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ORIGINAL ARTICLE

ENTOMOEPIDEMIOLOGY OF CHAGAS DISEASE IN BELÉM, PARÁ, BRAZILIAN AMAZON: A RETROSPECTIVE ANALYSIS (2010–2023)

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ABSTRACT

Chagas disease is classified as a neglected disease and has a high prevalence in the Northern region of Brazil, with outbreaks primarily associated with oral transmission. Nevertheless, triatomines, the vectors of *Trypanosoma cruzi*, play a significant role in this transmission cycle. This study aimed to describe the occurrence of vectors in the municipality of Belém, based on previous reports from the local population. A retrospective descriptive study with a cross-sectional component was conducted from 2010 to August 2023, focusing on the occurrence of *T. cruzi* vectors causing Chagas disease in urban and peri-urban areas of Belém. Data were obtained from the Municipal and State Health Departments of Pará. The information was organized in Microsoft Excel spreadsheets, and spatial distribution maps were created using the open-source software QGIS®. During the study period, the following triatomine species were recorded: *Rhodnius pictipes*, *R. robustus*, *Triatoma rubrofasciata*, *Panstrongylus geniculatus*, and *P. lignarius*, totaling 145 specimens collected. The majority (71%) of the specimens belonged to *T. rubrofasciata*, predominantly collected indoors, with a higher incidence in January. In 2023, a *T. rubrofasciata* specimen was found in a residence in the Jurunas neighborhood, parasitized by *T. conorhini*. Of the total specimens collected, 11% tested positive for flagellates resembling *T. cruzi.* Between 2010 and 2022, 376 cases of acute Chagas disease were reported, with the highest incidence in the neighborhoods of Benguí, Jurunas, Marco, and Icoaraci.

KEY WORDS: Triatomines; entomological surveillance; vector control; Amazon.

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INTRODUCTION

Discovered by the renowned Brazilian sanitarian Carlos Chagas in 1909 during his investigation of parasitic fauna in malaria-endemic areas of Minas Gerais, Chagas disease emerged amidst studies on blood-feeding insects infesting low-income households, commonly known as "kissing bugs." While researching these insects in the region, Chagas identified a parasite in the vector's digestive tract. After extensive testing, he confirmed that this parasite could be transmitted to mammals and hypothesized that it could also infect humans. Chagas subsequently examined blood samples from the local population and detected the flagellate in a two-year-old girl (Chagas, 1909).

In the Brazilian Amazon, the first case was reported in the State of Pará in 1969 (Shaw et al., 1969). Today, Brazil's Legal Amazon is considered endemic for Chagas disease due to the rising number of human cases, including an increasing number of acute cases reported in the region, underscoring the disease's emergence as a public health issue (Coura et al., 2002). Triatomines are insects that may or may not be infected with *Trypanosoma cruzi*, the etiological agent of Chagas disease. Vectorial transmission occurs through the feces of these blood-feeding arthropods and was long considered the primary epidemiological mechanism, responsible for up to 80% of disease cases (Dias et al., 2016).

Studies conducted around Belém provide significant insights into the vector and the diseases etiological agent, confirming the presence of *Triatoma rubrofasciata* in Belém, first documented in 1910 (Deane, 1947). More recently, this species has been observed to be widely distributed across the city. In 1949, Deane and Damasceno reported naturally infected specimens of *Panstrongylus lignarius* carrying *T. cruzi* in the Utinga forest, Belém.

There is an observable increase in the frequency of these insects in urban environments, particularly in Brazil, where such encounters are more pronounced compared to other regions of the Americas (Carbajal-de-la-fuente et al., 2002). This study aims to provide new perspectives for managing Chagas disease in Belém, highlighting the critical role of entomological surveillance as an essential component in investigating this endemic disease.

MATERIAL AND METHODS

This is a retrospective descriptive study with a current cross-sectional component on the occurrence of triatomines, vectors of Chagas disease, in the municipality of Belém, covering the period from 2010 to August 2023. Through the collection and analysis of data, an entomo-epidemiological route of Chagas disease was mapped in the capital of Pará State, which has a territorial area of 1,059,466 km² and an estimated population of 1,506,420 (IBGE, 2022).

Entomological data were obtained by requesting specific information about triatomines found in Belém during the study period. Using Excel spreadsheets as a database, the locations where these insects were found were georeferenced within the municipality using QGIS®, an open-source Geographic Information System (GIS) application that allows for georeferenced data visualization, editing, and analysis. The data were recorded in structured spreadsheets with descriptive variables, including: 1) epidemiological variables (collection/capture date, location within the residence – intradomicile or peridomicile, full address including neighborhood, and geographic coordinates); and 2) demographic variables of the insect population (gender, species, number of specimens collected, and infectivity).

For temporal organization, the data were divided into two groups: retrospective data (2010 to 2022) and current occurrences (2023). This organization reflects the methodology of the study, which combines incidental findings of triatomine specimens and field investigations conducted in the collection areas.

The analysis of triatomine infectivity based on retrospective data was conducted at municipal and State entomology laboratories affiliated with the health surveillance services of the respective health departments. Entomological surveillance is supported by standardized capture and submission bulletins, prepared by trained entomology technicians and used at both municipal and State levels to routinely record entomological data. Data provided by municipal and State authorities were organized into spreadsheets summarizing annual collection totals.

However, individual bulletins, which include detailed capture addresses and geographic coordinates, remain archived at the respective institutions and were not available for each specimen analyzed (Table). Inclusion criteria for the dataset were triatomine specimens submitted to municipal or State health services that were properly identified by certified personnel; for viable specimens, natural infection testing was also performed. Exclusion criteria included specimens of other Hemiptera species with no epidemiological relevance, unviable samples for morphological identification, and records with incomplete data, such as missing neighborhood information or date of laboratory entry.

These curated data were subsequently used to create species distribution maps at the neighborhood level in Belém, employing the open-source software QGIS® for georeferenced data visualization, editing, and spatial analysis. It is important to note that the georeferenced database reflects triatomine occurrence at the neighborhood level and does not necessarily represent precise capture addresses or exact geographic coordinates for each vector encounter.

Table – Triatomine species found in the municipality of Belém, by encounter location and infectivity, period 2010 – August 2023.

Year	*Triatoma rubrofasciata* *n*=104					*Panstrongylus geniculatus* *n*=13					*Panstrongylus lignarius* *n*=2					*Rhodnius pictipes* *n*=23					*Rhodnius robustus* *n*=3				
	IN	PE	NI	IE	Infectivity (%)	IN	PE	NI	IE	Infectivity (%)	IN	PE	NI	IE	Infectivity (%)	IN	PE	NI	IE	Infectivity (%)	IN	PE	NI	IE	Infectivity (%)
2010	27	0	5	13	23%	0	0	2	1	100%						1	0	1	0	0%					
2011	14	0	45	1	100%	0	0	1	1	0%						1	0	0	1	0%					
2012	0	0	1	0	0%	0	0	2	2	0%						0	0	3	3	0%					
2013						0	0	1	0	0%															
2014																									
2015	0	0	1	0	0%	0	0	1	0	0%	0	0	1	0	0%	0	0	3	0	0%	0	0	1	0	0%
2016	2	0	0	0	0%	1	0	0	0	0%						1	0	0	0	0%					
2017	4	0	0	3	66%											0	0	1	1	0%					
2018																									
2019	3	0	0	2	50%	1	0	0	1	0%						1	0	0	1	100%					
2020	1	0	0	0	0%											1	1	0	0	0%					
2021																									
2022						0	1	0	1	0%															
2023	1	0	0	1	100%	3	0	0	3	66%	1	0	0	1	0%	9	0	0	7	57%	2	0	0	1	100%
Total	42	0	52	20		5	1	7	9		1	0	1	1		14	1	8			2	0	1	1	

Legend: IN: Intradomicile; PE: Peridomicile; NI: Not Infected; IE: Infection Examination

These curated data were subsequently used to create species distribution maps at the neighborhood level in Belém, employing the open-source software QGIS® for georeferenced data visualization, editing, and spatial analysis. It is important to note that the georeferenced database reflects triatomine occurrence at the neighborhood level and does not necessarily represent precise capture addresses or exact geographic coordinates for each vector encounter.

Infectivity analysis was performed by compressing the abdomen of triatomines to collect feces, which were mixed with saline solution, placed on slides, and observed under an optical microscope at 400× magnification. This technique, introduced in 1914 with the xenodiagnosis (XD) method, proved useful for parasitological diagnosis of Chagas disease by infecting triatomines with a blood meal and waiting 90 days to investigate *Trypanosoma* in their fresh feces or intestinal content (Carvalheiro et al., 2009). Although XD is no longer used for diagnosis, triatomine dissection remains a method for detecting *Trypanosoma*. However, active searches in households, although a routine practice by municipal entomology teams, were not recorded in the bulletins.

For more recent prospective occurrences, infectivity analysis was conducted at the Chagas Disease Laboratory of the Evandro Chagas Institute. Parasitic characterization was performed using polymerase chain reaction (PCR) in collaboration with the Parasitology Laboratory at the University of São Paulo (USP), specifically PCR with Fluorescent Fragment Length Barcoding (PCR-FFLB) for *Trypanosoma* identification (Hamilton et al., 2008). Although specific quality control tests were not independently performed by the authors for species identification, all entomological procedures were conducted under a rigorous institutional routine.

The professionals responsible for specimen identification and diagnostic analyses are certified staff members working in structured entomology laboratories that operate in accordance with established biosafety and quality assurance standards. In addition, according to State Technical Note No.01/2021 from the Pará State Health Department, 10% of the triatomine samples submitted by municipalities and regional health centers are subjected to monthly quality control verification at the State Reference Laboratory. This procedure ensures the continuous validation and reliability of the species identification, microscopy, and molecular (PCR) analyses performed.

Disease case records were accessed through the TABWIN tool, developed to tabulate data from the Notifiable Diseases Information System (SINAN).

RESULTS

Between 2010 and August 2023, a total of 145 triatomine specimens were recorded in the municipality of Belém through passive surveillance. These specimens belonged to the species *Rhodnius pictipes*, *R. robustus*, *Panstrongylus geniculatus*, *P. lignarius*, and *Triatoma rubrofasciata*, all of which were adults, with no nymphs reported (Figure 1).

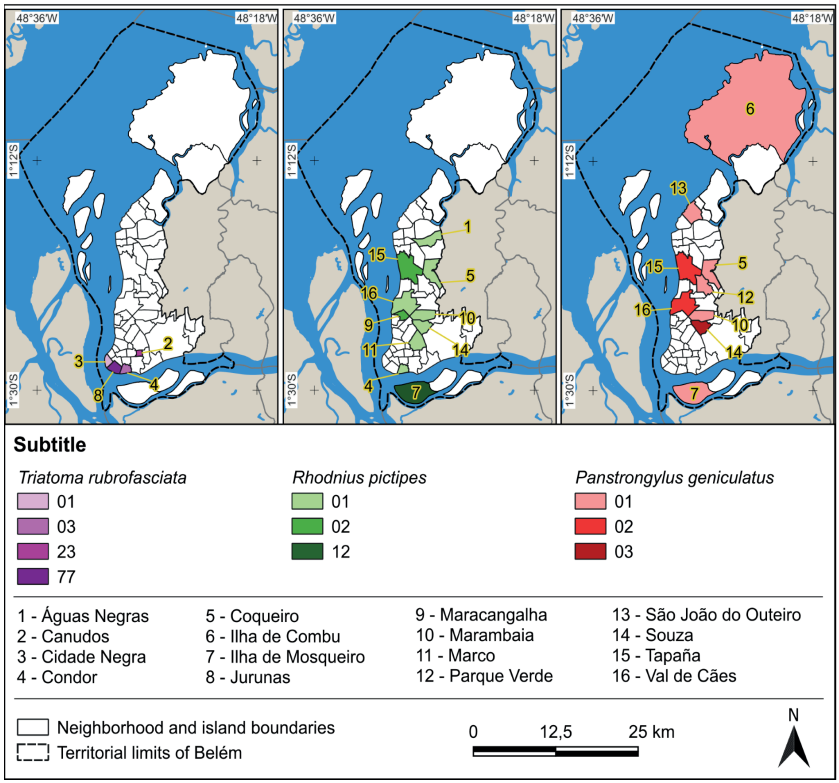

Figure 1. Occurrence of the species *Triatoma rubrofasciata*, *Rhodnius pictipes,* and *Panstrongylus geniculatus* in the municipality of Belém, State of Pará, from 2010 to August 2023.

The historical series revealed significant gaps in the notification of entomological data in 2014, 2018, and 2021, years in which no sightings of triatomines were reported to municipal or State health authorities. Regarding the locations where the insects were found, 51% were collected indoors, and 2% were found in peridomestic areas. However, 47% of the records lacked information about the capture site or presented incomplete data.

A low number of specimens underwent examination, and a lower percentage tested positive for parasites. This situation is partly due to the improper handling and storage conditions of the insects sent to the reference entomology laboratory, which frequently render them unviable for parasitological analysis.

To address this issue, it is crucial to implement an integrated strategy that includes ongoing education for health professionals and collaborators involved in the collection, transport, and delivery of specimens. Emphasis should be placed on the importance of proper handling from the moment of capture to laboratory submission. Additionally, the development and dissemination of standardized protocols are essential to ensure correct specimen storage, utilizing appropriate containers and effective preservation techniques.

Distribution of Triatomines in Belem

An analysis of the historical series reveals that the predominant species in the municipality of Belém was *T. rubrofasciata*, with 104 specimens captured, all through passive surveillance and in the adult stage. The neighborhood with the highest number of encounters with this vector was Jurunas, representing 77 specimens collected during the analyzed period. Notably, this species appeared in only four neighborhoods within the metropolitan region, three of which are located in port areas.

Triatoma rubrofasciata (De Geer, 1773) has a high prevalence in port areas and is typically a vector of *Trypanosoma conorhini*, which infects *Rattus rattus* (black rats), as this insect is closely associated with rats (Lent & Wygodzinsky, 1979). *T. rubrofasciata* is known to transmit at least two parasites: *T. cruzi* in Latin America and *T. conorhini* globally (Dujardin et al., 2015).

In 2023, a *T. rubrofasciata* specimen was reported inside the kitchen of a residence in the Jurunas neighborhood (Figure 2). The insect was captured alive in August. The homeowner reported that this was the second time a specimen of this species had been found in her house. In the infectivity test (fresh smear examination), the specimen tested positive for trypanosomatids. Using PCR analysis, the species *T. conorhini* was identified.

Additionally, two specimens of *Panstrongylus lignarius* and three specimens of *Rhodnius robustus* were reported, all found on Combú Island. One *P. lignarius* specimen was found indoors (in the bedroom). The variety of triatomine species allows them to be classified into wild and domestic species, with an intermediate category of peridomestic species. These species are occasionally attracted indoors but do not effectively colonize houses and thus feed on humans only occasionally (Waleckx et al., 2015).

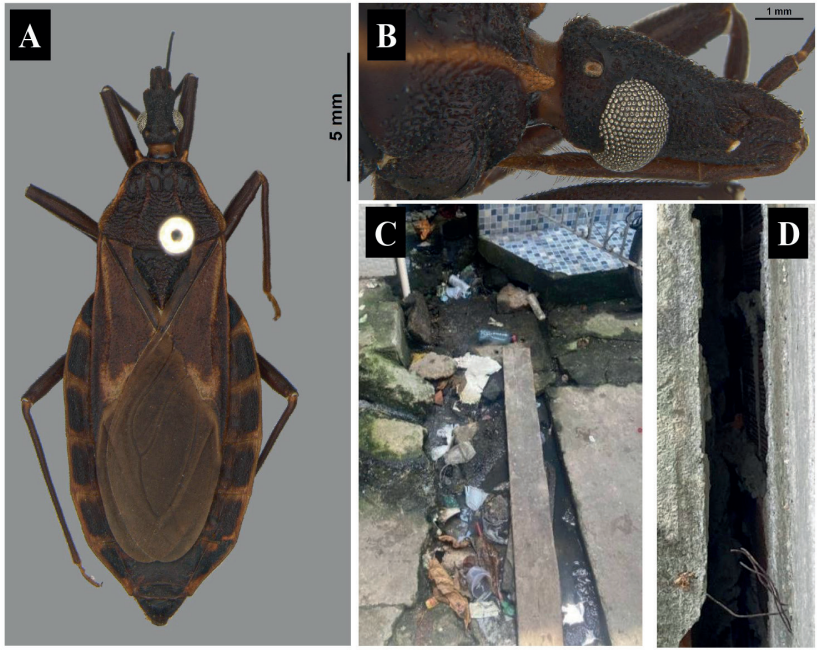

Figure 2. (A) Dorsal view of a female *Triatoma rubrofasciata* specimen; (B) Detail of the diagnostic characteristic of *T. rubrofasciata*; (C) Areas near the capture site; (D) Crevice used as a shelter by the food source associated with the captured specimens.

Reported cases of acute Chagas disease (ACD) from 2010 to 2022

From 2010 to 2022, 376 cases of acute Chagas disease were confirmed in the municipality of Belém, encompassing various neighborhoods. Among the 71 official neighborhoods, 38 localities reported ACD cases, including the districts of Icoaraci, Outeiro, and Mosqueiro, as well as peri urban areas such as Ilha de Cotijuba, Ilha do Combú, and Ilha Grande. Notably, the neighborhoods with the highest incidence were Jurunas and Benguí, followed by Marco and Icoaraci. Most cases occurred in the second semester of the evaluated years, aligning with the *açaí* harvest season when fruit handling and consumption intensify (Nascimento et al., 2021).

DISCUSSION

Although most reports indicate that *P. geniculatus* typically inhabits wild environments, this species exhibits intrusive behavior in human dwellings, particularly in deforested intermediate areas. It is attracted to artificial light from urban and rural buildings, increasing the risk of *T. cruzi* transmission (Vivas et al., 2021).

The presence of this species colonizing pigsties in peridomestic areas was reported in the municipality of Marajó, Pará (Valente, 1999). Regarding *P. geniculatus*, this species has been repeatedly associated with orally transmitted outbreaks. This hypothesis could be verified by comparing the post-feeding characteristics of this species and its natural infection rates with those of other species that invade human dwellings (Vivas, et al., 2021).

Rhodnius pictipes (Stål, 1872) is a wild species typically found in palm trees and bromeliads. However, adult specimens are often found inside homes or captured near light sources (Galvão & Gurgel-Gonçalves, 2014). This characteristic has been confirmed, as 100% of palm trees studied through a convenience sampling method harbored triatomines (Jesus, 2019). Although it primarily exhibits wild habits, this species has been found in artificial biotopes (human dwellings), suggesting a potential trend toward domiciliation (Serra et al., 1980; Silva & Silva, 1990).

A study conducted in Recife reported the presence of a *T. rubrofasciata* specimen in an urban area near the city's port (Silva et al., 2015). Another study conducted in a boarding house in downtown São Luís, Maranhão, found that most *T. rubrofasciata* specimens were infected with *T. conorhini*. However, two specimens were naturally infected with T. *cruzi* (Brazil et al., 1985).

Knowledge about this species, its behavior, and its biology remains scarce, as does understanding its role in disease transmission in urban areas. Therefore, it is crucial to emphasize that the presence of this vector in homes poses a risk and concern for local health services.

The presence of triatomines in both urban and peri urban areas of Belém correlates with all neighborhoods that reported disease outbreaks. For instance, in the Jurunas neighborhood, 35 cases of Chagas disease were recorded between 2010 and 2022, of which 28 were identified as orally transmitted according to SINAN notification forms. These outbreaks likely resulted from the sale of *açaí* without adherence to proper sanitary practices. However, a direct causal link remains unconfirmed, requiring in-depth epidemiological and entomological investigations. Such studies should include rigorous evaluations of affected individuals during and after outbreaks, complemented by detailed entomological surveys rather than relying solely on anecdotal evidence of insect sightings.

In the eastern rural Amazon, anthropogenic landscape disturbance increases the likelihood that palm tree crowns are infested by Chagas disease vectors, with higher vector densities per palm tree and per unit area. Vectorial infection by *T. cruzi* appears more common in disturbed landscapes compared to preserved ones (Santos et al., 2021). This highlights the importance of mapping entomo-epidemiological routes in urban areas to answer key questions: where, when, and why triatomine specimens are found in urban settings, and how they contribute to the high number of acute Chagas disease cases in Pará. Furthermore, it underscores the need to develop viable control measures and preventive strategies tailored to the Amazon region, particularly for oral transmission outbreaks.

This study represents an initial effort to characterize the triatomine species found in urban and peri-urban areas of Belém, Pará, emphasizing the need for new control methodologies. Addressing the uncertainties surrounding Chagas disease dynamics in the Amazon is crucial, especially considering the interplay between vector ecology, human activity, and environmental changes.

This study highlights significant findings regarding triatomine occurrence and Chagas disease cases in urban and peri-urban areas of Belém from 2010 to August 2023. A total of 145 triatomine specimens were identified, representing five species: *R. pictipes*, *R. robustus*, *T. rubrofasciata*, *P. geniculatus*, and *P. lignarius*. Notably, *R. pictipes* was detected across multiple neighborhoods, while *T. rubrofasciata* was predominantly associated with port areas. *P. geniculatus* was documented in neighborhoods such as Tapanã, Coqueiro, and Parque Verde, whereas *P. lignarius* and *R. robustus* were exclusively observed on Combú Island.

An important finding was that 11% of the examined triatomines carried flagellates in their digestive tract, underscoring their potential role in disease transmission. Additionally, 376 cases of acute Chagas disease were reported during the same period, with a notable concentration in the neighborhoods of Benguí, Jurunas, Marco, and Icoaraci. The relationship between the presence of triatomines and Chagas disease outbreaks in these areas emphasizes the urgent need for enhanced surveillance and control measures. These results provide critical insights into the dynamics of triatomine distribution and Chagas disease transmission in Belém, reinforcing the importance of targeted entomo-epidemiological investigations to mitigate public health risks.

No inferential statistical analyses were performed to correlate the occurrence of triatomines with reported cases of Chagas disease. However, the discussion highlights the presence of Chagas disease cases in neighborhoods where infected triatomines were also found. Most urban cases of Chagas disease in Belém are attributed to vector–oral transmission, mainly associated with the consumption of contaminated *açaí* pulp. Further studies are needed to determine whether the *açaí* prepared and commercialized in the city is already contaminated with triatomine feces, or if contamination occurs locally, within establishments that process and sell the fruit in neighborhoods where infected vectors have been reported.

Based on the findings of this study, we emphasize the need to advance investigations that integrate entomological surveillance, genetic analyses of triatomines, and continuous epidemiological monitoring in Belém and adjacent regions. Future studies should prioritize systematic surveys in urban and peri-urban areas with a history of acute cases and the presence of vectors, as well as the molecular characterization of triatomine populations and their associated trypanosomatids. The implementation of standardized protocols for insect collection, preservation, and laboratory analysis, coupled with the training of local teams, represents an essential step toward enhancing diagnostic capacity and strengthening public health responses. Moreover, investigations into the risk of oral transmission, particularly in the processing of fruits such as *açaí*, will be crucial to support preventive strategies and reinforce integrated surveillance of Chagas disease in the Amazon region.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

USE OF THE ARTIFICIAL INTELLIGENCE

The authors declare that no artificial intelligence (AI) tools were used in the writing, editing, analysis, or preparation of this manuscript. All aspects of the study, including data interpretation and manuscript development, were conducted exclusively by the authors.

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