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Spatial analysis of the natural infection index for Triatomines and the risk of Chagas disease transmission in Northeastern Brazil

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ABSTRACT

This study aimed to analyze the spatial pattern of natural infection index (NII) for triatomines and the risk of Chagas disease transmission in an endemic area of Northeastern Brazil. An ecological study was conducted, based on 184 municipalities in five mesoregions. The NII for triatomines was evaluated in the Pernambuco State, Brazil, from 2016 to 2018. Spatial autocorrelations were evaluated using Global Moran Index (I) and Local Moran Index (II) and were considered positive when $I > 0$ and $p < 0.05$, respectively. In total, 7,302 triatomines belonging to seven different species were detected. *Triatoma brasiliensis* had the highest frequency (53%; n = 3,844), followed by *Triatoma pseudomaculata* (25%; n = 1,828) and *Panstrongylus lutzi* (18.5%; n=1,366). The overall NII was 12%, and the higher NII values were *P. lutzi* (21%) and *Panstrongylus megistus* (18%). In the mesoregions of Zona da Mata, Agreste, Sertao, and Sertao do Sao Francisco, 93% of triatomines were detected indoors. The global spatial autocorrelation of I to NII was positive (0.2; p = 0.01), and II values calculated using BoxMap, MoranMap, Lisa Cluster Map were statistically significant for natural infections. With regard to the risk areas for the presence of triatomines, Zone 2 (the Agreste and Sertao regions) presented a relative risk of 3.65 compared to other areas in the state. Our study shows the potential areas of vector transmission of Chagas disease. In this study, the application of different methods of spatial analysis made it possible to locate these areas, which would not have been identified by only applying epidemiological indicators.

KEYWORDS: Chagas disease. Epidemiology. Triatomine. Spatial analysis.

INTRODUCTION

The species of triatomines responsible for the transmission of Chagas disease has a wide distribution and can easily adapt to household environments. The colonization of triatomines occurs due to constant changes in the natural environment caused by anthropic activities that have led to an imbalance in the ecosystem and a modification of vector behavior[1-3].

From the South of the United States, where 11 species have been found[4], to the South of Argentina and Chile, where 156 triatomine species (153 living and three fossils) that are responsible for the transmission of Chagas disease have been found[5-8]. Eight species of the *Triatoma* genus, especially *Triatoma rubrofasciata*, have been found in Africa, the Middle East, Southeast Asia, and the Western Pacific[5,9]. In Latin America, triatomines represent a substantial public health problem, as these insects transmit *Trypanosoma cruzi* (Chagas, 1909) in Bolivia, Argentina, Mexico, Brazil, Chile, Ecuador, Peru, Paraguay, Uruguay, Venezuela, Colombia, the Guianas, and throughout Central America[1,10-12].

In 2006, Brazil received an international certification for eradicating the transmission of the disease caused by *Triatoma infestans* (Klug, 1834)[13]; however, other species of triatomines can maintain the transmission cycle of disease[9]. In this country, 65 species of triatomines have been identified[14], including *Panstrongylus megistus* (Burmeister, 1835), *Triatoma sordida* (Stal, 1964), *T. brasiliensis* (Neiva, 1911), and *Triatoma pseudomaculata* (Corrêa & Espínola, 1964), and the main vector is *T. cruzi*[15-17]. In the Northeastern region, *T. brasiliensis* and *T. pseudomaculata* colonize both households and surrounding areas, thus maintaining the endemic cycle[18,19]. Moreover, Silva *et al*. collected triatomines in the Pernambuco State from 2013 to 2015 and recorded 10 species; however, only *T. brasiliensis* and *P. lutzi* (Neiva & Pinto, 1923) were positive for the *T. cruzi*[20] disease.

These findings indicate the need for eco-epidemiological studies that evaluate the transmission of *T. cruzi* by natural species, including studies that map regions according to the risk of vector transmission to better develop control strategies for public health. It is fundamental to gain knowledge regarding the distribution of triatomine fauna in endemic areas for Chagas disease in order to create relevant medical and public health guidelines. Therefore, this study aimed to analyze the spatial pattern of the natural infection index (NII) for triatomines and the risk of Chagas disease transmission in an endemic area of Northeastern Brazil.

MATERIALS AND METHODS

Study design

This was an ecological study that evaluated the distribution of triatomines, in an endemic area, for Chagas disease, namely the Pernambuco State, which is located in Northeastern Brazil[17].

Study area

Pernambuco State has a territorial extent of 98,076,001 km^2, with an estimated population of 9,616,621 as of 2020[21]. This state has 184 municipalities and a state district (the island of Fernando de Noronha) and is divided into five mesoregions[21] (Figure 1).

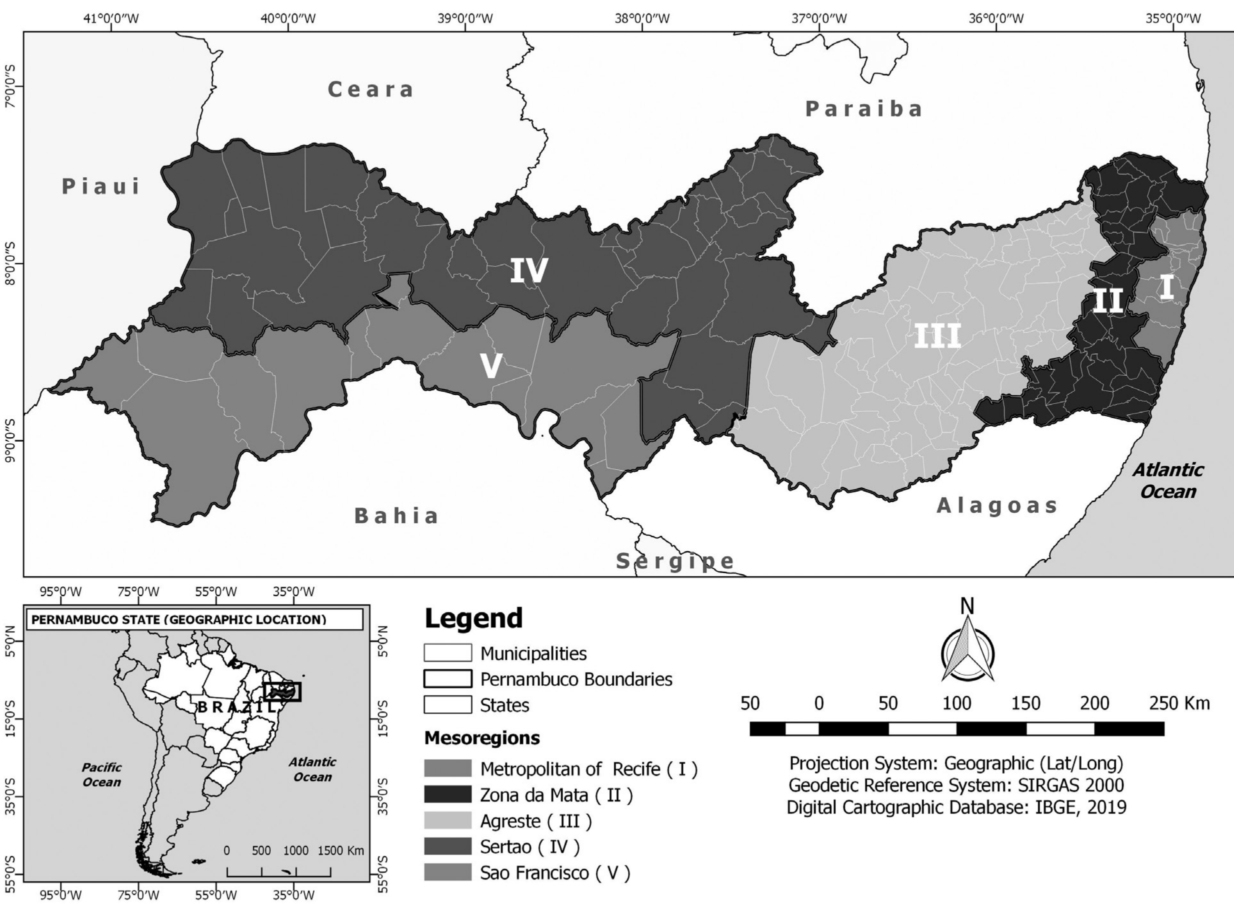

Figure 1 - Map of the mesoregions of the Pernambuco State.

The Recife Metropolitan Area comprises 14 municipalities and has approximately 4,103,780 inhabitants, with an average urbanization rate of 86%. This region has a tropical and humid climate, and the predominant biome is the Atlantic Forest[22]. Zona da Mata region comprises 43 municipalities and has approximately 1,318,264 inhabitants, with an average urbanization rate of 62%. This region has a humid climate, and the predominant biome is the Atlantic Forest[22].

The Agreste region comprises 71 municipalities and has approximately 2,172,963 inhabitants, with an average urbanization rate of 68%; the climate is humid in areas closer to the coast and arid in areas closer to Sertao. The biome presents a transition from the Atlantic Forest to the Caatinga[22].

The Sertao do Sao Francisco region comprises 15 municipalities, has approximately 434,713 inhabitants, with an urbanization rate of 47%, and a semi-arid climate[22]. Finally, the Sertao region has 41 municipalities and approximately 571,071 inhabitants, with an urbanization rate of 26%. The predominant vegetation is the Caatinga, reflecting the hot and dry climate of the region[22].

The Fernando de Noronha District was excluded from the current evaluation because it is an archipelago and does not present a relationship of contiguity or spatial adjacency with the municipalities of the state.

Study population and variables

From January 2016 to December 2018, triatomines were collected from houses/intradomicile and their vicinity/peridomicile (chicken coops, animal pens, stone walls, fences, rubble) by health agents of the respective municipalities. The insects were collected manually by municipal officers in household environments, in both urban and rural areas, using entomological tweezers. When required, lanterns and dislodging agents (Piriza 2%) were used. In the Pernambuco State, for two long decades, the insecticide Alpha-cypermethrin and the dislodging agent Piriza® have been used[17].

All insects were identified taxonomically and underwent a parasitological examination in entomology laboratories in one of the 12 health regions in Pernambuco State. All infected triatomines and 40% of non-infected insects were analyzed in the Laboratory for Endemic Diseases in Recife Municipality[17].

The triatomines were identified at the species level in accordance with the classifications presented by Lent and Wygodzinsky[23], Galvão[6] and Galvão and Gurgel-Gonçalves[14]. Parasitological research was performed using abdominal compression and subsequent parasitological studies of the fresh contents of the intestines. Parasite phenotypes (i.e., flagellates similar to *T. cruzi*, identified as natural infections by *Trypanosoma* sp.) were identified through observation under an optical microscope using Giemsa-stained insect feces. These insects arrived in the laboratory intact, but almost lifeless upon arrival due to the distances between laboratories[17,23].

The following variables were considered in this analysis: municipalities, number of captured insects and their life stages/characteristics (males, females, nymphs and not classified according to biological phase), collection sites (homes or surrounding areas), triatomine species, and number of infected insects. The NII was calculated according to the following formula developed by the Pan-American Health Organization and Brazilian Ministry of Health in 2003[24]. The NII = (total number of infected triatomines/total number of insects examined) × 100.

The population data and the cartographic base of the municipalities were obtained in shapefile format from the 2010 Demographic Census of the Brazilian Institute of Geography and Statistics (IBGE), which provides data in the form of a geographic projection system[17] (latitude/longitude) and geodetic system (SIRGAS 2000); these data are available on the IBGE website[22].

Data management and analysis

Spatial autocorrelation was evaluated using Global Moran Index (I), which tests the spatial dependence among observations. $I > 0$ indicates that standard deviations from the average are positive, thus showing direct correlation (clustering) between neighbors. When $I < 0$, standard deviations indicate negative or inverse correlations (i.e., dispersion). Finally, when $I = 0$, there is no spatial autocorrelation (i.e., randomness is observed)[25].

In addition, the local indicators produce a specific value for each area, thus allowing for the identification of groupings. Local Moran Index (II) (also known as Local Indicator for Spatial Autocorrelation [LISA]) is a spatial association indicator that evaluates the existence of clusters in the spatial arrangement of a given variable. This allows the detection of municipalities with spatial dependencies that are not illustrated through global indices[26]. Four types of maps were used in the Moran autocorrelation test: i) a map of raw NII; (ii) a BoxMap map showing clusters with high–high patterns (i.e., areas where a specific municipality and its neighbors show high NII values), low-low patterns (i.e., areas where a specific municipality and its neighbors show low NII values), low-high patterns (i.e., areas where a specific municipality has a low NII and its neighbors have a high NII), and high-low patterns (i.e., areas where a specific municipality has a high NII and its neighbors have a low NII); (iii) a MoranMap map showing statistically significant high-high, low-low, low-high, and high-low clusters; and (iv) a LISA Cluster Map indicating the magnitude of the p-value for each municipality, with statistical significance defined according to a two-sided threshold of $p < 0.05$.

Spatial relative risk analysis was performed using spatial scan statistics. A Poisson model was used wherein the number of events in each area was considered to be distributed according to the population at known risk. The scan window coverage radius was set at 50% of the total population[26].

We used the following software programs for processing, analyzing, and calculating the spatial autocorrelation indicators and constructing thematic maps: Microsoft Excel (Microsoft, Seattle, WA, USA), SPSS statistical software (version 21.0, IBM, Armonk, NY, USA), QGIS (version 3.10.8, QGIS, Rimouski, Quebec, Canada), GeoDa (version 1.14.0, Geoda, Tel Aviv, Israel), and SaTScan (version 9.6, SaTScan, Boston, MA, USA).

Ethics statement

The study was approved by the Research Ethics Committee of the Hospital Complex HUOC/PROCAPE, Pernambuco State (CAAE 88154818.6.0000.5192).

RESULTS

In total, 7,302 triatomines (seven species) were captured in 99 different municipalities. The triatomines were primarily found in intradomicilary areas, including 6,001 adults (3,249 males and 2,752 females), 1,276 nymphs, and 25 triatomines were unclassified because they arrived at the laboratory in a condition that prevented identification, so they were only classified in terms of biological phase. Table 1 shows that the average NNI was 12% among the five infected species of triatomines; the most frequently infected species were *P. lutzi* (21%) and *P. megistus* (18%).

Three species of triatomines, *T. brasiliensis*, *T. pseudomaculata* and *P. lutzi* were distributed in a greater number of municipalities than the other species in the study (Figure 2). The highest NNI values were found in the Agreste, Sertao do Sao Francisco, and Sertao mesoregions. The presence of a single infected *R. nasutus* specimen was noteworthy in Sertao.

The Agreste, Sertao, and Sao Francisco regions had the highest frequency of triatomines found inside households (Figure 3A). Among these regions, triatomines were collected in greater numbers in the Agreste and Sertao regions (Figure 3B). Zone 2 (i.e., west of the Agreste and east of Sertao) presented a relative risk of 3.65 compared to other areas of the state (Figure 4).

The following municipalities had the highest NII values (Figure 5): Zona da Mata, Agreste, Sertao, and Sertao do Sao Francisco. The I value was positive (0.2) and statistically significant ($p = 0.01$), indicating the existence of spatial correlation. In the BoxMap, the municipalities with the highest rates of infection were concentrated in the Zona da Mata, Agreste, and Sertao areas (Figure 5B). These areas showed statistical significance in the MoranMap and LISA Cluster Map, thereby identifying infection clusters in the Agreste and Sertao regions (Figures 5C and 5D).

DISCUSSION

In this study, seven species of triatomines were found in four mesoregions of the Pernambuco State. The highest NII values were detected in three mesoregions, and natural infections were detected in five species of triatomines. The results of the study suggest that the risk of infected

Table 1 - Natural infection index for *Trypanosoma* sp. based on triatomines examined in the Pernambuco State from 2016 to 2018.

Species	Total insects examined		Intradomicile		Peridomicile		Positive	
	N	%	N	%	N	%	N	%
T. brasiliensis	3,844	53%	3,585	93.50%	259	6.50%	395	10.50%
T. pseudomaculata	1,828	25%	1,733	95%	95	5%	159	8.70%
P. lutzi	1,366	18.50%	1,233	90.50%	133	9.50%	285	21%
P. megistus	162	2.20%	142	87.50%	20	12.50%	29	18%
T. sordida	45	0.60%	41	92%	4	8%	-	-
T. petrocchiae[1]	44	0.50%	34	77.50%	10	22.50%	-	-
R. nasutus[2]	13	0.20%	11	84.50%	2	15.50%	1	7.50%
Total	7,302	100%	6,779	93%	523	7%	869	12.00%

[1]*T. petrocchiae* (Pinto & Barreto, 1925); [2]*R. nasutus* (Stal, 1859)

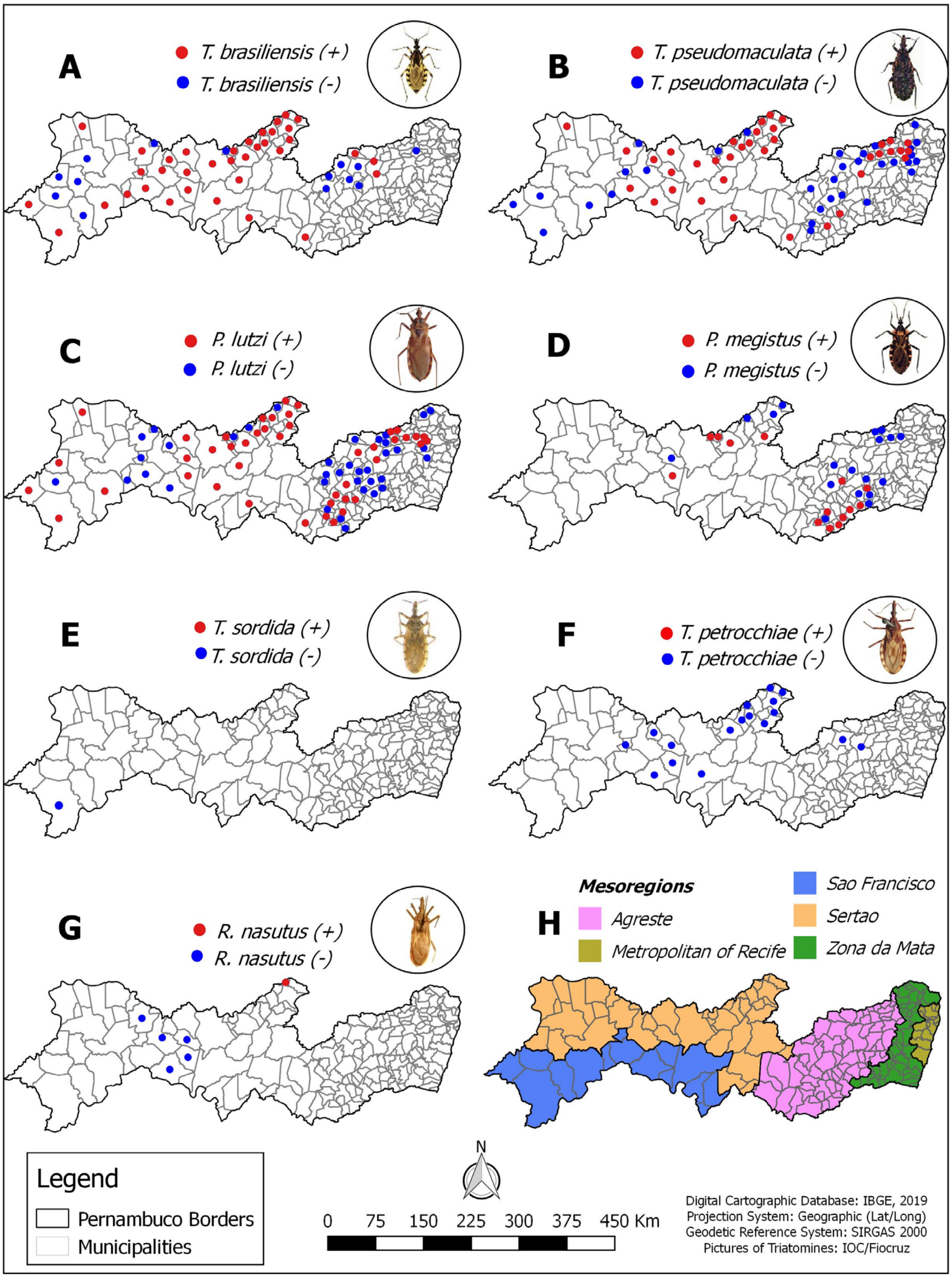

Figure 2 - Locations from where triatomines species were captured in the Pernambuco State, Northeastern Brazil, 2016–2018

triatomines is approximately three times higher in the Agreste and Sertao regions than in the other areas studied here.

We detected in this study a higher presence of adult males compared to adult females or nymphs. The presence of nymphs among the collected species characterizes the adaptation of triatomines to household environments with regard to consolidating the colonization process. The nymphs may have been found in fewer numbers due to the low sensitivity of the method. In Brazil, this process is directly related to the endemicity of Chagas disease[27,28].

The *T. brasiliensis and T. pseudomaculata* species were the most frequent in the study, indicating their vector potential in endemic regions, especially in or near households in rural areas[2,29]. In our study, these vectors were naturally infected in the Agreste and Sertao regions; these results are consistent with results from other studies conducted in Northeastern Brazil[29,30].

Ceccarelli *et al*. showed that the number of triatomines in Argentina post-2000 has almost tripled compared to

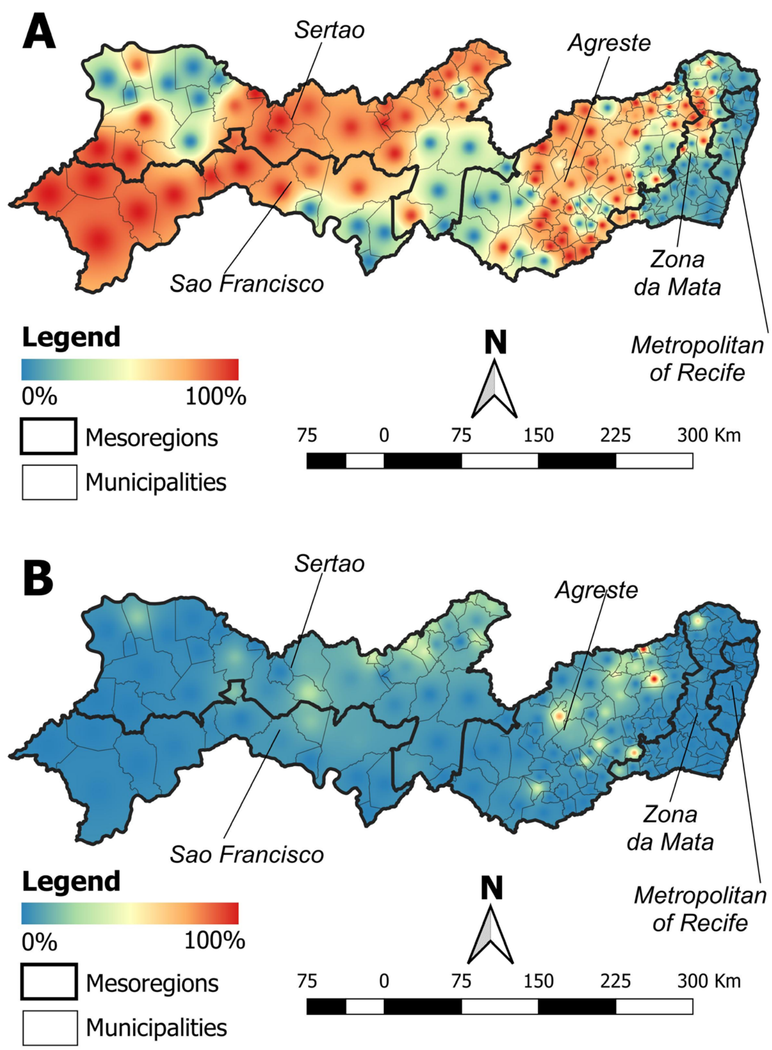

Figure 3 - Prevalence map of triatomines captured in the intradomicile and peridomicile in the Pernambuco State, Northeastern Brazil, 2016–2018: A) triatomines captured in the intradomicile; B) triatomines captured in the peridomicile.

the numbers indicated in the records prior to 2000, which were mainly collected in the dry and humid areas of Chaco (approximately 15,000 insects were collected in 100 years). Although the *T. infestans* species was present in Argentina, there was no record of *P. megistus* in this country[31].

In our study, 7,302 vector insects were collected between January 2016 and December 2018; however, we detected only a small number of *P. megistus*. This result corroborates results of previous studies conducted in the Northeastern region[29,30] and differs from studies conducted in the Southeastern[32] and Southern regions of Brazil[16]. This may be related to the chemical control used to eliminate triatomines in the household environments in the various regions[33]; however, the NNI for *Trypanosoma* sp. (18%) shows its vector capacity.

More than 1,500 triatomines were collected in a previous study conducted in the United States. The triatomines were predominantly collected in Texas and showed an NII of 54%; the two most frequently detected species were *Triatoma gerstaeckeri* (Stål, 1859) and *Triatoma sanguisuga* (Leconte, 1855)[4]. The triatomine species found in the United States are almost exclusively wild[4], unlike those found in Central America[33] and South America[31,34].

In these regions, triatomines are found indoors; this is a similar result to our findings showing that 6,779 (93%) triatomines were collected inside homes. In our study, the highest NII rates, with regard to *Trypanosoma* sp., were found in *P. lutzi* (21%) and *P. megistus* (18%); however, *T. brasiliensis*, *T. pseudomaculata*, and *P. lutzi* showed the greatest spatial distribution. Barreto *et al.*[35] previously identified that *P. lutzi* was responsible for the highest rate of natural infections among the triatomines captured, although this species was found to only represent a small proportion of triatomines in Northeastern Brazil as a whole[35]. The highest occurrence of *P. megistus* was recorded in the Paraná State, with an infection rate by *T. cruzi* of 19.7%[16]. The

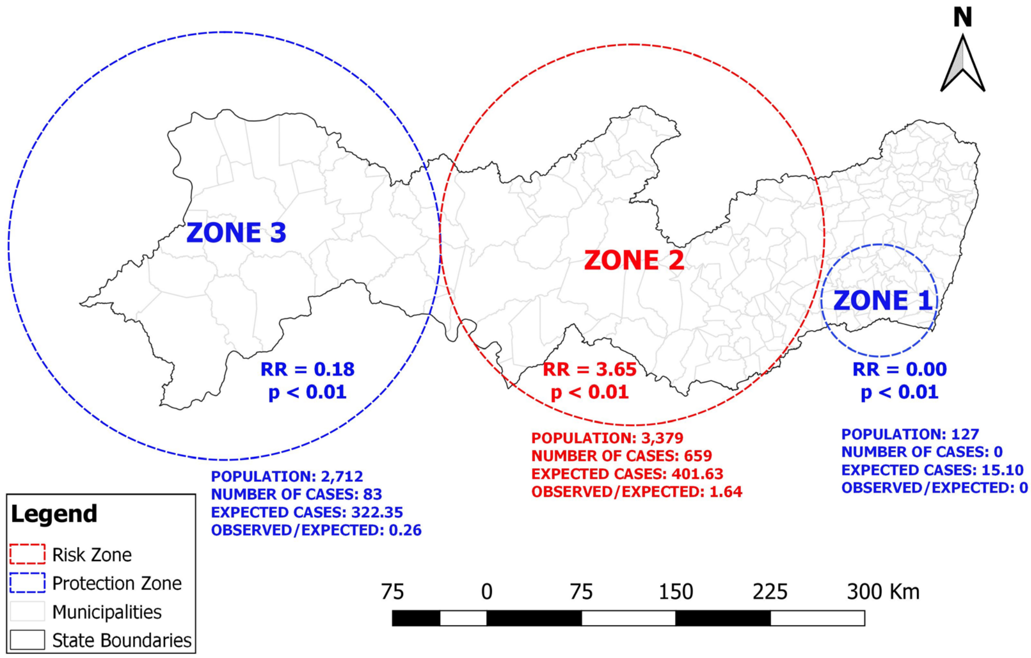

Figure 4 - Spatial cluster classification: natural infection index for triatomines in the Pernambuco State, Northeastern Brazil, 2016–2018.

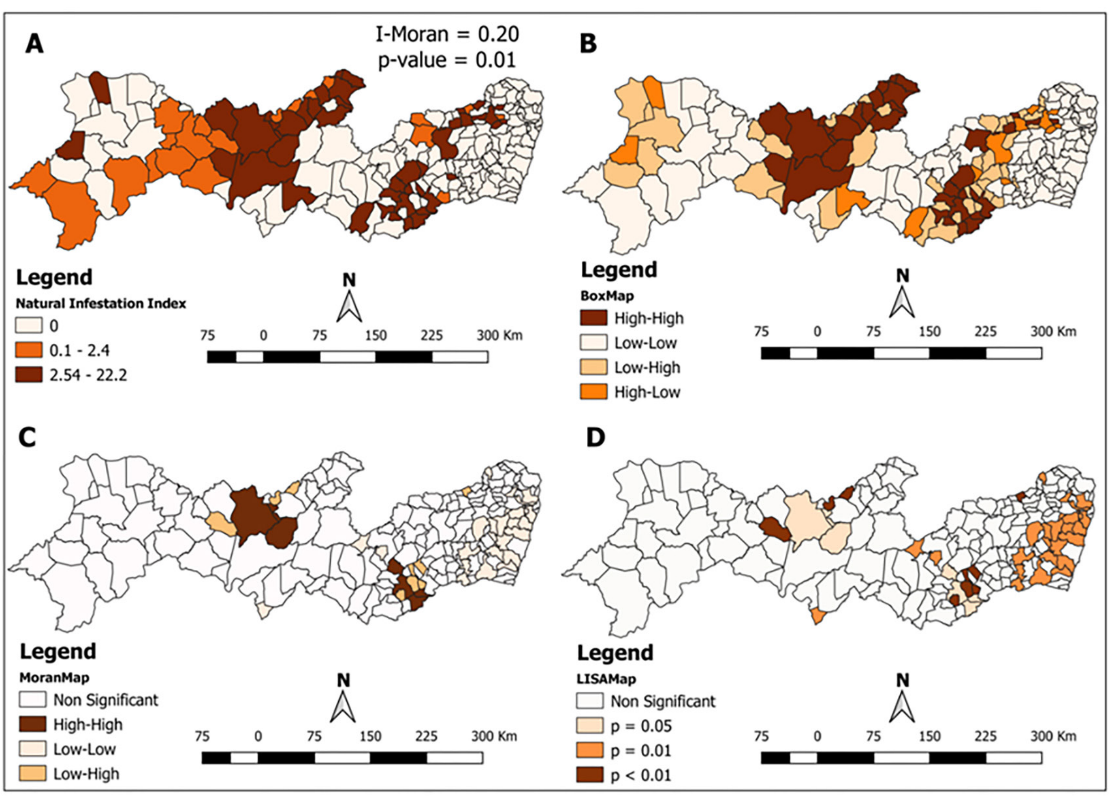

Figure 5 - Moran autocorrelation map showing natural infection index in the Pernambuco State, Northeastern Brazil, 2016–2018.

findings of these studies corroborate the present findings by showing that *P. lutzi* and *P. megistus* have vector importance in an endemic area of Chagas disease in Brazil[17,35].

Silva *et al*.[20] demonstrated the presence of eight species of triatomines distributed across Pernambuco, although natural infection was identified exclusively in *T. brasiliensis* and *P lutzi*. In our study, five naturally infected species were found, thus representing three additional species (*T. pseudomaculata*, *P. megistus*, and *R. nasutus*) compared to those identified in the 2013-2015 study. *T. sordida* was identified in only one municipality, but it was not infected. Only one specimen of *R. nasutus*, which was present in five other municipalities, showed natural infection. *T. petrocchiae* was the most colonized species in areas surrounding houses; however, no specimens were infected.

Between 2011 and 2014, 140 triatomines of four different species (100 *Triatoma vitticeps* [Stal, 1859], 25 *Panstrongylus geniculatus* [Latreille, 1811], eight *P. megistus*, and seven *Triatoma arthurneivai* [Lent and Martins,1940] specimens) were identified in Southeastern Brazil, with a highly heterogeneous spatial pattern evidenced in urban areas[32]. Among the insects collected in a previous study, *P. megistus* did not show a record of natural infection with *Trypanosoma* sp.[32] (unlike in the present study). More specifically, in our study, the species showed natural infection in the hinterland and wilderness areas. This condition may explain why infested households were located closer to open fields.

P. lutzi was found to be spread across four mesoregions; the Metropolitan region was the only region with no records of this species. Previous studies[17,29] conducted in Northeastern Brazil have reported the presence of this species mainly in the semi-arid regions of the Agreste region, with its absence noted in the coastal areas; these findings are similar to our findings, and the species is still considered wild[2,17]. In contrast, *R. nasutus* was found in municipalities in Sertao, with a single record of positivity in our study. This finding is consistent with previous studies demonstrating its presence in semi-arid areas and the Caatinga biome[17,29].

Only one specimen of *T. sordida* was collected in the mesoregion of Sertao do Sao Francisco. However, no record of positivity was found in this species, despite it being the most frequently detected species in the arid and semi-arid regions of Brazil[29], as well as in Argentinian and Bolivian Chaco[31,36]. It is possible that this finding is due to the fact that *T. sordida* is considered a secondary species in Northeastern Brazil, except in the Bahia State, in which there is a high frequency of this species[19]; this state borders the Pernambuco State municipality where this species was found. The macroecology of Chagas disease vectors is based on the distribution of geographical regions that present suitable environmental conditions for most triatomine species and are considered areas with an elevated risk of the disease due to the high number of infections with *T. cruzi* in the human population.

Spatial analysis conducted in our study identified a spatial autocorrelation in the Agreste and Sertao regions and a three-fold increased risk of finding naturally infected triatomines in these two areas than in other areas of the state. The vector geographical distribution is associated with regions of poverty and its transmission occurs with greater frequency in populations residing in poorly built, finished and maintained houses, commonly observed in rural areas of endemic countries in Latin America[37], and it is not found in large urban centers that exist in the coastal and forest regions of the Pernambuco State[17]. The physiogeographic characteristics of these areas (Zone 2) are favorable for the presence of the triatomine species typical of the semi-arid territory and the Caatinga. The activities that constitute the primary sector of the economy of the Sertao and Agreste regions (i.e., livestock and subsistence agriculture) are conducted in an area with a high density of triatomines, thereby enabling the presence of these vector species in households and surrounding niches[30,35]. The municipalities with higher mortality rates also showed the highest rates of chronic diseases, with patterns of homogeneity observed in the Zona da Mata and Sertao[38] regions.

Our study demonstrates the need for health services to be alerted regarding the possible occurrence of acute cases of Chagas disease in the Agreste and Sertao regions. Thus, gaining knowledge of the spatial dynamics of these vectors is of paramount importance. This study also highlights the use of geotechnologies, especially in analyses involving environmental and epidemiological factors, wherein spaces are integral to the processes of disease diffusion; this allows for better characterization and quantification of exposure and its possible results with regard to health consequences[35]. Therefore, our findings contribute to the understanding of the structure of vector eco-epidemiology in space, allowing physicians and research scientists to interpret the distribution of health events and associated information with respect to the evaluation and prioritization of control programs for Chagas disease.

Information from the surveillance of Chagas disease vectors currently contributes to the identification of the epidemiological status of a given area. However, the findings of the study should be interpreted with caution, owing to the limitations due to the use of secondary data, which may lead to inconsistencies in the quantity and quality of information processing. However, to minimize the limitations of the study, duplication, incompleteness, and inconsistency were analyzed in the notification process and controlled by cleaning the database. Quality control is carried out by the Laboratory for Endemic Diseases at the central level of triatomines, sent by the laboratories of the municipalities. The impact was that, for the first time, a study with both spatial analysis and risk of areas of natural infection by *T. cruzi* was carried out, and these findings are important for decision-making in the entomological surveillance governmental program.

CONCLUSION

Our study shows potential areas of vector transmission of Chagas disease in the Pernambuco State. In this study, the application of different spatial analysis methods made it possible to locate these areas, which would not have been identified only by applying epidemiological indicators. Seven species of triatomines were found in the state. However, the highest NNI values were detected in three mesoregions. The most infected species were *P. lutzi* (21%) and *P. megistus* (18%), whereas the species with the greatest spatial distribution were *T. brasiliensis*, *T. pseudomaculata*, and *P. lutzi.* We conclude that there is an approximately three-fold increased risk of infected triatomines in a cluster of two areas (the Agreste and Sertao regions) than in the other regions evaluated in our study.

AUTHORS' CONTRIBUTIONS

CAM contributed to the design, collection, data analysis, interpretation and writing of first and subsequent drafts of the paper; MBA and ZMM contributed to the design, interpretation and writing of first and subsequent drafts of the paper; ALSO contributed with data analysis, interpretation and writing of first and subsequent drafts of the paper; SMM contributed with writing of first and subsequent drafts of the paper; WAOJ contributed with the interpretation and writing of first and subsequent drafts of the paper.

CONFLICT OF INTERESTS

The authors report no conflict of interests. The authors are solely responsible for the content and writing of this article.

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