient between the locations considered in the study, a significant difference ($p < 0.05$) was observed, underlining the municipality of Caraúbas, with 100% domiciliation in 2013 (Table 3).

All municipalities in the study showed contaminated specimens during the analyzed period (Table 3).

The distribution of colonization rates and domestic infestation shows the progression of these values between the years 2009 and 2012, denoting the discontinuous control of entomological indicators throughout the analyzed period. There was a statistical difference ($p < 0.05$) between the rates of domestic and peridomestic infestation over the period analyzed, so that the peridomicile had a higher infestation rate in all years of the study (Graphic 1).

Discussion

After more than four decades of fighting Chagas' disease vector transmission in Brazil, the setting investigated still has a wide distribution of triatomines and is among the regions originally at risk for vector transmission, along the states of Alagoas, Bahia, the Federal District, Goiás, among others, a condition explained by the number of autochthonous and potentially vector species[7], such as *T. brasiliensis*[4-8] and *T. pseudomaculata*[9] and the emergence of "new species", such as *P. lutzi*[10], all of which are found in the field of this study.

The prevalence of nymphs among the specimens captured in the region is the characteristic

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Table 1. Trypanosoma cruzi captured and positive synanthropic triatomines corresponding to the Western Mesoregion (RN), Brazil, from 2008 to 2013.

Species	Intradomestic				Peridomestic			
	Male	Female	Nymph	Infected	Male	Female	Nymph	Infected
Triatoma brasiliensis	317	226	401	4	502	414	1,199	5
Triatoma pseudomaculata	116	86	127	2	566	363	996	5
Rhodnius nasutus	15	1	1	...	8	2	...	...
Panstrongylus megistus	6	2	...	...	2	...	...	...
Panstrongylus lutzi	24	5	2	...	6	3	1	1
Total Captured	478	320	531	6	1,084	782	2,196	11

Source: Chagas Disease Control Program of the Second Regional Health Administration of Rio Grande do Norte.

Table 2. Values of intra- and peridomestic triatominal densities and rate of natural infection by captured species, related to the municipalities of the Western Mesoregion (Brazil), from 2008 to 2013.

Species	Intradomestic triatomine density	Peridomestic triatomine density	Natural infection index *(%)*
Panstrongylus megistus	0.00017	0.00004	0.0
Panstrongylus lutzi	0.00055	0.00019	2.5
Rhodnius nasutus	0.00031	0.00021	0.0
Triatoma brasiliensis	0.01990	0.04490	1.2
Triatoma pseudomaculata	0.00690	0.04074	0.1

Source: Chagas Disease Control Program of the Second Regional Health Administration of Rio Grande do Norte.

of the adaptive action of triatomines to the artificial ecotope, consolidated in the domestic process[11,12], which did not show a declining artificial ecotope in most of the years considered and may be related to the invasion of this environment by females in fertile period[13].

The higher concentration of triatomines in the extradomestic space, reaffirmed by the significant difference between the means of the intradomestic and peridomestic densities was also a finding denoted in other studies[13], and can be explained by the focus on measures to combat the domiciled vectors[14] and by the appropriate conditions for agglomeration of colonies in the peridomicile promoted by careless cleaning of domestic annexes[15]. In addition, the occupation of this space can act as a defense mechanism for triatomines in response to the reduced species in the households through chemical control, which compose residues in the peridomestic space, where the insecticides do not perform satisfactorily[15-17] due to the effects of the climatic variations, of the direct incidence of solar rays and rainfall[18].

The occupation of the domestic environment, while showing less representativeness compared to the number of specimens detected, should not be ignored, indicating the need for routine surveillance in residences of locations with high triatomine burden in the peridomicile[14]. Studies indicate the risks of the transfer of insects from the extradomestic space to within households, related to the habits of individuals transporting wood and their own belongings from the wild environment to the household and its surroundings, which may contain triatomines, especially in the younger forms, and may cause passive dispersion of vectors[19]. In addition, the possibilities of transmission also go back to the development of activities such as cattle raising and the process of disorderly appropriation of the natural environment, followed by abrupt changes in vegetation and reducednumber of wild animals[20].

The high prevalence of *T. brasiliensis* and *T. pseudomaculata*[5] was also a finding that was repeated in the states of Sergipe, Ceará[3], Piauí[21-24] and Pernambuco[25], confirming the general panorama of Chagas' disease in the Northeast, with regard to *P. megistus* and *T. brasiliensis,*and P. megistus has decreased its occurrence practically throughout the Northeast, with *T. brasiliensis* being the main species, followed by *T. pseudomaculata*[13].

In any of the analyzed years, the study showed infestation rates higher than those of the North-

Table 3. Entomological indices of triatomines in the municipalities of the Western Mesoregion (NR), Brazil, from 2008 to 2013.

Municipalities	Indices* (%)	Year						$\overline{X}$
		2008	2009	2010	2011	2012	2013	
Apodi	DII	11.0	4.4	7.1	3.9	5.2	7.8	6.8
	CI	46.8	23.8	24.0	11.1	47.0	37.3	31.7
	NII	...	0.3	1.4	...	0.5	0.4	0.4
Campo grande	DII	37.3	4.9	6.1	38.2	47.7	17.8	25.5
	CI	54.0	7.4	43.7	40.0	34.4	31.8	35.2
	NII	...	...	0.8	...	...	...	0.1
Caraúbas	DII	11.0	2.9	3.7	2.7	11.5	5.2	6.0
	CI	100.0	60.0	66.6	50.0	37.5	100.0	69.0
	NII	...	2.2	...	...	...	...	0.3
Felipe guerra	DII	11.0	2.0	2.8	1.1	4.9	1.0	3.8
	CI	28.5	33.3	50.0		33.3	50.0	49.2
	NII	...	...	...	5.0	...	...	0.4
Gov. Dix Sept Rosado	DII	16.4	2.0	8.3	5.2	...	...	5.3
	CI	...	75.0	27.7	52.9	...	...	25.9
	NII	...	...	...	0.7	...	...	0.1
Janduís	DII	16.9	8.4	11.8	10.0	8.6	...	9.2
	CI	...	...	...	...	50.0	...	8.3
	NII	1.8	...	...	1.6	...	...	0.6
Messias Targino	DII	2.5	3.6	3.4	3.1	...	...	2.1
	CI	...	...	20.0	...	...	...	3.3
	NII	36.3	2.5	3.1	...	...	...	7.0
Mossoró	DII	1.1	2.5	1.1	3.4	2.9	6.5	2.9
	CI	11.1	35.1	46.1	38.4	68.7	57.1	42.8
	NII	...	...	...	...	...	...	0.0
Upanema	DII	15.0	10.0	2.5	7.4	5.7	11.0	9.3
	CI	30.7	100.0	35.1	40.0	66.6	75.0	61.6
	NII	...	1.5	...	...	...	...	0.3

*Indices: DII- Domestic Infestation Indices, CI – Colonization Indices, NII –Natural Infection Indices.
Source: Chagas Disease Control Program of the Second Regional Health Administration of Rio Grande do Norte.

east, in a survey conducted in the 1990s, similar to that of Ceará, in the same decade[3], and lower than the state of São Paulo[26], data that is reaffirmed in the stagnated intradomestic and peridomestic colonization and infestation indices during the years studied, with emphasis on this last index, higher than intradomestic invasion throughout the analyzed period. In this perspective, the reduced entomological indices related to the peridomicile of the region under study is an important challenge, since the infestation of the domestic environment is not due to insecticide's ineffectiveness, but elements such as the modification of natural areas, the use of material for the construction of annexes coming directly from the wild and without any specific treatment regarding the handling[25] and breeding of animals[27].

The region's colonization index neared that reported in Piauí[21] and exceeded the respective foci rates in the state of São Paulo[11], Minas Gerais[9] and Rondônia[28], a condition that may have been caused by reduced food demand[28,29], modification of wild environments[29] through the deforestation or realization of fires and reduced fauna through predatory hunting, practices inherent to the field of study.

Although it represented the highest expression among the triatomines found in the intradomestic environment, since it resembles the reality of the state of Piauí[21], the *T. brasiliensis* species had a peculiar behavior in the region evaluated, considering that the extradomestic space was its preferred environment. It persists as a concern and priority in Public Health in the areas of its occurrence[23] and is characterized by great capacity for infestation, colonization[8,13] and reinvasion of the home by wild foci[24], establishing itself as a priority target of vector control campaigns

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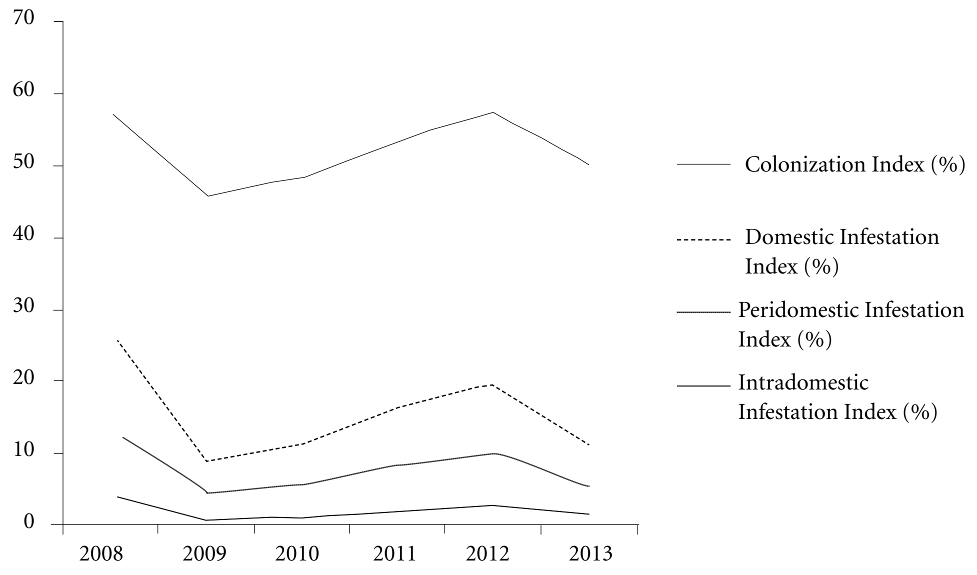

Graphic 1. Distribution of the entomological indices of the Western Mesoregion (RN), Brazil, from 2008 to 2013.

Source: Chagas Disease Control Program of the Second Regional Health Administration of Rio Grande do Norte.

among municipalities of states such as Piauí[21]. In this setting, considering the classification of this species as the main species[13] and considering the high seroprevalence of Chagas' disease, highlighting the Western mesoregion[5], the role of *T. brasiliensis* as a vector disseminator of *T. cruzi* infection is reaffirmed.

The species *T. pseudomaculata*, characteristic of the extradomestic space[30,31], as in other settings investigated, was predominant in the peridomicile[13,21,22,31-33], although the number of nymphs captured in the intradomestic environment surpassed that of Ceará[31], Piauí and Paraíba[21,31]. The condition of occupation of *T. pseudomaculata* restricted to the peridomicile determines the difficulty for the traditional chemical control, since the insecticides demonstrate a transient effect in a situation in which the habitat corresponds to the extradomestic space, so that reinfestation becomes a common process[24,34], attributed to the great pressure of recolonization by this species[24]. However, its classification as a second species in intradomestic space triatominal density, right after *T. brasiliensis*, reinforces its progressive adaptation to the artificial ecotope, as reported in other studies[3,35]. Habits such as passive firewood management for daily use and timber transportation are considered as possible facilitating factors for the residence of *T. pseudomaculata*[21], in addition to, in the situation of a larger number of specimens, this species colonizing the interior of households and take over the spot of *T. brasiliensis*. In addition, it was found that certain man-made structures were occupied by both species, indicating that *T. pseudomaculata* is gradually becoming dominant[16], maintaining infestation and colonization levels both in the household and in the peridomicile, with great invasive potential and is difficult to control[13]. Therefore, the adaptation of *T. pseudomaculata* to the intradomestic behavior raises the need for further investigation, given that the approximation of vectors with human living spaces increases the risk of transmission of Chagas disease[22].

The study shows the persistence of triatomines capable of transmitting *T. cruzi* in the region, so that the natural infection index was similar to that found in the same state in the 1980s[3], although the proportion of infected triatomines has been low, as shown in another study[36], and is lower than that observed in the state of Piauí[6] and in Minas Gerais[11,37].

Among the infected species detected in the intradomestic environment, it is worth noting that, although it evidenced lower intradomestic and peridomestic density than *T. brasiliensis*, *P. lutzi* showed the highest rate of natural infection among the species considered and was distributed in almost all of the locations of the study, deserving attention for the increased number of captures in Brazil[38], as it can easily form colonies inside the households[13] and has one of the highest rates of natural infection in other locations[21,38].

T. brasiliensis also showed representativeness and is the main vector in the transmission of *T. cruzi* in Northeast Brazil[8,9,21] and greater power of synanthropy[8,39,40].

The *T. pseudomaculata* species also showed infected captured specimens, although some studies consider that the risk for vector transmission through this species is low when compared to *T. brasiliensis*[41] due to its low conversion rate for metacyclic trypomastigotes[32,40,42], the infecting form of vertebrates[32]. On the other hand, while some studies show a low natural rate of infection[13,43], a survey carried out in the state of Bahia showed that all samples of *T. pseudomaculata* collected for seroprevalence responded positively to the *T. cruzi* test, which denotes the representativeness of this species in the transmission in regions where the presence of the vector is clear[10].

The *P. megistus* species, despite being a highly dispersed agent in the Brazilian territory, with a recognized potential for infestation and colonization of households[21] and high levels of infection[21,44,45], showed low density in the evaluated region, besides not having evidenced no positive species for *T. cruzi*[10,21], differently from the State of Ceará and Bahia in the 1990s and the Federal District[10,13,46]. We would like to add that this was the only species among the ones found that did not have any nymph captured in the intradomestic environment. However, this finding does not disqualify the epidemiological importance of the species for the area, since the presence of adults in the peridomestic environment can trigger the establishment of colonies in the intradomestic space and maintenance of the parasite's transmission cycle[13].

Similarly, the *R. nasutus* species had a reduced number of specimens captured and none of the triatomines evaluated were contaminated, as also shown by the literature[23]. However, it is worth mentioning that two-thirds of the numbers detected were in the intradomestic environment, a finding that diverged from other reported realities[8,13,21], showing that, although this species does not frequently colonize households, a possible household invasion may occur from wild environments, increasing the risk of household vector transmission without actual colonization[47-49].

A significant statistical association between triatomine distribution and study sites was found, similar to the findings in the Federal District[47] and in Piauí[8]. Several factors may explain this situation, from the assiduity of the surveillance actions of the endemic agents in the municipalities[9], encompassing the whole rural area characteristic of each location, the environmental conditions, the state and organization of households and the community's commitment to surveillance of triatomines[8].

The lack of declining infestation and colonization rates in most of the years addressed in the study can be explained by the appearance of other diseases, such as dengue[26,50] and American visceral leishmaniasis[26], which triggered the redirection of activities in the area of education in health and interfered with the actions of notification of triatomines by populations[51]. In addition, low prevalence rates of Chagas' disease in some regions and political-administrative issues related to systematic control programs[46] have contributed to the disrupted follow-up of entomological surveys over the years[26,46].

Findings of the study demonstrate that the main obstacle to be overcome in the Chagas disease vector transmission control is continuous surveillance, assuming that household invasions from the extradomestic environment will always be possible[11]. In this context, community participation in the triatomine detection process is required[47] in order to decentralize of entomological surveillance actions by the field teams[48], a measure with a significant impact, as demonstrated in some regions[11,13,47,49-51].

Final considerations

The focus setting demonstrated a still critical area for the proliferation of Chagas' disease, given the numbers of captured triatomines and the potential vectors detected in the region, such as *T. brasiliensis*, representing the highest intradomestic density among captured triatomines; *P. lutzi*, characterizing the highest rate of natural infection; and *T. pseudomaculata*, which has entered households, translating its adaptive action to the various ecotopes of human experience. It is also worth noting that this study faxed some limitations, which started from the lack of data referring to some locations, a factor that may have underestimated the actual indexes analyzed.

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The recognition of the entomological triatomine-related indicators in the region facilitated the approximation with the distribution of vectors and the vulnerability of communities to the *T. cruzi* infection, and was a relevant tool for the design of control and surveillance actions that avoid the possibility of propagation of the most popular species and prevent the recrudescence of the extirpated species.

We suggested the systematic follow-up of entomological surveillance by field teams, as well as the involvement of local communities in the detection, recognition and consequent notification of vectors in the intradomestic and peridomestic spaces and in the reorganization of the surroundings of households, highlighting the appropriation of measures that promote popular education in health as the strengthening element of this process.

Collaborations

MAF Barreto, MAF Cavalcanti, CM Andrade, EGC Nascimento and WO Pereira worked on the design, outline, data analysis and interpretation, writing, critical review and approval of the version to be published.

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