Environmental influence on *Triatoma vitticeps* occurrence and *Trypanosoma cruzi* infection in the Atlantic Forest of south-eastern Brazil

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Abstract

Trypanosoma cruzi requires a triatomine insect vector for its life cycle, which can be complex in different enzootic scenarios, one of which is the unique transmission network in the Atlantic Forest of south-eastern Brazil. In Espírito Santo (ES) State, highly infected *Triatoma vitticeps* are frequently reported invading domiciles. However, triatomines were not found colonizing residences and mammals in the surrounding areas did not present *T. cruzi* infection. To date, the biotic and abiotic variables that modulate *T. vitticeps* occurrence and *T. cruzi* infection in ES State are still unknown. The aim of this study was to identify the environmental variables that modulate their occurrence. Local thematic maps were generated for two response variables: *T. vitticeps* occurrence and *T. cruzi* infection. The following explanatory variables were tested: climate (temperature, relative air humidity and rainfall), altitude elevation, mammalian species richness as well as soil and vegetation types. Spatiotemporal distribution patterns and correlation levels between response and explanatory variables were assessed through spatial statistics and map algebra modelling. The central and southern mesoregions presented higher *T. vitticeps* and *T. cruzi* distributions and can be considered transmission hotspots. The explanatory variables that can explain these phenomena were relative air humidity, average temperature, soil type, altitude elevation and mammalian species richness. Algebra map modelling demonstrated that central and southern mesoregions presented the environmental conditions needed for *T. vitticeps* occurrence and *T. cruzi* infection. The consideration of environmental variables is essential for understanding the *T. cruzi* transmission cycle. Cartographic and statistical methodologies used in parasitology have been demonstrated to be reliable and enlightening tools that should be incorporated routinely to expand the understanding of vector-borne parasite transmission.

Introduction

In recent years, Earth's biodiversity has been threatened due to global warming, landscape fragmentation, habitat loss and industrial exploration of plant and animal species (Pecl *et al.*, 2017; Lewin *et al.*, 2018). Understanding the impact of these issues is crucial since the lack of knowledge associated with diverse and recurring environmental changes affect complex ecosystems with

emergence of infectious diseases a result (Lewin *et al*., 2018). The south-eastern Brazilian Atlantic Forest is a biome that still sustains precious biodiversity (Bovendorp *et al*., 2017), including diverse species and their parasites, such as various species of the haemoflagellate protozoan *Trypanosoma*, their triatomine vectors and mammalian hosts (Lisboa *et al*., 2000; Lisboa *et al*., 2006; Monteiro *et al*., 2006; Dario *et al.*, 2017a; Dario *et al*., 2017b).

Loss of habitats and food resources has driven wild taxa into closer human vicinity. In the Brazilian south-eastern Espírito Santo (ES) State, domiciliary invasion in rural areas by infected insect species, mainly *Triatoma vitticeps,* is frequently reported from different municipalities (Santos *et al*., 2006; Dario *et al*., 2017b; Dario *et al*., 2018), which especially occurs in the mountainous regions (Leite *et al*., 2011). *Triatoma vitticeps*, in addition to presenting high infection rates (Santos *et al*., 2006; Dario *et al*., 2018), is capable of harbouring four discrete typing units (DTUs) of *T. cruzi*: TcI, TcII, TcIII and TcIV (Dario *et al*., 2017b; 2018) and also *T. dionisii* (Dario *et al*., 2017b), a *Trypanosoma* species considered restricted to bats. In 2012, a two-year-old patient in Guarapari Municipality died after having acquired Chagas disease by putting his hand in his mouth after handling a *T. viticeps* specimen that had been crushed. It was demonstrated that the patient presented a mixed *T. cruzi* infection by DTUs TcI, TcII, TcIII and TcIV and also *T. dionisii* (Dario *et al*., 2016). This peculiar enzoonotic scenario, in which a unique vector displays high *T. cruzi* infection rates and high DTU diversity, raised a series of questions regarding the reservoir system (mammalian species and landscape physiognomy) and the variables that determine the *T. vitticeps* and the *T. cruzi* distribution.

The occurrence of triatomine and *T. cruzi* infection may present different transmission patterns that can be spatially and temporally influenced (Roque *et al*., 2008). This shows that environmental variables play an important role in the vector distribution (Parra-Henao *et al*., 2016) and consequently also for *T. cruzi* transmission. By revealing hidden transmission patterns, spatial analysis has been increasingly demonstrated to be a powerful tool in the study of vector-borne diseases (Bavia *et al*., 2005; Kitron *et al*., 2006; Xavier *et al*., 2012; Parra-Henao *et al*., 2016; Ferro e Silva *et al*., 2018; Miranda *et al*., 2019) but the technique is still not used routinely. However, this approach would be useful as climatic conditions and landscape changes influence triatomine spatial distribution (Gurgel-Gonçalves & Cuba, 2009; Pereira *et al*., 2013; Ibarra-Cerdeña *et al*., 2014; Parra-Henao *et al*., 2015; Dias *et al*., 2016; Ferro e Silva *et al*., 2018). Moreover, little is known about the influence of environmental variables on *T. cruzi* infection and transmission. Most studies report observations under laboratory conditions or explore just one type of environmental variable in the analysis (Jansen *et al*., 2020). So far, only a minority has attempted to establish correlations of biological variables applying spatial conditions.

Gurgel-Gonçalves *et al*. (2012), through ecological niche modelling, proposed *T. vitticeps* occurrence in ES State by multitemporal remotely sensed imagery and climatic data. In addition, Leite *et al.* (2011) reported its occurrence in locations with irregular mountains, but none of these studies included a *T. cruzi* infection/transmission analysis. There are only scarce data concerning the environmental variables that may determine *T. cruzi* dispersion in detail. It is necessary to understand the role of the environment in influencing the high rates of *T. vitticeps* infected by *T. cruzi*, currently considered a re-emerging parasite. Most of the triatomines found inside residences are adult specimens, while the presence of nymphs is rare. This shows that *T. vitticeps* must have acquired the infection in the sylvatic environment and invaded the residences due to natural/artificial factors.

The aim of this study was to evaluate the environmental variables that modulate *T. vitticeps* occurrence and *T. cruzi* infection in the ES Atlantic Forest. We hypothesized that the distribution and infection by *T. cruzi* in *T. vitticeps* correlate with biotic and abiotic variables testing soil and vegetation types, mammalian species richness, altitude elevation and climate (temperature, rainfall and relative air humidity). Vegetation and soil types are directly related to land use and land cover that ultimately determine the plant composition (food supply and habitat) and mammalian species diversity. Climate variables can interfere with the triatomine life cycle (evolution and activity), where higher temperatures can accelerate or delay its development. A direct relationship between loss of mammalian species richness and selection of resilient, mammalian competent *T. cruzi* reservoirs has already been described (Xavier *et al*., 2012). This selection increases *T. cruzi* circulation and raises the chance of the vector becoming infected and passing into the peri-domestic and domestic environments. Altitude can influence the triatomine distribution since ES State is characterized by different altitude elevations, which can impact the presence of triatomines and the parasites (de Fuente-Vicente *et al*., 2017). This article also aims to answer open questions about the ecology of *T. cruzi* and *T. vitticeps* regarding the importance of mixed infection in the course of transmission, how the vector and the infection can be selected or adapted due to climate change and whether there is a possibility of triatomine colonization in different households. To accomplish these goals, we employed a multidisciplinary approach, including spatial and statistical modelling.

Materials and methods

Study area

Espírito Santo State is located in the coastal south-eastern region around the geographical coordinates of 20°16'S, 40°17'W. According to the Brazilian Institute of Geography and Statistics (IBGE), the state covers an area of 46,074,447 km² and has an estimated population of 4,018,650 inhabitants (IBGE, 2019). The state borders the Atlantic Ocean to the east, Bahia State to the north, Minas Gerais State to the west and northwest, and Rio de Janeiro State to the south. ES is divided into four mesoregions and 78 municipalities (Figure 1): the Northwest with 17 municipalities, the North Coastal with 15 municipalities, the Central with 24 municipalities and the South with 22 municipalities (IBGE, 1990, 2016).

The area of ES State is entirely located in the Atlantic Forest biome, with forest at lower altitudes and open vegetation higher up. In 2019, it was estimated that the state had 581,163 acres of remnant forest areas corresponding to 12.6% of the state (SOS Mata Atlântica, 2019). The Atlantic Forest is included in an ecological corridor project, the so-called '*Central Corridor of the Atlantic Forest*', which aims to integrate their conservation units with those in southern Bahia State (Lamas *et al*., 2006). Two main climatological types dominate: tropical rains and a humid mesothermal climate.

Triatoma vitticeps collection and *Trypanosoma cruzi* infection data

The *T. vitticeps* occurrence and *T. cruzi* infection data analysed in this study were published by Dario *et al*. (2018), who also included *T. cruzi* molecular characterization. This work was carried out in the Atlantic Forest in collaboration with the *Núcleo de*

Entomologia e Malacologia from Secretaria de Estado da Saúde do Espírito Santo (Nemes - SESA/ES) and *Instituto de Medicina Tropical* from the Federal University of Espírito Santo between June 2010 and May 2012.

Triatomine species are frequently found inside domiciles. When found there, residents are advised to capture the insect carefully and take it/them to the nearest triatomine information post (PIT) if available or to contact a municipal health agent for collection. *Triatoma vitticeps* identification was performed according to Lent and Wygodzinsky (1979). The triatomine digestive tract was dissected, diluted in saline solution (0.85%) and examined for flagellate forms similar to *T. cruzi.*

Cartographic data

For map construction, the ES regional divisions into mesoregion and municipality maps were acquired from IBGE (http://www.ibge.gov.br). All the constructed maps in this study are in the geodetic reference World Geodetic System 84 (WGS 84). *Triatoma vitticeps* occurrence and *T. cruzi* infection data were georeferenced by decimal degree coordinates by the Integrated System of Geospatial Bases of Espirito Santo State (Sistema Integrado de Bases Geoespaciais do Estado do Espírito Santo - GEOBASES) at the Central Institute of Research, Technical Assistance and Rural Extension (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural - INCAPER) coordinate database.

Climatic data

The following climatic data were addressed: minimum, average and maximum temperatures, rainfall and relative air humidity. Weather information was obtained from the National Meteorology and Geophysics Institute (*Instituto Nacional de Meteorologia* - INMET) and the Centre for Weather Forecast and Climatic Studies (*Centro de Previsão de Tempo e Estudos Climáticos* - CPTEC). The data were collected from the following five meteorological stations located in ES State: Vitória, São Mateus, Sooretama, Venda Nova do Imigrante and Jerônimo Monteiro. Based on the locations where *T. vitticeps* had invaded households, been collected by residents and delivered to a PIT or a health service post, climatic data for a 180-day time series were obtained and the average value of each climatic variable calculated using Microsoft Excel® software. Climatic maps were generated using the interpolation method by inverse distance weighting (IDW) of each entry of climatic variable data from the five meteorological stations. Climatic values for each *T. vitticeps* occurrence were extracted using the QGIS Point Sampling Tool plugin (https://plugins.qgis.org/plugins/pointsamplingtool/). All these steps were performed in the geographic information system (GIS) application on QGIS Noosa software version 3.6.2 (https://www.qgis.org).

Environmental data

The environmental variables chosen for analysis were soil/vegetation types and altitude elevation (Table 1) obtained from the Geobase, INCAPER institut.

Mammal species richness data

Mammalian genus occurrence data in ES State were obtained from the Global Biodiversity Information Facility (GBIF) and the Distributed Information System for Biological Collections: the Integration of Species Analyst and SinBiota (*Sistema de Informação Distribuído para Coleções Biológicas: a Integração do Species Analyst e do SinBiota* - speciesLink) databases (Table 1). ArcGIS v. 9.3 (ESRI, Redlands, CA, USA) was used to calcu-

late the species richness by generating convex polygons (vectors) for each mammalian species column in the attribute table. Each polygon created was exported to a new layer that generated individual shapefiles and assembled them into a unique shapefile in which the columns created (the attributes table of each shapefile assembled) were joined into a single line. This allowed the determination of the total species richness within each area investigated, which was calculated from a new column created in the unique shapefile. The sum of all values was inserted into this new column, thereby generating the quantitative species richness within each area.

Model structure and analyses scale

Environmental and mammalian species richness shapefile maps that did not include climatic variables were transformed into matrix format (raster). For the analysis, a 10-km radius scale analysis was defined for *T. vitticeps* occurrence, *T. cruzi* infection and each explanatory variable mean value estimation by generating 10-km radius buffer maps around each point of *T. vitticeps* occurrence. Through the ArcGis v. 9.3 join function, *T. vitticeps* occurrence was counted and the mean values of each explanatory variable estimated within each buffer. Finally, the values obtained were transferred to a table for statistical analysis (Tables S1 and S2). For the *T. cruzi* infection analysis, occurrence was classified as 0 for uninfected and 1 for infected *T. vitticeps* specimens.

Table 1. The complete set of response and explanatory variables used in statistical, spatial statistical and spatial models.

Response variable	Class	Data type
Triatoma vitticeps	Occurrence *T. cruzi* infection	Discrete
Explanatory variable		
Soil*	Rock outcrop Yellow argisol Red argisol Red-yellow argisol Haplic cambisol Haplic gleisol Yellow latosol Red-yellow latosol Quartzarenic neosol	Nominal
Vegetation*	Recovery Fisheries management Management Mosaic/corridor Inventory Sustainable use fomentation Environmental education UC-US create UC-indef create Protected area	Nominal
Elevation*	Altitude	Discrete
Temperature°‡	Maximum Average Minimum	Discrete
Rainfall°‡	Average	Discrete
Humidity air°‡	Relative	Discrete
Mammal species richness§^	Average	Discrete

*GEOBASES - https://geobases.es.gov.br/); °CPTEC - https://www.cptec.inpe.br/; ‡INMET - http://www.inmet.gov.br/portal/); §speciesLink - http://www.splink.org.br/); ^GBIF - https://www.gbif.org/pt/).

Statistical modelling

The correlations between each explanatory variable were performed for each variable excluding correlations that presented values higher than 0.8. Response variable histograms and boxplot graphs were used to observe the data distribution pattern. In case of a non-normal data distribution, the adjustment adequacy test, available in the fitdistrplus package (Delignette-Muller and Dutang, 2015), was used to identify the distribution type determined by the best value of the Akaike Information Criterion (AIC).

After the correlation test and selection of the variables, the datasets were analysed by multiple linear regression; the logistic model; the Poisson regression model; the negative binomial model; the multiple regression (Box-Cox transformation); the generalized linear model (gamma distribution); and the discriminant analysis (Figure 2). The models were evaluated by ANOVA to determine their use in the statistical modelling. The comparison criterion between the models was based on the minor AIC and residual deviance values, except for discriminant analysis. The hit rate was calculated for the regression models to discriminate the chance of the model hitting the occurrence of the response variable. The correlations between the variables that explained the response variables (*T. vitticeps* and *T. cruzi* infection) were considered when they presented a statistical significance at $P \le 0.05$. All statistical analyses were performed using R software (version 3.5.0).

Spatial statistical modelling

Global and local Moran's indexes (Figure 2) were applied to verify the existence of spatial autocorrelation between the distribution of *T. vitticeps* occurrence and *T. cruzi* infection and to identify transmission hotspots. For the univariate global Moran's index (GMI) and the bivariate GMI, the neighbourhood matrix was defined using the weights manager tool queen-type contiguity adopted as criterion with the first-order contiguity set as the default (Luenam & Puttanapong, 2020). Regions with common borders were considered as neighbours. To demonstrate that the index value was not randomly obtained, the pseudo-significance test was performed with 999 permutations adopting $P \le 0$ for the null hypothesis to be rejected. The hypotheses tested were: H_o= no spatial dependence between the variables; and H_I= spatial dependence between variables. The index values can vary between -1 and 1 and are interpreted as follows: i) -1= spatial dependence with regular distribution; ii) 0= no spatial dependence; 1= spatial dependence with the presence of a cluster. This result can also be visualized as a scatter diagram and displayed as a two-dimensional graph.

As spatial dependence was identified in GMI, univariate and bivariate local Moran's index (LMI) analyses (Figure 2) were performed to determine the local spatial association pattern. In addition to showing the dispersion diagram, as in GMI, the LMI produces the significance map and the cluster map where it is possible to observe the presence or absence of spatial dependence. The GMI and LMI were calculated using GeoDa software version 1.12 (GeoDa Center for Geospatial Analysis and Computation, Arizona State University, Tempe, AZ, USA).

Map algebra modelling

The map algebra was applied to map areas of *T. vitticeps* occurrence and *T. cruzi* infection spatial correlation between the biotic and abiotic variables. By this methodology, it is possible to classify and identify areas with high, medium and low occurrences of given data and their direct or indirect correlation types with the studied environmental area. For this analysis, it was necessary to convert *T. vitticeps* and *T. cruzi* infection shapefile into heat maps (raster format), and they were built within a 400-m² resolution. An exploratory analysis (Figure 2) of the data was carried out to verify the types of correlation between the responses together with the explanatory variables. For the correlation maps, arithmetic operations (addition - for direct correlation; and subtraction - for indirect correlation) were performed (Figure 2) resulting in direct and indirect correlation maps between response and explanation variables, respectively. A final map was created with the subtraction of the direct and indirect correlation maps to visualize the areas of high, medium (transition) and low occurrence of *T. vitticeps* and *T. cruzi* infection (Figure 2). Map algebra modelling was performed using the raster calculator function in QGIS software version 3.6.2.

Results

In this study, 350 *T. vitticeps* specimens were analysed, 241 of which were found to be infected by *T. cruzi* with a distribution in 23 of 78 ES municipalities. The Central mesoregion had the high-
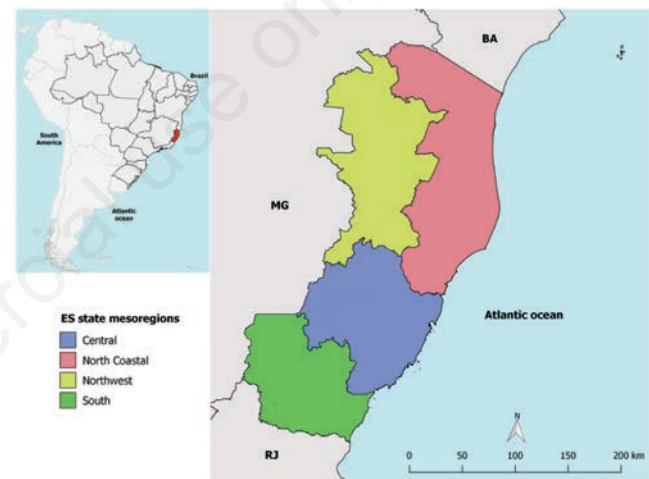

Figure 1. The political divisions of Espírito Santo State. MG, Minas Gerais State; BA, Bahia State; RJ, Rio de Janeiro State.
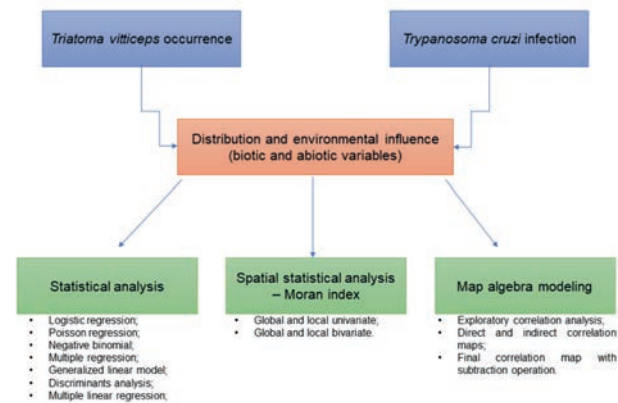

Figure 2. Methodological flowchart for modelling *Triatoma vitticeps* occurrence and *Trypanosoma cruzi* infection in the Atlantic Forest.

est *T. vitticeps* occurrence and *T. cruzi* infection (16/24 municipalities), followed by the South mesoregion (05/22 municipalities). The Northwest and North Coastal mesoregions presented only one municipality occurrence each in their 17 and 15 municipalities, respectively (Figure 3).

Statistical modelling

To test the hypothesis that *T. vitticeps* and *T. cruzi* distribution and infection are correlated with the biotic and abiotic variables, a correlation test was first performed. Strong correlations among the explanatory variables were observed between maximum temperature *versus* minimum and average temperatures, and between average and minimum temperatures. These results indicate that only one of these variables should remain for statistical modelling, and the average temperature was maintained in the analysis.

Considering that the data presented a normal distribution, *T. vitticeps* occurrence data were submitted to a linear multiple regression model to investigate which variables would explain their occurrence in ES. The linear multiple regression model was not adjusted for the data since the coefficient of multiple determination value (R^2) was equal to 0.18 and the determination-adjusted coefficient (R^{2a}) equal to 0.16. Although the model did not show significant predictions or give an average response to the response variable, the linear multiple regression showed which variables were significant (P-value 3.499e-11). This means that at least one explanatory variable contributed significantly to the model, and the results showed that rainfall, mammal species richness, vegetation and soil types were significant (P≤0.005). Since the data did not present a non-normal distribution, the response variable distribu-

tion was determined to define which nonlinear model would be used. According to the distribution histogram and boxplot graph (Figure S1), *T. vitticeps* distribution values presented a non-normal distribution with a strong positive asymmetry. Because of this distribution, an analysis using the R fitdistrplus package for fitting distributions was performed to verify the discrete distribution of
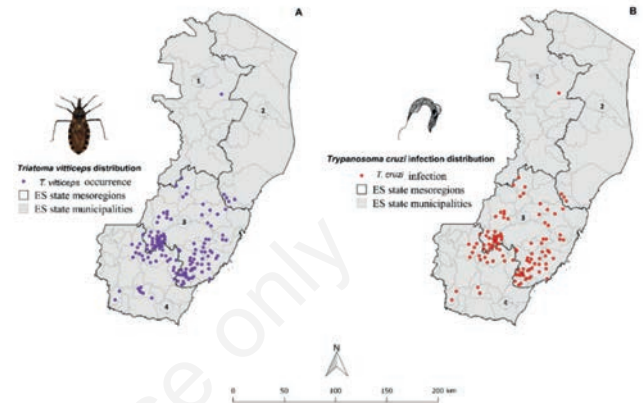

Figure 3. *Triatoma vitticeps* (A) and *Trypanosoma cruzi* (B) distribution in Espírito Santo State mesoregions represented by numbers. The numbers on the map represent: 11-Northwest; 2-North Coastal; 3-Central; and 4-South.

Table 2. Statistical modelling of *Triatoma vitticeps* occurrence and *Trypanosoma cruzi* infection.

Statistical model	Data type	AIC value	R-adjusted	Residual deviation	Hit rate
Logistic regression*	Classification	358.02	0.2180	338.02	76%
Multiple regression (Box-Cox transformation)	Count	669.84	0.2380	133.90	-
Negative binomial regression*	Count	2172.50	0.2210	355.97	65%
Generalized linear model (gamma distribution)	Count	2320.90	0.0080	202.54	-
Poisson regression*	Count	2940.10	0.1971	1624.40	-
Quasi-Poisson regression*	Count	NA	0.1971	1624.40	-
Discriminant analysis	Classification	-	-	-	64%
Multiple linear regression°	Normal distribution	-	0.1600	-	-

*Generalized linear models; °Multiple determination R^2=0.18; F test=3.499e-11.

Table 3. Significant explanatory variables for *Triatoma vitticeps* occurrence and *Trypanosoma cruzi* infection.

Statistical model	Data type	Akaike value	Significant variable	
			Direct	Indirect
Logistic regression	*T. cruzi* infection (classification)	398.16	Average temperature (4.25e-05) and relative air humidity (2.21e-08)	Altitude elevation (0.023231) and soil type (0.000478)
Negative binomial regression	*T. vitticeps* occurrence (count)	2208.4	Relative air humidity (2e-16)	Mammal species richness (3.23e-05) and soil type (0.000339)
Quasi-Poisson regression	*T. vitticeps* occurrence (count)	NA	Relative air humidity (<2e-16)	Mammal species richness (1.5e-05) and soil type (0.00446)
Multiple regression (Box-Cox transformation)	*T. vitticeps* occurrence (count)	669.84	Relative air humidity (0.000125), altitude elevation (0.023983)	Mammal species richness (2.46e-11)
Generalized linear model (gama distribution)	*T. vitticeps* occurrence (count)	2320.9	Average temperature (0.000166) and mammal species richness (1.68e-08)	-

the data. The observation data shown in Figure S2 were above the dashed line and outside the grey area and it was therefore not possible to determine which distribution the data fitted better. However, the adjustment suitability test using the AIC could do this and showed that the data presented a negative binomial distribution (Figure S3). Given the distribution analysis of *T. vitticeps* and the linear relationship of the data, we verified which of the following models best fitted the analyses: the logistic model, the Poisson regression model, the negative binomial regression model, the multiple regression (Box-Cox transformation), generalized linear model (gamma distribution) and discriminant analysis. The comparisons between the models were based on the AIC and residual deviance to determine which model fitted best considering the level of significance ($P \le 0.05$). The logistic model and discriminant analysis were applied for *T. cruzi* infection classification, in which *T. vitticeps* specimens were classified as 1 when the specimen was positive for *T. cruzi* infection and 0 when negative. Poisson regression, negative binomial, multiple regression and generalized linear models were applied to *T. vitticeps* occurrence data. Table 2 shows that the AIC values indicated that logistic regression, which considers *T. cruzi* infection together with discriminant analysis, would be the best model for the classification analysis. The main significant explanatory variables ($P \le 0.05$) that could best explain *T. vitticeps* occurrence and *T. cruzi* infection were found to be relative air humidity, average temperature, soil type, altitude and mammalian species richness (Table 3). While relative air humidity and soil type explained *T. vitticeps* and *T. cruzi* infection in almost all tested models, the former was directly correlated and the latter inversely proportional. Altitude elevation was found indirectly correlated with *T. cruzi* infection and directly correlated with *T. vitticeps*

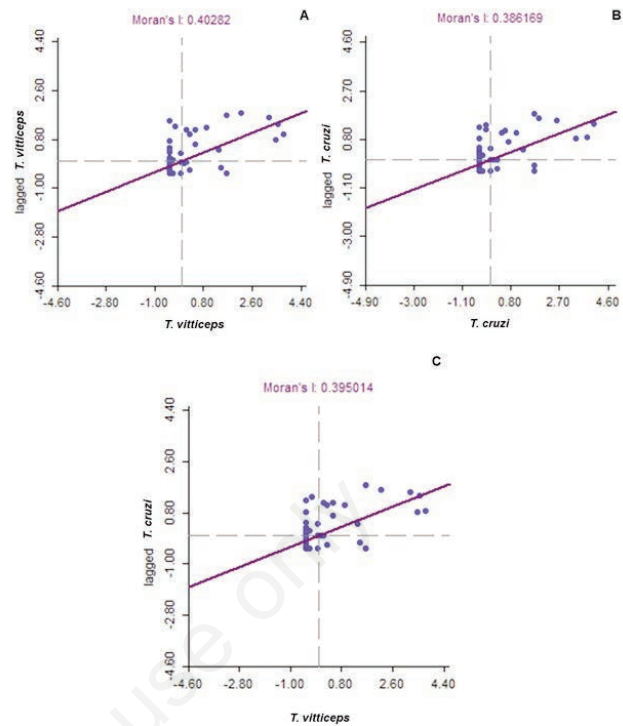**Figure 4. Univariate and bivariate global Moran's indexes scatter diagrams of *T. vitticeps* and *T. cruzi* correlations. A) *T. vitticeps* occurrence; B) *T. cruzi* infection; C) *T. vitticeps*/*T. cruzi* correlation.**
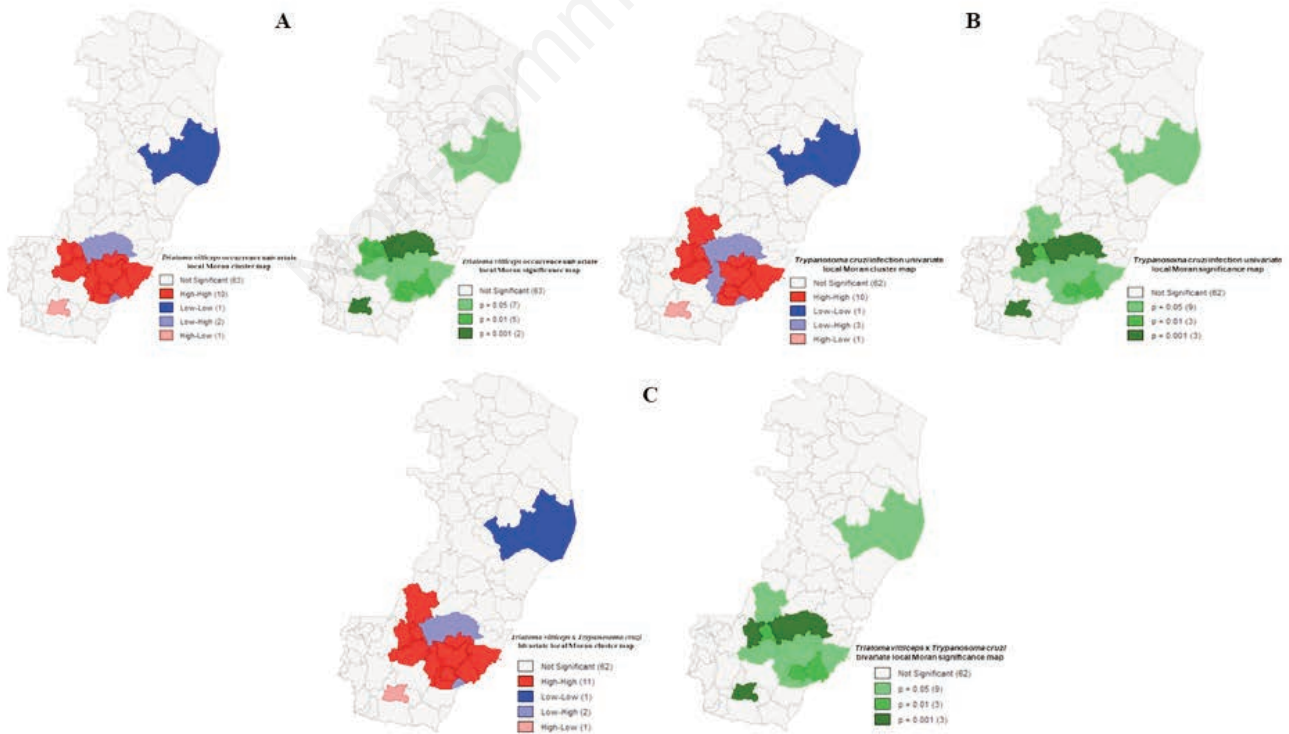

Figure 5. Significance and cluster maps of univariate and bivariate local Moran's index. A) *T. vitticeps* occurrence; B) *T. cruzi* infection; C) *T. vitticeps*/*T. cruzi* correlation.

occurrence, while the average temperature was directly correlated with *T. cruzi* infection. Mammalian species richness might inversely explain *T. vitticeps* occurrence, although in the generalized linear model (gamma distribution), the variable presented a direct correlation.

Spatial statistical modelling

As seen in Figure 4, the spatial statistical analysis showed a relationship between *T. vitticeps* and *T. cruzi* in areas with the same occurrence as in neighbouring areas (univariate). Figure 4C shows the relationship between the presence of *T. vitticeps* in one area and the presence of *T. cruzi* in the neighbouring area (bivariate), while the univariate and bivariate GMI values obtained for *T. vitticeps* occurrence (I=0.40282; P≤0.05), *T. cruzi* infection (I=0.386169; P≤0.05) and their correlation (I=0.395014; P≤0.05) demonstrated a positive or direct spatial autocorrelation indicating that these variables occurred in clustered patterns in ES State (Figure 4).

The maps of the LMI univariate and bivariate analyses pointed to the existence of statistically significant clusters of *T. vitticeps* occurrence. Figures 5A-C show the local univariate and bivariate Moran's index results with a P≤0.05. In the municipalities presented in grey shades in Figure 5, the classification was not significant within 999 interactions. Thus, the municipalities in the Central, South and North Coastal mesoregions were spatially autocorrelated (Figure 5A). In the North Coastal mesoregion (n=15), one municipality was classified as Low-Low autocorrelation for all three spatial statistical analyses (Figure 5A-C), in the Central mesoregion (n=24), eight municipalities were classified as High-High and two as Low-High autocorrelations, in the South mesoregion (n=22), two municipalities were classified as High-High autocorrelations and one as a High-Low. With respect to *T. cruzi* spatial autocorrelation, nine municipalities were classified as High-High and two as Low-High in the Central mesoregion. In the South mesoregion, one municipality was classified as including areas with High-High, High-Low and Low-High spatial autocorrelations. Regarding *T. vitticeps/T. cruzi* spatial autocorrelation, nine municipalities in the Central mesoregion were classified as High-High spatial autocorrelations and two as Low-High. In the South, two municipalities had High-High and one municipality High-Low spatial autocorrelations.

Given these results, the boundary municipality areas between the Central and South mesoregions can be said to have hotspots for *T. vitticeps* occurrence and *T. cruzi* infection. It is important to highlight that most of the hotspots were found within the Central mesoregion. In these cases of High-Low and Low-High spatial correlations, the variable pattern in each location was different from that of its neighbour, and distinct spatial regimes were observed in some areas in both mesoregions.

Map algebra modelling

According to the exploratory analysis, four explanatory variables showed direct correlations for *T. vitticeps* occurrence and *T. cruzi* infection (Table 4): average temperature, relative air humidity, vegetation and soil types. It is important to note that average temperature and relative air humidity were observed to have this same correlation in the statistical modelling. From the spatial point of view, the areas with the greatest direct correlation between the variables showed a focal pattern between the Central and South mesoregion borders (Figure 6). In addition, minimum and maximum temperatures, rainfall, altitude elevation and mammalian species richness presented indirect correlations with *T. vitticeps* occurrence and *T. cruzi* infection (Table 4). In the spatial analysis, the indirect correlations between the response and explanatory variables were associated with increased *T. vitticeps* occurrence and *T. cruzi* infection areas since the areas in red in Figure 6 were more frequently observed. Therefore, the occurrence area related to the response variable was expanded, with more widespread observations within the Central mesoregion; however, the main occurrence related to the response variables remained between Central and South mesoregions borders.

In the spatial correlation maps of *T. vitticeps* occurrence and *T. cruzi* infection (Figure 6), the Central mesoregion presented the greatest occurrence of both variables, and these correlations decreased in areas closer to the coastal area. It was also noticed that the high-correlation areas presented in the map algebra modelling were the same as those presented in the bivariate LMI. Although the observation was greater within the Central mesoregion, we emphasize that many of these observations were seen at their limits within the South mesoregion borders. This suggests that these areas present the ideal environmental conditions for *T. vitticeps* occurrence and *T. cruzi* infection in the ES Atlantic Forest. Although there were observations of *T. vitticeps* occurrence and *T. cruzi* infection in the Northwest mesoregion (Figure 6), the environmental conditions, according to map algebra modelling, did not favour this.

Table 4. Direct and indirect correlations between response and explanatory abiotic and biotic variables.

Explanatory variable	Correlation	
	T. vitticeps occurrence	*T. cruzi infection*
Maximum temperature	Indirect	Indirect
Average temperature	Direct	Direct
Minimum temperature	Indirect	Indirect
Rainfall	Indirect	Indirect
Relative air humidity	Direct	Direct
Altitude elevation	Indirect	Indirect
Mammal species richness	Indirect	Indirect
Vegetation type	Direct	Direct
Soil type	Direct	Direct

Discussion

We feel that it can be ruled out *T. cruzi* infection in *T. vitticeps* specimens in the ES State Atlantic Forest was acquired in the domiciliary environment, since this region is not endemic for Chagas disease (Sessa *et al.*, 2002) and no domiciliary colonies were found to be infected in the samples studied. According to our results, *T. vitticeps* occurrence and *T. cruzi* infection are concentrated in the Central and South ES mesoregions, as observed in the hotspots determined by both the spatial statistic and the map algebra modelling. This can be explained by the biotic and abiotic conditions observed in the areas indicated by statistical analyses.

The ecology of a multihost parasite, as *T. cruzi* is, involves multiple factors, which includes different hosts and vectors under different environmental transmission conditions. Spatial analysis for the study of vector-transmitted parasites allows us to evaluate how the environment can modulate a given transmission cycle and to anticipate possible epidemiological risks ahead of changing climate conditions. For studies involving environmental data of spatial phenomena and the transmission of vector-borne parasites, spatial statistical and related methodologies and analyses can be used. The advantages of using statistics in parasitology are that it allows the determination of the significance of each explanatory variable and its real influence on the phenomenon studied. However, a potential difficulty of interpretation in statistical analysis lies in the absence of visual evidence of the results, in this case, the areas of occurrence of the object of study. This is not the case in spatial statistics, where it is possible to envision parasite distribution patterns and transmission hotspots and to identify the environmental variables that influence these factors from the degree of spatial association present in the dataset. In this study, we applied map algebra modelling, which allowed us to evaluate the environmental influence and the occurrence area of parasite, host and vector together. Map algebra has proved very useful in studies of parasitology because it does not work with discrete and artificial geographic limits but with continuous areas that permits a clear visualization of the epicentre of the phenomenon studied and, by interpolation, its area of influence on its surroundings. Additionally, the results are easy to interpret. The disadvantage of
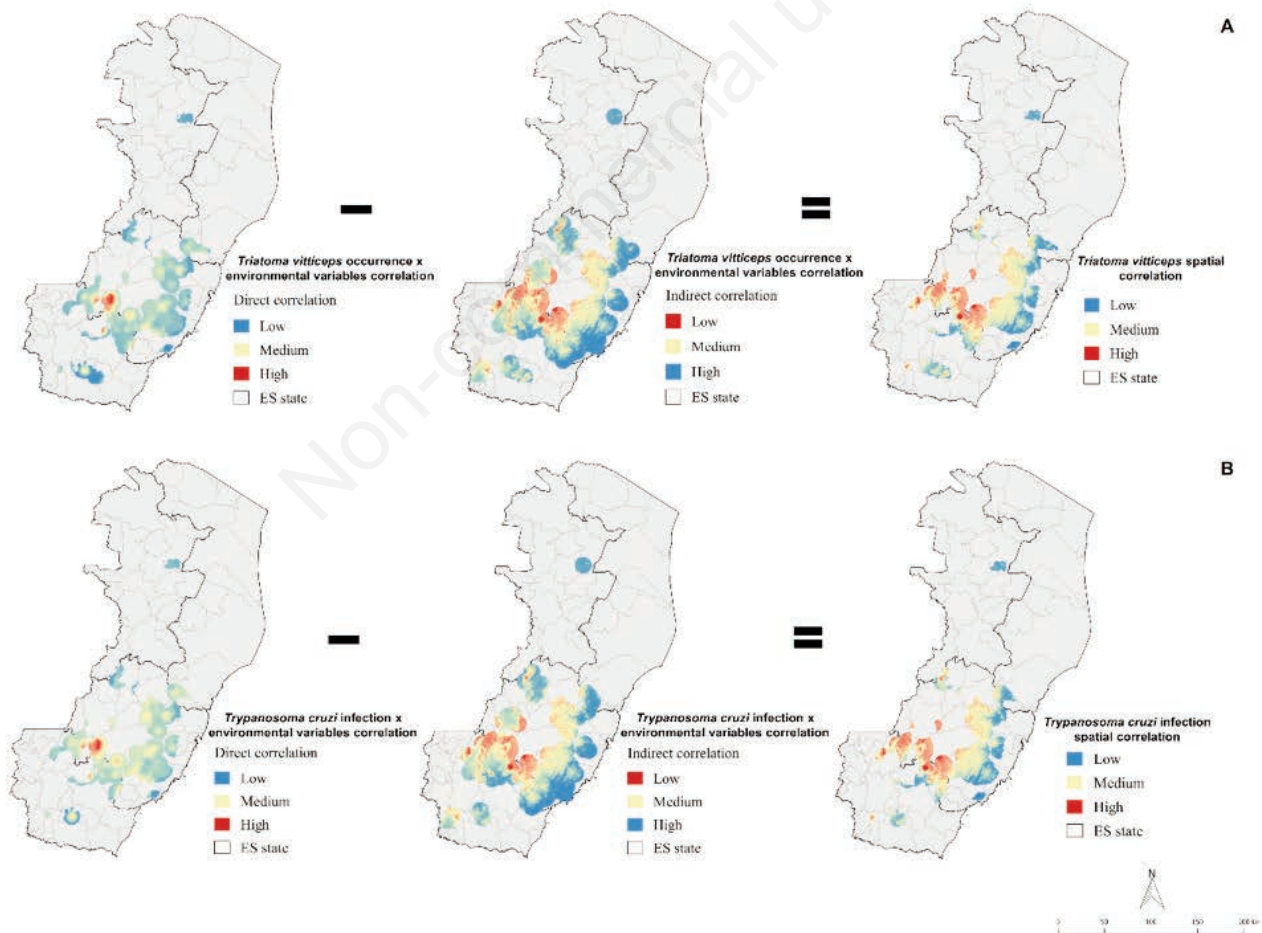

Figure 6. Spatial correlation of *Triatoma vitticeps* and *Trypanosoma cruzi* between environmental variables using map algebra modelling. A) *T. vitticeps* spatial correlation; B) *T. cruzi* infection spatial correlation.

this methodology is the need for a high degree of accuracy in data collection and preparation required.

In the multidisciplinary context, few studies have applied statistical modelling by GLM to analyse triatomine distributions in the environment and degree of *T. cruzi* infection of the insect (Suarez-Davalos *et al*., 2010; Grijalva *et al*., 2012; de Souza *et al*., 2015; Carbajal-de-la-Fuente *et al*., 2017; Espinoza Echeverria *et al*., 2017). Only two studies have applied GLM in the *T. cruzi* infection context, *i.e.* Xavier *et al*. (2012) and Fernández *et al*. (2019). This is the first study to analyse triatomine occurrence and *T. cruzi* infection together and their correlation with the environment. Additionally, comparing a broad range of statistical analyses was the best method for this type of study because the response variable data presented different types (count and classification).

Triatomines are highly variable in the sense that each species is capable of adapting to different environmental conditions and climatic variations. In *T. vitticeps* ecological niche modelling Gurgel-Gonçalves *et al*. (2012) showed that its distribution correlated with annual variables, such as mean temperature, mean diurnal range, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation of the wettest month, precipitation of the driest month and the normalized difference vegetation index (NDVI) values for the ES Atlantic Forest environment. These results revealed environmental areas suitable for species occurrence but did not indicate how each variable influenced its distribution. In addition, the authors did not evaluate other variables, such as relative air humidity, altitude and mammalian fauna. During the modelling, we observed that the higher the relative humidity, the greater the *T. vitticeps* occurrence. This could explain why this species is so well adapted to the Atlantic Forest and not capable of surviving in other Brazilian biomes. Although the precipitation variable was not significant in our study, a previous investigation performed in Minas Gerais State showed that rainfall was the most important variable for the occurrence of *T. vitticeps* (de Souza *et al*., 2010). The Atlantic Forest presents microhabitats that influence *T. vitticeps* biology, which is probably determined by the whole set of variables of these microhabitats.

According to Barrozo *et al*. (2017), relative humidity is important in the selection of *T. vitticeps'* resting places and indeed the general location of triatomines, as has been observed for *Rhodnius prolixus* at room temperature (Wigglesworth *et al*., 1934; Barrozo *et al*., 2003; 2017). We demonstrate here that relative air humidity together with average temperature is related to *T. vitticeps* infection by *T. cruzi*. Additionally, relative humidity favours heat exchange between a host (the emitter), in this case warm-blooded animals, and the thermoreceptors of triatomines since humid air has a higher thermal conductivity and better heat capacity than dry air (Barroso *et al*., 2017). This means that relative humidity can induce the contact of triatomines with mammals thereby increasing their chance of *T. cruzi* infection as well as the number of individuals becoming infected with *T. cruzi*.

Contrary to the effect of real-time air humidity, the relationship between temperature and *T. cruzi* infection has been more thoroughly investigated, especially regarding how ambient temperature can influence *T. cruzi* infection in triatomines (Asin & Catalá, 1995; Pérez-Morales *et al*., 2017; de Fuentes-Vicente *et al*., 2018). Under laboratory conditions, *T. cruzi* has been shown to develop faster in *T. infestans* as the temperature increased (Asin and Catalá, 1995), while increased metacyclogenesis with increasing temperature has been observed in *R. prolixus* specimens (Tamayo *et al*., 2018). This observation could explain the high *T. cruzi* infection rates and high metacyclic rates in *T. vitticeps* in our study area.

The abiotic variable altitude showed a significant indirect relationship to *T. cruzi* infection in *T. vitticeps*. In a study from Oaxaca State, Mexico, altitude was described as influencing the *T. cruzi* infection rate in *T. mazzotti* and *T. phyllosoma* negatively, *i.e. T. cruzi* infection rates decreased at higher altitudes (Ramsey *et al*., 2000). Since temperature and altitude elevation are connected, it is possible that they could influence the probability of triatomine infection due to species differences in thermotolerance and thermopreference (de Fuentes-Vicente *et al*., 2018).

Mammalian species richness showed an indirect correlation with *T. vitticeps* occurrence in the statistical modelling and with *T. cruzi* infection in the map algebra modelling. This means that the lower the mammalian species richness, the greater the chance of encountering triatomines in the residential environment, and the greater the number of triatomines infected by *T. cruzi.* This correlation can be explained by the fact that the environmental area has suffered degradation due to human actions and thereby influenced mammalian species richness negatively. However, Leite *et al*. (2011) suggest that less degraded areas can maintain larger triatomine populations and, consequently, have higher dispersion and domiciliary invasion rates in the Atlantic Forest, and Xavier *et al*. (2012) found that dogs had higher *T. cruzi* infection rates in areas with lower mammal species richness and abundance. The only clear finding is that lower mammalian species richness favours triatomines contacting mammalian species that can become infected with *T. cruzi*, resulting in triatomines feeding on these hosts. From this correlation, we can affirm the phenomenon of the amplification effect (Ostfeld & Keesing, 2000; Schmidt *et al*., 2012; Simpson *et al*., 2012) in the area examined in the present study.

The soil type showed an indirect correlation in the statistical modelling for *T. vitticeps* occurrence and *T. cruzi* infection, but it is unclear how much this influences the epidemiological scenario. The single study in which soil types were analysed showed that *T. dimidiata* presented morphological changes according to type of soil in El Salvador (Carmona-Galindo *et al*., 2020). This was not the case in our study. One possible explanation for this is change of land cover, *e.g.*, deforestation of land to become used for agriculture, could influence the correlation in the study area. In addition, almost all *T. vitticeps* specimens in this study were collected inside domiciles, that had been invaded by adult stage insects that could fly in from outside. We do not know from how far they came because we do not know the flying abilities of these insects. However, it is likely that they came from far away since the mammals in the peridomestic areas did not demonstrate infection with *T. cruzi,* unlike the triatomines, which presented high rates of *T. cruzi* infection.

Spatial autocorrelation is an important tool when referring to geographical analysis (Chen, 2013). A way to calculate the spatial autocorrelation is using Moran's index, which is a Pearson's correlation between neighbours (Moran, 1948). This methodology can show how an event is occurring, is distributed and indicates hotspots in a specific area; it is an important method for evaluating disease surveillance because it can identify geographical areas with certain spatial distributions (Elliott and Wartenberg, 2004; Robertson and Nelson, 2014). The use of this approach in studies concerning spatiotemporal analysis of infectious neglected diseases has increased in recent years. Using univariate GMI and LMI have shown that several diseases present cluster distribution patterns including hotspots (Machado *et al*., 2017; Osei & Stein, 2017; Mandal *et al*., 2018; Salimi *et al*., 2018; Tewara *et al*., 2018; Alene *et al*., 2019; Nuñez-González *et al*., 2019; Okunlola *et al*., 2019; Vivaldini *et al*., 2019). Bivariate GMI and LMI have only been applied to evaluate spatial autocorrelation and clusters

between visceral leishmaniasis incidence and conditions of vulnerability (Rocha *et al.*, 2018).

In relation to triatomines, *T. cruzi* and Chagas disease, spatial autocorrelation by Moran's *I* has not been used frequently. Only one study evaluating Chagas disease in Ecuador (Nuñez-González *et al.*, 2019) reported cluster distributions and hotspots throughout the country. This work was the first to apply Moran's index for the analysis of *T. vitticeps* and *T. cruzi*. The results demonstrated the presence of spatial clusters of *T. vitticeps* and *T. cruzi* infection in the Atlantic Forest, especially in the ES Central and South mesoregions. These areas must be constantly monitored because *T. vitticeps* is constantly found in the domiciliary environment and, as a consequence, contributes to the risk of infection suffered by the inhabitants.

Xavier *et al.* (2012) demonstrated that map algebra modelling is an efficient tool for prediction studies of vector-borne parasite transmission. The difference in the map algebra regarding the type of representation does not have strict limits and therefore allows for the analysis of continuous space and the possibility of multi-spatial analysis. This is different from other spatial methodologies, *i.e.* the Moran's index, that performs at most bivariate analyses, while the representation of the unit of analysis is regionally limited. The results observed in this analysis confirm the results observed by spatial statistics and other statistical analyses since the significant variables and the regions indicated by the spatial statistical analysis were almost the same as those indicated by the map algebra modelling.

Limitations of this study presents include difficulties in implementing and working with statistical programmes in entomological surveillance routinely, the climatic and environmental data surveys can present gaps during the studied period and validation of the related variables in the field can be difficult. Map algebra is easy to apply and the results easy to interpret as the final product is visualized and therefore usful for health agents when monitoring parasite transmission and performing spatiotemporal analysis. Thus, the best prevention measures can be taken as health agents can see and target priority areas for monitoring.

Conclusions

Study of the environment is essential for understanding the transmission cycle of a given parasite species, especially that of multihost parasite species. In this case, it was possible to explain why *T. vitticeps* and *T. cruzi* infections occurred in the ES Central and South mesoregions due to favourable conditions demonstrated by statistical analysis and map algebra modelling. Statistical methodologies should be incorporated routinely to expand the understanding of vector-borne parasite transmission, while the cartographic tools are reliable and make it possible to understand the environmental variables that modulate the transmission cycles within nature. Multidisciplinary studies are essential and should be used more often in regard to vector-borne parasites.

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