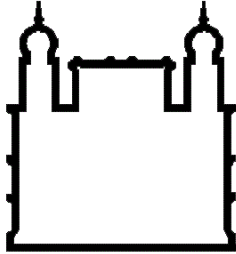


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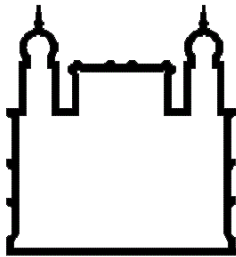
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GILMAR RIBEIRO-JR

**SALVADOR
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Tese apresentada ao Curso da Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa como avaliação parcial para obtenção do título de doutor.

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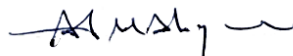
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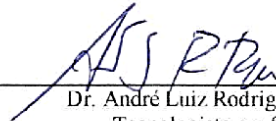
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RESUMO

VULNERABILIDADE PARA TRANSMISSÃO VETORIAL DE *Trypanosoma cruzi* NO ESTADO DA BAHIA.

A doença de Chagas é um dos principais problemas de saúde pública em todo planeta, em especial, para a América Latina, com cerca de 70 milhões de pessoas sob risco de infecção e, aproximadamente, 5,7 milhões de indivíduos infectados por *Trypanosoma cruzi* em 2010. Apesar desse quadro, pouco se conhece sobre as áreas com vulnerabilidade para a transmissão vetorial da Chagas na Bahia, informação relevante e estratégica para orientar as ações de vigilância e combate ao agravo. O objetivo principal dessa pesquisa foi classificar os municípios da Bahia quanto ao grau de vulnerabilidade para doença de Chagas. Os objetivos alcançados estão apresentados na forma de quatro artigos científicos, três deles já publicados e um na forma de manuscrito: O primeiro artigo avalia os padrões alimentares e taxa de infecção por *T. cruzi* de triatomíneos capturados na Bahia entre 2012 e 2014 e mapeia a ocorrência de triatomíneos infectados, alimentados em humanos e animais domésticos. No segundo artigo, apresentamos o TriatoScore, um novo indicador para a classificação de triatomíneos e territórios geográficos. No terceiro, demonstramos as alterações espaço-temporais na distribuição e frequência dos principais triatomíneos sinantrópicos do Estado da Bahia em dois períodos (A:1957-1971 e B:2006-2019). No quarto, ainda na forma de manuscrito, utilizamos a Análise Multicritério de Decisão (AMD), levando em consideração indicadores entomológicos baseado no TriatoScore; socioeconômicos, percentual de imóveis na zona rural vivendo em situação de extrema pobreza; demográficos (densidade demográfica); e dados epidemiológicos: (a) dispensação de benzonidazol; (b) autorização de internação hospitalar; (c) óbitos por doença de Chagas; (d) doença de Chagas auto referido; (e) residência e naturalidade de pacientes detectados na triagem sorológica na Hemoba, para classificar os municípios mais vulneráveis para a doença de Chagas na Bahia. Na avaliação dos 5906 triatomíneos coletados na Bahia entre 2012 e 2014, identificamos 15 espécies de vetores de *T. cruzi* já descrita no Estado, presentes em 127 dos 417 municípios da Bahia. As análises moleculares de 695 triatomíneos revelaram 10% de infecção por *T. cruzi*, sendo essas taxas maiores nas espécies complexo *T. brasiliensis*. Constatamos que a maioria dos triatomíneos se alimentou de sangue de aves (74,2%), outras fontes alimentares foram cães (6%), gatos (0,5%) e humanos (1%), sendo observada a ocorrência de triatomíneos infectados com *T. cruzi* e alimentados com sangue humano foram detectados dentro das casas. A análise geoespacial revelou ampla distribuição de triatomíneos infectados por *T. cruzi* na Bahia, além disso, triatomíneos alimentados em cães, humanos e gatos foram observados principalmente na região Nordeste do Estado. No segundo artigo apresentamos o “TriatoScore”, um indicador

entomológico que se baseia em informações sobre o comportamento ecológico e a biogeografia de distribuição de espécies de triatomíneos. Os índices mais elevados foram maiores em municípios dominados por ecorregiões secas a semiáridas, quando comparados a aqueles dominados por campos de savana ou, especialmente, ecorregiões de mata atlântica. (3) No terceiro artigo, realizamos uma análise comparativa dos dados entomológicos nas bases de dados disponíveis, tendo sido observado mudanças na distribuição espaço-temporal dos vetores no Estado. Das 26 espécies de triatomíneos já registradas no Estado, 18 foram encontradas no período A e 20 em B. *P. megistus* foi a espécie mais frequente nos municípios no período A (38%), enquanto no período B, foram *T. sordida* (38%) e *T. pseudomaculata* (35%). Destacamos que no período B a maioria dos triatomíneos (91%) foi capturada no peridomicílio. Dentro das casas, e as espécies com maior abundância relativa foram *T. sordida* (51%), *T. brasiliensis* (28%) e *T. pseudomaculata* (15%), enquanto no peridomicílio houve predomínio de *T. sordida* (86%). Observamos acentuada redução de *T. infestans* no período B, com último foco residual detectado em 2015 no município de Novo Horizonte. No quarto artigo, na forma de manuscrito, revelamos a existência de municípios com alta vulnerabilidade para doença de Chagas em quase todo o território da Bahia, com exceção do Sul do Estado, que foi classificado como de baixa vulnerabilidade. Ao estratificarmos os resultados por Mesorregião, as principais áreas vulneráveis estavam localizadas nas regiões Centro-Sul, Vale do São Francisco, Metropolitana de Salvador e em especial na Região Centro-Norte, que apresentou maior proporção de municípios considerados como de alta vulnerabilidade. Os municípios com alta vulnerabilidade apresentaram maior pobreza na zona rural, baixa densidade demográfica e maior frequência de espécies sinantrópicas epidemiologicamente relevantes. A associação da AMD e geoestatística auxiliou a identificarmos 80 municípios (19%) classificados como de alta vulnerabilidade para a doença de Chagas, apesar disso, o risco de transmissão de *T. cruzi*, em suas diferentes formas, continua presente em todo Estado.

Palavras Chaves: Doença de Chagas, *Trypanosoma cruzi*, Triatomíneos, Bahia, Vulnerabilidade, Biologia Molecular, Geoestatística.

ABSTRACT

VULNERABILITY OF *Trypanosoma cruzi* VECTOR BORNE TRANSMISSION IN THE STATE OF BAHIA.

Chagas' disease is one of the central public health problems worldwide, especially in Latin America, with approximately 70 million people at risk of infection and about 5.7 million individuals infected by *Trypanosoma cruzi* in 2010. Despite these circumstances, little information is available about areas vulnerable to vector transmission of *T. cruzi* in Bahia, a piece of relevant and strategic information to guide surveillance actions and fight against the disease. The main objective of this research was to classify the Municipalities of Bahia according to the degree of vulnerability to Chagas' disease. We group the achieved Objectives into four articles, three published in a peer-review scientific journal. We describe through the first paper the prevalence of *T. cruzi* infection and bloodmeals and map between 2012 and 2014 *T. cruzi*-infected triatomines fed on humans and domestic animals. In the second article, we develop and describe TriatoScore, a new indicator to classify triatomines and geographic territories. In the third, we demonstrate space-time changes in the distribution and frequency of the main synanthropic triatomines in Bahia during two periods (A:1957–1971 and B:2006–2019). In the fourth, unpublished manuscript, we used the Multicriteria Decision Analysis (MDA) considering multiple indicators: Entomological (TriatoScore), Socioeconomic (percentage of properties in rural areas living in extreme poverty), Demographics (demographic density), and Epidemiological data: (a) Dispensation of benznidazole; (b) Hospital Admission Authorization; (c) Deaths caused by Chagas disease; (d) Self-reported Chagas disease; (e) residence and place of birth of patients detected in the serological screening at Hemoba; to classify the most vulnerable municipalities for Chagas disease in Bahia. In the first paper, when evaluating the 5906 triatomines collected in Bahia between 2012 and 2014, we identified 15 species of *T. cruzi* vectors, present in 127 of the 417 municipalities of Bahia. Molecular analysis of 695 triatomines revealed ~10% *T. cruzi* infection, with these rates higher in *T. brasiliensis* complex species. We observed most triatomines fed on avian (74.2%). Other triatomine blood meals were dogs (6%), cats (0.5%), and humans (1%), and the occurrence of infected triatomines was observed with *T. cruzi* and fed with human blood were detected inside house. Geospatial analysis revealed a wide distribution of *T. cruzi* infected in Bahia, and Triatomine fed on dogs, humans, and cats were described in the Northeast Region. Through the second paper, we present TriatoScore. An entomological score based on information on ecological behavior and biogeography of

triatomine species. TriatoScore values were higher in municipalities of Dry to Semi-arid Ecoregions comparing those dominated by Savannah Grasslands or Atlantic Forest Ecoregions. (3) In the third article, we conducted a comparative analysis of entomological data in available databases, observing changes in the space-time distribution of vectors in Bahia. Of the 26 triatomine species registered, 18 were in Period A while 20 in B. *P. megistus* was the most frequent species in the municipalities of Bahia in Period A (38%) and *T. sordida* (38%), *T. pseudomaculata* (35%) in Period B. We describe *T. sordida* (51%), *T. brasiliensis* (28%), and *T. pseudomaculata* (15%) as the high relative abundance species inside houses. *T. sordida* predominated in peridomicile (86%). We observed a marked decrease in the spatial distribution of *T. infestans* in Period B, with the last residual focus detected in 2015 in the municipality of Novo Horizonte. In the fourth article, we reveal areas with high vulnerability to Chagas' disease in the entire territory of Bahia, except South Region, which ranked as a low vulnerability area. When we stratified the results by Mesoregion, the main vulnerable areas were located in Center-South, São Francisco Valley, Metropolitan Region of Salvador, and especially in the Center-North Region, which had a higher proportion of municipalities considered as highly vulnerable. The high-vulnerability municipalities held high values of poverty in rural areas, low demographic density, and high municipal values of TriatoScore. The association of AMD and geostatistics helped us identify 80 municipalities (19%) classified as highly vulnerable to Chagas disease. Despite that, the risk of *T. cruzi* transmission, in its different forms, continues present throughout Bahia.

Keywords: Chagas disease, *Trypanosoma cruzi*, Triatomines, Bahia, Vulnerability, Molecular Biology, Geostatistics.

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LISTA DE SIGLAS E ABREVIATURAS

AIH	Autorização de Internação Hospitalar
BA	Bahia
BHC	Hexaclorobenzeno
°C	Graus Celsius
CA	Califórnia
CCZ	Centro de Controle de Zoonoses
CID	Classificação Estatística Internacional de Doenças e Problemas Relacionados com a Saúde
CMIA	Quimiluminescência
DC	Doença de Chagas
DIVEP	Diretoria de Vigilância Epidemiológica
DNA	Ácido desoxirribonucleico
DTU	<i>Discrete Typing Unit</i>
ELISA	Imunoensaio enzimático
EUA	Estados Unidos da América
FIOCRUZ	Fundação Oswaldo Cruz
Hab.	Habitantes
HAI	Hemaglutinação indireta
HEMOBA	Fundação de Hematologia e Hemoterapia da Bahia
IBGE	Instituto Brasileiro de Geografia e Estatística
IDH	Índice de Desenvolvimento Humano
IFA	Imunofluorescência indireta
IFN	Índice de Infecção Natural
IgG	Imunoglobulina G
IGM	Instituto Gonçalo Moniz
IL	Illinois
km	Quilômetros
km ²	Quilômetros quadrados
LACEN	Laboratório Central de Saúde Pública
LPBM	Laboratório de Patologia e Biologia Molecular
µL	Micro litro

ng	Nano grama
nm	Nano metro
OMS WHO	Organização Mundial de Saúde / World Health Organization
OPAS	Organização Pan-americana da Saúde / Pan American Health
PAHO	Organization
pb	Pares de bases
PBS	<i>Phosphate Buffer Saline</i>
PCR	Reação em Cadeia da Polimerase
PIB	Produto Interno Bruto
PIT	Posto de Informação de Triatomíneo
PNCDCCh	Programa Nacional de Controle da Doença de Chagas
Prof.	Professor
RMS	Região Metropolitana de Salvador
SESAB	Secretaria da Saúde do Estado da Bahia
SIAB	Sistema de Informação de Atenção Básica
SIH	Sistema de Informações Hospitalares
SIM	Sistema de Informação de Mortalidade
SMS	Secretária Municipal de Saúde
SPSS	Statistical Package for the Social Sciences
SSA	Salvador
SUS	Sistema Único de Saúde
SUVISA	Superintendência de Vigilância em Saúde
SVO	Serviço de Verificação de Óbitos
TC	<i>Trypanosma cruzi</i>
UD	Unidade Domiciliar
UV	Ultravioleta

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1. INTRODUÇÃO

A doença de Chagas humana (DCH) representa um sério problema de saúde pública mundial, com maior impacto, significado social e implicações econômicas para os pacientes e os países da América Latina (LIDANI *et al.*, 2019). A transmissão nos países endêmicos é principalmente vetorial, causada pelo protozoário *Trypanosoma cruzi* (Chagas 1909), sendo transmitido por triatomíneos (Hemiptera: Reduviidae) infectados (CHAGAS, 1916).

Publicações da Organização Mundial de Saúde (OMS) estimaram cerca de 70 milhões de pessoas sob risco de infecção e, aproximadamente, 5,7 milhões de indivíduos infectados por *T. cruzi* em 2010 na América Latina (WHO, 2015). No Brasil, estima-se a prevalência agrupada de portadores da doença de Chagas em 4,2%, variando de 4,4% na década de 1980 a 2,4% após 2000 (WHO, 2015). Considerando essas prevalências, as estimativas mais recentes são de ~1,3 a 3,2 milhões de pessoas infectadas só no Brasil, com ~321 e ~409 mil infectados que provavelmente, irão desenvolver as formas digestiva e cardíaca do agravo (DIAS, J. C. *et al.*, 2016). Esse cenário ilustra as elevadas morbidade e mortalidade dos portadores (COURA; VINAS, 2010), causando impacto financeiro não somente ao sistema público de saúde de um País, mas também, à previdência social (HOTEZ *et al.*, 2012; LEE *et al.*, 2013).

A transmissão de *T. cruzi* é historicamente associada à zona rural, tipo de moradia e condições insalubres na residência, cenários que favorecem a colonização de triatomíneos no interior das residências (GURTLER; YADON, 2015; PARRA-HENAO *et al.*, 2016), entretanto, em nível nacional, os dados disponíveis apontam para o risco de transmissão, independentemente da colonização do vetor (PINTO *et al.*, 2009; SILVEIRA; DIAS, 2011). Vale destacar também a transmissão por alimentos contaminados, em especial na Região Norte do País, e nos países não endêmicos, predominam a forma de transmissão congênita, transfusão sanguínea e transplante de órgãos.

Os triatomíneos são insetos essencialmente silvestres, no entanto, algumas espécies possuem populações capazes de se adaptar às modificações antrópicas na paisagem natural, podendo gradualmente ser encontrado no ambiente domiciliar (LENT; WYGODZINSKY, 1979; COSTA *et al.*, 2015; GALVAO; JUSTI, 2015). Os triatomíneos invadem as residências em geral na forma adulta (BARBU *et al.*, 2013; WALECKX *et al.*, 2015) e eventualmente ocorre colonização (BERN *et al.*, 2019). No caso de colonização intradomiciliar, a ocupação acontece geralmente em residências construídas com precárias

condições e técnicas de edificação rudimentares, como as casas de *taipa* ou *pau-a-pique*, onde as frestas, típicas deste tipo de construção, oferece abrigo para os vetores de *T. cruzi* (WALTER *et al.*, 2005; GURTLER; YADON, 2015).

A ocorrência de um triatomíneo no ambiente intradomiciliar, seja de forma intrusiva ou por colonização, expõe os moradores das residências às picadas desses insetos, que podem causar reações alérgicas (ANDERSON; BELNAP, 2015) e, aumentam o risco de transmissão de *T. cruzi* ao homem e também aos seus animais domésticos (CORDOVEZ; SANABRIA, 2014; SALDANA *et al.*, 2015; WALECKX *et al.*, 2015). Mais recentemente, devido à ocupação de remanescentes florestais nas grandes metrópoles tem sido observada a ocorrência de vetores infectados em casas e apartamentos (RIBEIRO-JR; GURGEL-GONÇALVES, 2015).

No Brasil, as ações de combate aos principais vetores de *T. cruzi* iniciaram de forma organizada com o *Programa Nacional de Controle da Doença de Chagas* (PNCDCh) a partir de 1975 (SILVEIRA, 2011). Neste período, a espécie *T. infestans* possuía ampla distribuição em Unidades Domiciliares (UDs) pelo País, além de alta infestação no ambiente intradomiciliar. Esse vetor triatomíneo possui como característica marcante a alta sinantropia e a capacidade de proliferação com altas densidades em ambiente domiciliar (GUARNERI *et al.*, 2000). Tais características fizeram com que *T. infestans* fosse considerado o principal vetor da DCH no País (PASSOS; SILVEIRA, 2011; SILVEIRA, 2011; SILVEIRA; DIAS, 2011). Entretanto, por se tratar de uma espécie exótica no Brasil, provavelmente com origem nos vales andinos da Bolívia (GARCIA *et al.*, 2013; PANZERA *et al.*, 2014), *T. infestans* demonstra pouca competência em se adaptar as características naturais dos biomas nacionais, sendo restrito, portanto, aos ambientes antrópicos (ecossistema urbano) e considerado exclusivamente doméstico no Brasil (SILVEIRA, 2011; SILVEIRA; DIAS, 2011).

A partir de 1991, o Brasil integrou junto com os países do Cone Sul (WHO, 1991; 2000; BRASIL, 2001), algumas iniciativas para eliminar o principal vetor doméstico no país – *T. infestans*. As ações massivas e sistemáticas de tratamento químico surtiram efeito e, em abril de 2006 (DIAS, 2007), o Brasil recebeu da OPAS a “*Certificação Internacional de Eliminação da Transmissão da Doença de Chagas por Triatoma infestans*”.

Na Bahia, já foram descritas diversas áreas endêmicas para a transmissão vetorial de *T. cruzi* (GONÇALVES, 1912; PONDÉ, 1946; COSTA, 1955; SILVA, 1966; BARRETT *et al.*, 1979; BARRETT *et al.*, 1980; MAGUIRE *et al.*, 1986; HAIDAMAK *et al.*, 2016; SILVEIRA *et al.*, 2016), associadas, principalmente, às espécies *T. infestans* e *Panstrongylus*

megistus (Burmeister, 1835) (COSTA, 1955; LEAL *et al.*, 1965; SHETLOCK; SERAFIM, 1972; PIESMAN *et al.*, 1985; MAGUIRE *et al.*, 1986; ALMEIDA *et al.*, 2009).

As primeiras pesquisas, iniciaram com os esforços do pesquisador Pirajá da Silva logo após a descrição de *T. cruzi* por Carlos Chagas em 1909 (SILVA, 1966; FALCÃO, 2008). Nos anos seguintes foram realizados esforços científicos na Bahia para identificação dos triatomíneos que catalogaram 18 espécies de triatomíneos até a década de 1970 (NEIVA, 1914; SHETLOCK; SERAFIM, 1972; ALMEIDA, 2015). Recentemente, após diversas descrições e revisões, foram reconhecidas as ocorrências de 26 espécies diferentes de triatomíneos (SOUSA *et al.*, 2020). Neste cenário, observa-se que diversos fatores podem alterar a distribuição e abundância relativa das espécies mais relevantes em transmitir *T. cruzi* ao homem, ao longo do tempo. Sendo assim, as análises comparativas de dados epidemiológicos (SHETLOCK; SERAFIM, 1972; MENDONCA *et al.*, 2015), do nível de domiciliação (SHETLOCK; SERAFIM, 1972; REBELO *et al.*, 1998; ALMEIDA *et al.*, 2008; ALEVI *et al.*, 2015; MENDONCA *et al.*, 2015) e da distribuição espacial dos vetores (SHETLOCK; SERAFIM, 1972; REBELO *et al.*, 1998; PHILLIPS *et al.*, 2006; ALMEIDA *et al.*, 2008; COSTA; PETERSON, 2012; GURGEL-GONCALVES *et al.*, 2012; ALEVI *et al.*, 2015; MENDONCA *et al.*, 2015) são estratégias válidas para a vigilância epidemiológica e para o delineamento estratégico das ações de vigilância e combate às espécies sinantrópicas de triatomíneos (SHETLOCK; SERAFIM, 1972; REBELO *et al.*, 1998; PHILLIPS *et al.*, 2006; ALMEIDA *et al.*, 2008; MARTINEZ-IBARRA *et al.*, 2008; COSTA; PETERSON, 2012; GURGEL-GONCALVES *et al.*, 2012; ALEVI *et al.*, 2015; MENDONCA *et al.*, 2015).

Por exemplo, *T. infestans* é um vetor competente com grande capacidade vetorial. Estima-se que no passado, entre as décadas de 1960 e 1980 (DIAS; SCHOFIELD, 1998), mais de 80% dos casos da doença de Chagas, no Brasil, ocorreram devido à transmissão vetorial por esta espécie (MENDONCA *et al.*, 2015). Nos locais onde existem diversos vetores competentes, reconhecer a distribuição geográfica, avaliar o nível de domiciliação dos triatomíneos e estabelecer uma classificação epidemiológica que reflète a capacidade relativa das espécies na transmissão de *T. cruzi* é estratégico para a vigilância dos triatomíneos sinantrópicos (SARKAR *et al.*, 2010; BERN *et al.*, 2011; CURTIS-ROBLES *et al.*, 2015).

Além das informações dos triatomíneos, para o efetivo delineamento de áreas vulneráveis à transmissão vetorial de *T. cruzi* por triatomíneos sinantrópicos, é necessário também avaliar determinantes sociais, fatores econômicos e biológicos (KJOS *et al.*, 2013;

GORCHAKOV *et al.*, 2016), pois as variáveis ambientais e sociais são capazes de influenciar no estilo de vida das populações, tornando-as mais vulneráveis a se expor à infecção por *T. cruzi* a partir de transmissão vetorial (GARCIA *et al.*, 2015a; GARCIA *et al.*, 2015b; GORCHAKOV *et al.*, 2016).

Desta forma, o objetivo deste estudo foi analisar a ocorrência e distribuição dos triatomíneos, determinar a taxa de infecção por *T. cruzi* e os padrões alimentares nos triatomíneos pesquisados na Bahia e classificá-los quanto à importância epidemiológica de transmitir *T. cruzi* ao homem e seus animais domésticos por meio de um novo indicador entomológico, alternativo e reprodutível. E por fim, a partir de dados vetoriais, dados sociais, econômicos e epidemiológicos do PNCDCh realizar a classificação dos municípios baianos quanto ao grau de vulnerabilidade para doença de Chagas e assim, definir regiões geográficas prioritárias para a vigilância do agravo no Estado na Bahia.

2. REVISÃO DE LITERATURA

2.1. Histórico da doença de Chagas

A Tripanossomíase Americana, mais conhecida como doença de Chagas (DCH), foi descoberta por Carlos Ribeiro Justiniano das Chagas (Figura 1), em 1909, na cidade de Lassance, situada na Região Norte do Estado de Minas Gerais.



Figura 1 – Registros históricos das atividades do Pesquisador Carlos Chagas.

Legenda: A esquerda observa-se o médico e a primeira paciente descrita com a doença – Berenice; B. à direita, a casa de Berenice. Fonte: <https://chagas.fiocruz.br/historia/a-descoberta/>

Em Lassance, Carlos Chagas coletou alguns insetos conhecidos popularmente como “barbeiros” que estavam se alimentando em indivíduos que viviam em condições precárias de habitação. Ele enviou alguns exemplares para o médico e professor Oswaldo Cruz, que realizou exames e isolou um parasita coletado nas fezes do inseto. Em 14 de abril de 1909, Carlos Chagas descobriu em uma criança de dois anos de idade chamada Berenice os mesmos protozoários que havia descoberto anteriormente nos triatomíneos e em outros mamíferos (Figura 2). Em seguida, discorreu sobre o agente etiológico da tripanossomíase americana, sua biologia no hospedeiro vertebrado e invertebrado, seus reservatórios e diversos aspectos da patogenia e sintomatologia da doença (CHAGAS, 1916).

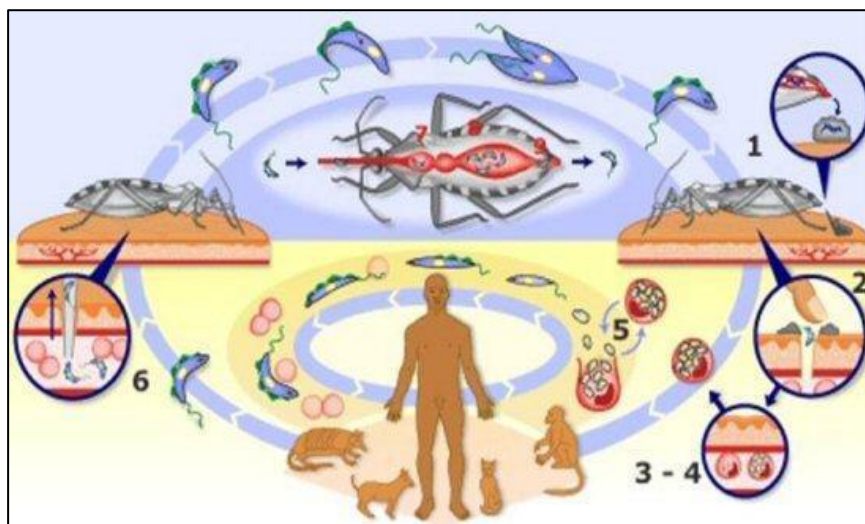


Figura 2 – Ciclo biológico de *Trypanosoma cruzi* (Chagas, 1909).

Legenda: 1. Repasto sanguíneo e defecação do triatomíneo; 2. Contaminação por *T. cruzi* pela manipulação das fezes; 3-4. Replicação intracelular do *T. cruzi*; 5. Lise de células parasitadas pelo *T. cruzi* e liberação dos parasitas na corrente sanguínea do hospedeiro vertebrado (parasitemia); 6. Novo repasto de triatomíneo; Fonte: WHO;

2.2. Epidemiologia

A infecção por *T. cruzi* ocorre desde o extremo sul dos Estados Unidos, se estendendo por toda a América Central e do Sul, em áreas endêmicas e não endêmicas (Figura 3) (CHAGAS, 1916; WHO, 2000; LÓPEZ-VÉLEZ *et al.*, 2020). Na década de 1990, foi descrita a estimativa de que ~18 milhões de pessoas estavam infectadas por este parasita, e que ~40 milhões de indivíduos estavam expostos ao risco de adquirir infecção em pelo menos 15 países. Em 2000, ocorreram 21 mil mortes por doença de Chagas e 200 mil novos casos registrados. No ano de 2005, de acordo com estimativas da Organização Pan-Americana de Saúde, 20% da população da América Latina estava sob risco de infecção (neste caso, 109 milhões de indivíduos) e cerca de 7,7 milhões de pessoas estavam infectadas (RASSI A. JR *et al.*, 2010) (Figura 4).

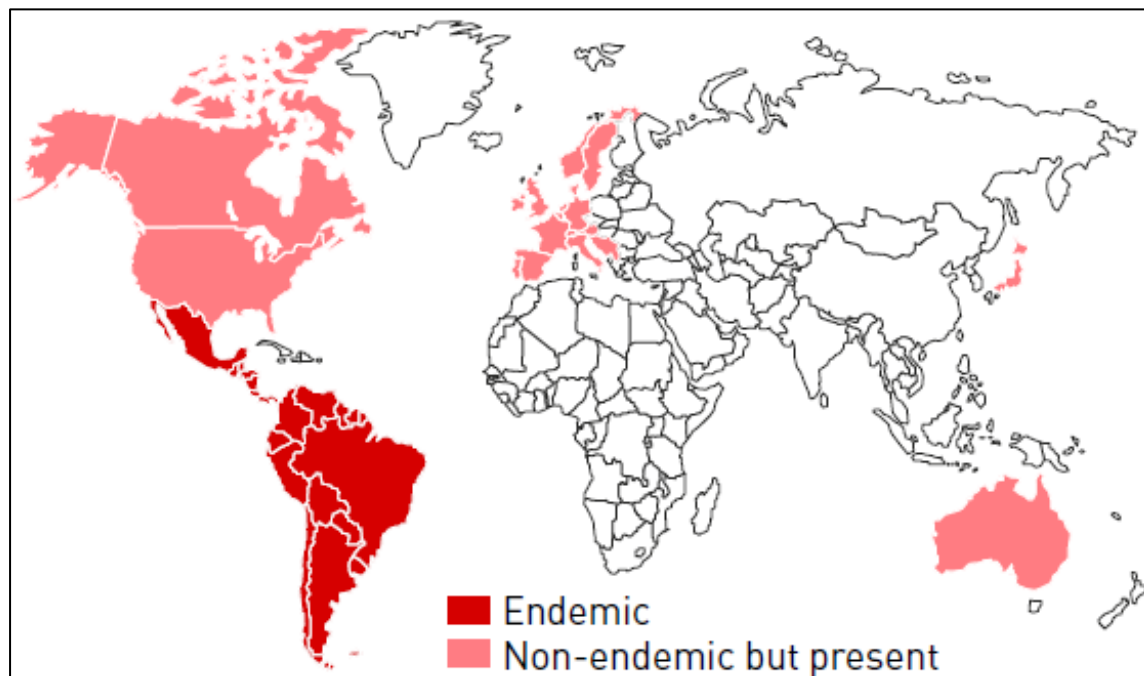


Figura 3 – Distribuição espacial da doença de Chagas nos diferentes continentes em relação à endemicidade. Fonte: WHO.

No Brasil, entre 1975 e 1980, a estimativa era de 6,5 milhões de pessoas infectadas, reduzindo para aproximadamente 4,6 milhões (MARTINS-MELO *et al.*, 2012a; MARTINS-MELO *et al.*, 2012b). Entretanto, a taxa de mortalidade devido a DC permanece elevada, com cerca de seis mil mortes anuais (WHO, 2000; RASSI A. JR *et al.*, 2010; HOTEZ *et al.*, 2012; MARTINS-MELO *et al.*, 2012a).

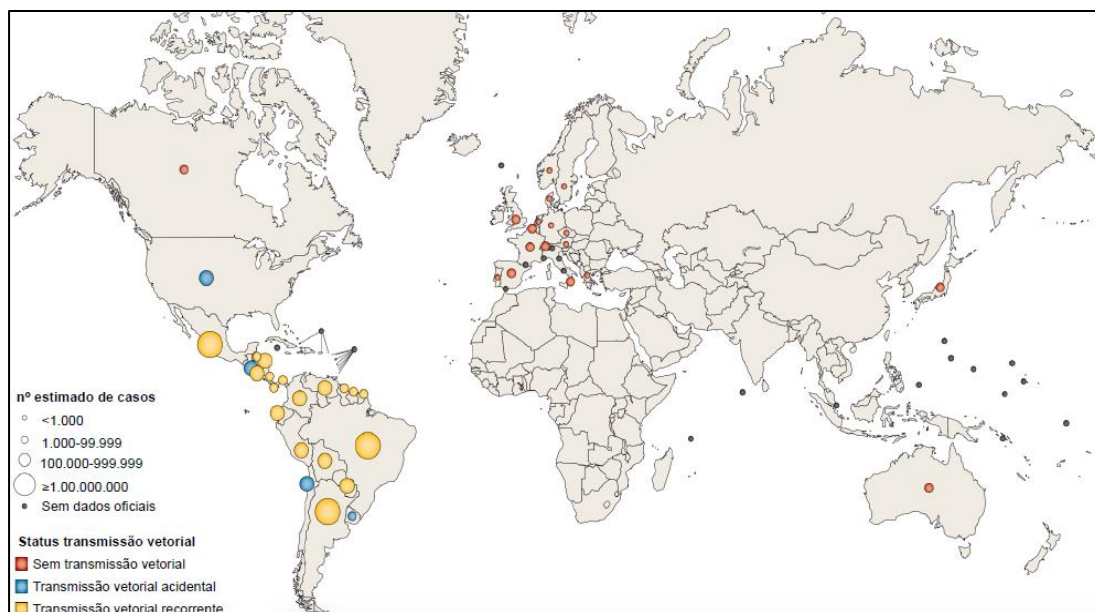


Figura 4 – Distribuição espacial da doença de Chagas nos diferentes continentes e situação da transmissão vetorial.
Fonte: WHO.

A média de idade da ocorrência de infecção nas áreas de intensa transmissão é aos quatro anos de idade e, pelo menos, 85% dessas infecções ocorrem em crianças menores de dez anos (COURA, 2007). No final da década de 1990 foi observada uma acentuada redução da doença de Chagas chegando a 96% só no Brasil, entre crianças entre 7 a 14 anos de idade (DIAS; SCHOFIELD, 1999).

No último inquérito sorológico, realizado no Brasil entre 2001-2008 em 104.954 crianças com idades até cinco anos, foi demonstrado que, em 11 crianças (0,01%) houve a positividade por provável transmissão vetorial (OSTERMAYER *et al.*, 2011).

Por outro lado, casos agudos da doença de Chagas continuam sendo registrados (ABAD-FRANCH *et al.*, 2014a; ABAD-FRANCH *et al.*, 2014b; RIBEIRO *et al.*, 2019) e há uma urgente necessidade de estudos para avaliar a prevalência e incidência da infecção por *T. cruzi* nestes locais, e para implementar estratégias mais eficazes de controle vetorial (MARTINEZ *et al.*, 2011; SHIKANAI-YASUDA; CARVALHO, 2012).

Trypanosoma cruzi pode ser transmitido vetorialmente por ~150 espécies de insetos triatomíneos hematófagos (MONTEIRO *et al.*, 2018). Dentre os vetores transmissores de *T. cruzi* no Brasil, podemos citar principalmente as seguintes espécies: *Triatoma infestans* Klug, 1834, *Panstrongylus megistus* (Burmeister, 1835), *Triatoma sordida* Stal, 1859, *Triatoma pseudomaculata* Correia e Espínola, 1964, e *Triatoma brasiliensis* (Neiva, 1911) (Figura 5).

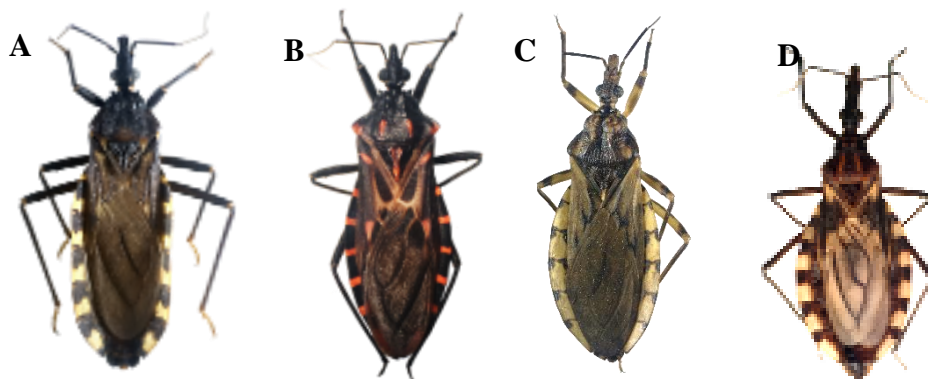


Figura 5 – Exemplos de insetos triatomíneos.

(A) *Triatoma infestans* (Klug, 1834); (B) *Panstrongylus megistus* (Burmeister, 1835); (C) *Triatoma sordida* (Stål, 1859); (D) *Triatoma brasiliensis* (Neiva, 1911). Fonte: FIOCRUZ/IGM/LPBM.

Nos primeiros estudos realizados em áreas endêmicas na região de Minas Gerais e, na Bahia, foi observado que os triatomíneos viviam em florestas, e de lá saíam para ocupar as casas de pau-a-pique e sem reboco, além do peridomicílio das residências (PONDÉ, 1946).

Os triatomíneos tornam-se infectados a partir do repasto sanguíneo em animais mamíferos, a exemplo de humanos, previamente infectados com as formas tripomastigotas circulantes (CHAGAS, 1916; DIAS, 1993). Esses parasitos, quando ingeridos, diferenciam-se para a forma epimastigota. Sob esta forma, aderem nas membranas peri-microvilares do intestino médio posterior e então se multiplicam no trato digestório do triatomíneo. Com o decorrer da infecção, os protozoários que alcançam as paredes da ampola retal diferenciam-se para as formas tripomastigotas metacíclicas, infectantes para o hospedeiro vertebrado, sendo eliminado pelas fezes e/ou urina, geralmente enquanto o triatomíneo se alimenta. No homem e nos outros vertebrados apresenta-se no sangue periférico sob a forma de tripomastigota e nas células dos tecidos sob a forma amastigota (CHAGAS, 1916; DIAS, 1993), conforme observado na Figura 6.

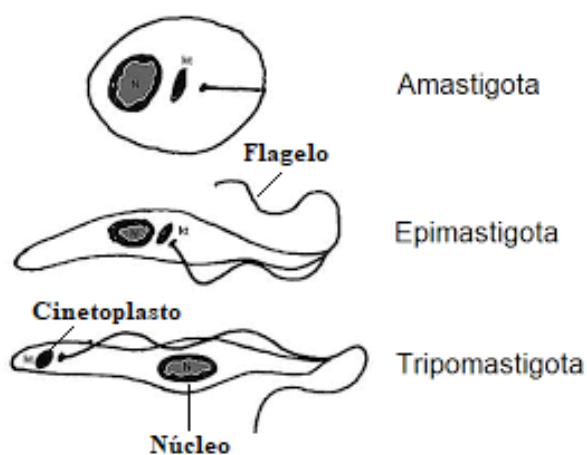


Figura 6 – Formas evolutivas de *T. cruzi*. Fonte: adaptado de (Rossi et al., 2003).

A forma mais conhecida de transmissão de *T. cruzi* é a via vetorial, neste tipo de transmissão, um triatomíneo infectado, ao realizar repasto sanguíneo, evacua fezes com formas infectantes de *T. cruzi* que, ao penetrarem na corrente sanguínea do indivíduo, iniciam a infecção. Entretanto, outras formas de transmissão são importantes, como a via transfusional e materno infantil. Até a década de 1990, os indivíduos submetido a transfusão sanguínea corriam risco de se infectar com sangue contaminado por *T. cruzi*, pois não havia triagem sorológica dos doadores nos hemocentros (DIAS; SCHOFIELD, 1999).

Existe ainda o risco da transmissão vetorial-acidental ou vetorial-oral de *T. cruzi*, associado ao consumo de alimentos contaminados, como consequência da aproximação dos vetores de *T. cruzi* da população local, levando consigo, o risco da transmissão oral - a exemplo do surto oral de Santa Catarina, no sul do Brasil (STEINDEL *et al.*, 2008) e em diversas partes da América Latina (ALARCON DE NOYA *et al.*, 2010; BENITEZ *et al.*, 2013).

Nesse contexto epidemiológico, a transmissão de *T. cruzi* pela via vetorial ainda é a forma mais prevalente em áreas endêmicas, acontecendo a partir de triatomíneos infectados (DIAS, J. C. *et al.*, 2016). Paralelamente, existe a transmissão por transfusão sanguínea, por transplante de órgãos, através do consumo de alimentos ou bebidas contaminados, acidentes laboratoriais, infecção induzida ou criminal, e a forma vertical ou materno-infantil, da mãe para o filho ou filha. Hoje, decorrente do sucesso das técnicas de controle vetorial, a transmissão congênita ganha destaque (MARTINS-MELO *et al.*, 2014), de forma que, tem se tornado a principal e mais persistente forma de parasitose nas regiões onde houve conquistas no controle das outras formas de transmissão.

A prevalência de doadores de sangue com DCH no Brasil, tomando como base, o ano de 1978, foi de 3,1% e, de acordo com os dados do Inquérito Nacional Sorológico, diminuiu para 0,9%, em 1995 (DIAS; SCHOFIELD, 1999). Dados recentes demonstraram que a prevalência em doadores da rede pública de hemoderivados no Brasil, das diferentes regiões geográficas, foi de 0,75%, ou seja, 6.036 casos para 804.923 doadores (DIAS; SCHOFIELD, 1998).

Na Bahia, um total de 3.084 casos foram registrados para 494.010 doadores analisados, com uma prevalência de 0.61%. A prevalência de *T. cruzi* em bancos de sangue varia de 0,06%-0,49% nos EUA; 5,3% no Paraguai e, maior que 5% na Bolívia (DIAS; SCHOFIELD, 1998). Vale ressaltar ainda o grande aumento do número de pessoas com infecção crônica por *T. cruzi* nos últimos anos nos EUA - devido ao alto índice de imigrantes de países do Cone Sul para o referido país estadunidense (WHO, 2000; BERN *et al.*, 2019).

2.3. Manifestações Clínicas

A doença de Chagas humana (DCH) apresenta duas fases: uma fase aguda e uma fase crônica (WHO, 2000). De maneira sucinta, é possível distinguir os seguintes padrões para cada uma destas fases:

I. Fase Aguda

Tem início após a infecção por *T. cruzi*. Nesta fase, existem manifestações de doença febril, que podem persistir por várias semanas (SVS-MS, 2006). Os sinais e sintomas podem desaparecer espontaneamente e evoluir para a fase crônica ou progredir para formas agudas graves; que podem levar ao óbito (CHAGAS, 1916). Raramente observa-se o “Chagoma de inoculação” e o “Sinal de Romana” (Figura 7) e neste caso, predomina-se o parasito circulante na corrente sanguínea sendo, muitas vezes, com quantidade expressiva de parasitos em circulação (PINTO *et al.*, 2009).

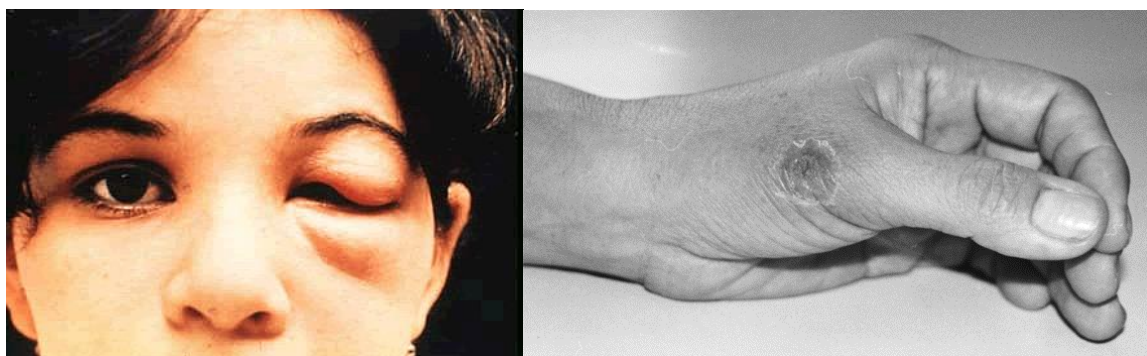


Figura 7 – Sinal Romana (A) e Chagoma de inoculação (B) de *T. cruzi*.
Fonte: <https://www.fcencias.com/2016/07/13/doenca-chagas-tripanosomiase-americana/>

II. Fase Crônica

Neste estágio, existem poucos parasitas circulantes no sangue. Inicialmente, é uma fase assintomática e sem sinais de comprometimento do coração e ou, do sistema digestório. Pode apresentar-se como uma das seguintes formas:

- **Forma indeterminada**

O paciente se apresenta geralmente com um quadro geral assintomático e sem comprometimento do aparelho circulatório (clínica, radiografia de tórax e eletrocardiograma normais) e, do aparelho digestório (avaliação clínica e radiológica normais de esôfago e cólon) (SVS-MS, 2006). A clínica deste paciente poderá continuar desta forma por toda sua vida ou, tardiamente, evoluir para a forma cardíaca, digestiva ou mista.

- **Forma cardíaca**

Em geral, o coração apresenta-se aumentado de tamanho e de peso, com dilatação das cavidades cardíacas podendo apresentar aneurisma apical. A dilatação ocorre em consequência da destruição das fibras cardíacas e substituição por tecido fibroso (Figura 8). O acometimento cardíaco frequentemente evolui para quadros de insuficiência cardíaca congestiva e, miocardiopatia dilatada. Este tipo de quadro ocorre em média em 30% dos casos crônicos relatados (SVS-MS, 2006) e é a maior responsável pela morbidade e mortalidade na doença de Chagas (BOCCHI *et al.*, 2009).

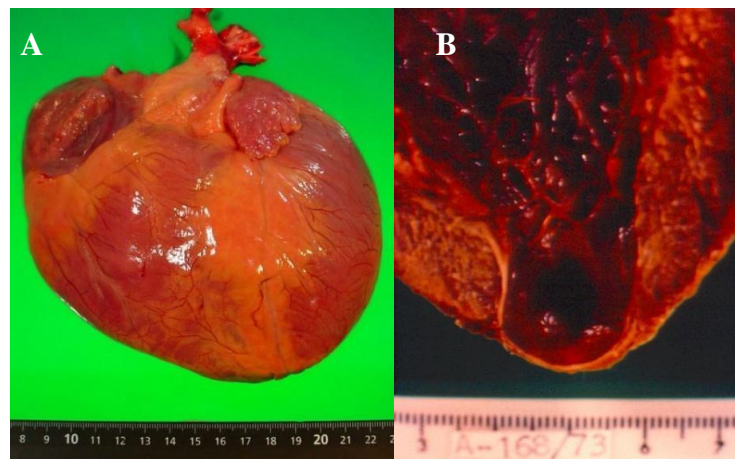


Figura 8 – Forma crônica cardíaca da doença de Chagas.
Legenda: A. Miocardite Chagásica. B. Aneurisma Apical. Fonte:

- **Forma digestiva**

É caracterizada pelo acometimento do aparelho digestivo sob a forma de megaesôfago ou megacólon (Figura 9). Observada em cerca de 10% dos casos crônicos da doença de Chagas (SVS-MS, 2006).

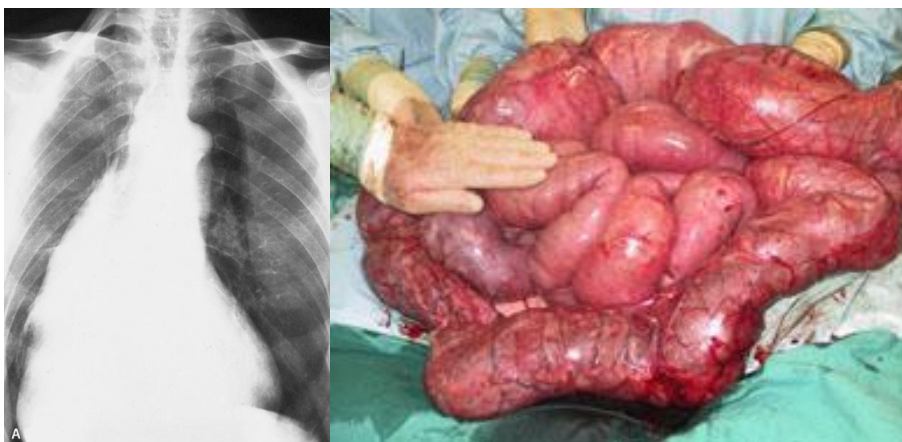


Figura 9 – Forma crônica digestiva da doença de Chagas.
Legenda: A. Megaesôfago. B. Megacólon. Fonte:

2.4. Entomologia e vulnerabilidade de transmissão vetorial

Triatomíneos são insetos que pertencem ao grupo dos percevejos (Hexapoda: Heteroptera), e que desenvolveram a habilidade de utilizar o sangue, principalmente de vertebrados, como recurso alimentar para crescimento (ecdise) e maturação dos seus ovos (ABAD-FRANCH; MONTEIRO, 2007; NOIREAU; DUJARDIN, 2010).

São insetos que apresentam crescimento hemimetábolo e apenas os adultos possuem asas, sendo as formas imaturas chamadas de ninfas (JURBERG; GALVÃO, 2006). Os adultos, enquanto formas aladas, possuem maior capacidade de dispersão do que as ninfas, que dependem da forma cursiva para dispersão. Vetores silvestres possuem mantêm maior capacidade de voo que espécies domiciliadas, e algumas espécies como o *T. infestans* foi registrada uma capacidade de voo de cerca de 200m (SCHOFIELD *et al.*, 1999).

O ciclo de vida dos triatomíneos, taxa de ovoposição, capacidade de replicação anual, capacidade de infestação domiciliar, coeficiente de infecção pelo *T. cruzi* e resistência ao jejum variam de acordo com a espécie estudada (RABINOVICH *et al.*, 2011), sendo esses importantes indicadores para avaliar a competência vetorial de uma espécie (BARRETO-SANTANA *et al.*, 2011).

I. Histórico de encontro de triatomíneos e o risco de transmissão de *T. cruzi* na Bahia

Os primeiros registros sobre triatomíneos na Bahia foram realizados em 1911 nas pesquisas de Pirajá da Silva e Carlos Chagas. Pirajá da Silva identificou triatomíneos (*Conorhinus megistus* = *Panstrongylus megistus*) provenientes da cidade de Mata de São João, a 68 km da cidade de Salvador. Após a confirmação da identificação dos insetos por Oswaldo Cruz, ainda no mesmo ano, Pirajá da Silva descreveu ainda a ocorrência de triatomíneos nas cidades de Feira de Santana, Candeias, São Francisco do Conde e na cidade de Salvador; provenientes do Asilo São João de Deus, na época, localizado no Solar Bela Vista, no Bairro de Engenho Velho de Brotas, locais onde em anos seguintes descreveu *T. cruzi* infectando esses triatomíneos (SILVA, 1966; FALCÃO, 2008).

Passados quase 40 anos, a DCH, agora mais bem conhecida por pesquisadores da região, se revelou como um sério problema de saúde pública, quando foi descrito que um número elevado de triatomíneos infectados por *T. cruzi*. Triatomíneos foram capturados simultaneamente ao registro de casos autóctones da cardiopatia chagásica na cidade do Salvador, no bairro de Engenho Velho de Brotas (PONDÉ, 1946). Duas espécies de triatomíneos, *Panstrongylus megistus* Burmeister, 1835 e *Triatoma rubrofasciata* De Geer,

1773, foram encontradas naturalmente infectadas por *T. cruzi*, no centro histórico da cidade, sendo associadas aos casos humanos. O último hemíptero mencionado se encontrava infectado por *Trypanosoma conorhini* Donovan, 1909, parasita dos ratos domésticos (GONÇALVES, 1912). Tal fato motivou o médico Octávio Mangabeira Filho a coordenar junto com outros pesquisadores (COSTA, 1955), uma campanha de combate a esses vetores com inseticidas. Foi constatada, em seguida, uma acentuada redução nas populações de triatomíneos nesses locais (LEAL *et al.*, 1965). Entretanto, persistiram alguns focos infestados por *P. megistus* responsável pela infecção de várias famílias residentes nos bairros de Acúpe de Brotas e Federação (SILVA, 1966).

No início da década de 1970, em um dos últimos trabalhos realizados na cidade de Salvador, foram examinados mais de 600 exemplares de *P. megistus* e *T. rubrofasciata*, de diversas áreas da cidade, principalmente o pelourinho, dentre os quais, 16% destes (96 insetos) estavam infectados por *T. cruzi* (SHERLOCK; GUITTON, 1974). Nas últimas décadas, *Triatoma tibiamaculata*, encontrada naturalmente em ninhos de marsupiais e roedores (SHERLOCK; GUITTON, 1974), vem sendo considerada como uma espécie intrusiva na cidade do Salvador (DIAS-LIMA; SHERLOCK, 2000; RIBEIRO *et al.*, 2015).

Em se tratando de pesquisas realizadas na Bahia, nos últimos 30 anos, muitos trabalhos foram publicados sobre doença de Chagas e, diversos pesquisadores se dedicaram a explorar a doença realizando estudos epidemiológicos (Bittencourt *et al.*, 1972; Hoff *et al.*, 1978; Barrett *et al.*, 1979a; Barrett *et al.*, 1980; Bittencourt, Mota, Ribeiro Filho, *et al.*, 1985; Piesman, Sherlock, Mota, Todd, *et al.*, 1985; Maguire *et al.*, 1986; Escolano *et al.*, 1989; Dias *et al.*, 2006; Dias, J. P. *et al.*, 2008); estudos clínicos (Da Silveira, 1976; Camara *et al.*, 1983; Maguire *et al.*, 1983; Mota *et al.*, 1984; Bittencourt *et al.*, 1990; Aras *et al.*, 2003; Goncalves *et al.*, 2011); estudos ecológicos e/ou epidemiológicos (Miles, 1976; Hoff *et al.*, 1978; Minter *et al.*, 1978; Bittencourt, Mota e Povia 1985; De La Fuente *et al.*, 2008), e ainda estudos entomológicos (Sherlock e Guitton, 1974; Sherlock e Muniz, 1974; Sherlock e Serafim, 1974; Barrett *et al.*, 1979a; Maguire *et al.*, 1986; Sherlock *et al.*, 1987; Almeida *et al.*, 2009; Carvalho-Costa *et al.*, 2010). Poucos estudos foram dedicados a estudar a dinâmica da doença de Chagas de forma geral no estado e, utilizando técnicas mais recentes para avaliar-se o risco associado em uma determinada localidade (Harry *et al.*, 2008; Harry *et al.*, 2009; Roux *et al.*, 2011b; a; Ribeiro *et al.*, 2015).

II. Vulnerabilidades para doença de Chagas no Estado da Bahia

O termo “vulnerabilidade” teve seu uso popularizado na década de 1980 e representa uma particularidade que indica “o estado de fraqueza, que pode se referir tanto ao comportamento das pessoas, grupos étnicos, áreas geográficas”, ou simplesmente grupos de possuem riscos individuais em comum (Dever *et al.*, 1988; Jackson e Chappuis, 2011). Mesmo conceitualmente impreciso, o termo *vulnerabilidade* amplia a compreensão dos múltiplos fatores que fragilizam os sujeitos no exercício de sua cidadania (Jaffery *et al.*, 1992; Rogers, 1997).

Em saúde, a utilização do termo “*vulnerabilidade*” começou no início dos anos 1980, com os estudos sobre a *síndrome da imunodeficiência adquirida - SIDA*, com a determinação dos “Grupos de Risco” (Berki *et al.*, 1977; Tereskerz e Jagger, 1997; Ma *et al.*, 2008). A adoção do conceito de *vulnerabilidade*, em substituição ao conceito de grupo de risco, aconteceu nesse processo; indicando a ampliação das chances e das formas de acometimento pela doença pela totalidade da população (Jaffery *et al.*, 1992; Rogers, 1997).

No caso da doença de Chagas, a vulnerabilidade de transmissão vetorial de *T. cruzi* está relacionada com a vulnerabilidade social (VINHAES *et al.*, 2014; MIRANDA *et al.*, 2018; RIBEIRO *et al.*, 2019), quando os indivíduos vivem em locais com hábitos ou condições de moradia domiciliares insalubres, o que propicia maior exposição aos triatomíneos e *T. cruzi*. Adicionalmente, as ações profissionais no âmbito das políticas de saúde e assistência social podem tanto reduzir quanto reforçar essas vulnerabilidades (VALDEZ-TAH *et al.*, 2015; LÓPEZ-VÉLEZ *et al.*, 2020).

Triatomíneos são insetos essencialmente silvestres, no entanto, algumas espécies possuem populações capazes de se adaptar às modificações antrópicas na paisagem natural, podendo gradualmente, se adaptar ao ambiente domiciliar (LENT; WYGODZINSKY, 1979; GALVAO; JUSTI, 2015). Os triatomíneos adentram no interior das residências na maioria das vezes sem colonizar, de forma intrusiva, porém em determinadas condições ocorre a colonização e domiciliação (BARBU *et al.*, 2013; RIBEIRO *et al.*, 2015; WALECKX *et al.*, 2015), principalmente em moradias vulneráveis (VALDEZ-TAH *et al.*, 2015), onde carecem medidas apropriadas de salubridade (GURTLER; YADON, 2015) ou áreas sob efeito do desequilíbrio ambiental (RICARDO-SILVA *et al.*, 2016).

O peridomicílio é o primeiro ambiente a ser colonizado por triatomíneos em uma Unidade Domiciliar (UD), especialmente os anexos destinados à manutenção de animais vertebrados como galinheiros, chiqueiros, canil, gatil, pocilga e curral (LENT;

WYGODZINSKY, 1979; POJO DE REGO *et al.*, 2006). Nestes espaços, os triatomíneos geralmente encontram recurso alimentar e abrigo contra predadores naturais, favorecendo ao estabelecimento de colônias peridomiciliares (ROSSI *et al.*, 2015). A colonização intradomiciliar, ou domiciliação, geralmente acontece em residências construídas com precárias condições salubres e técnicas de edificação rudimentares, a exemplo das casas de taipa, que possuem paredes com fendas e rachaduras, servindo como abrigo para os vetores, que a partir dali, utilizam os animais domésticos e o homem como recurso alimentar, e assim podem completar seu ciclo reprodutivo (RAMSEY *et al.*, 2005; GURTLER; YADON, 2015). A presença de animais domésticos no interior das residências também pode contribuir para manutenção de vetores no ambiente doméstico e favorecer a ocorrência de picadas ao homem (CECERE *et al.*, 1997; GURTLER *et al.*, 2014). Neste caso, o risco de transmissão de *T. cruzi* (Chagas, 1909) é aumentado, pois também aumenta a exposição dos moradores aos triatomíneos (BUSTAMANTE; TARLETON, 2014; KAPLINSKI *et al.*, 2015).

De forma geral, a ocorrência de um triatomíneo infectado no ambiente domiciliar representa risco de transmissão de *T. cruzi* ao homem e animais domésticos (CORDOVEZ; SANABRIA, 2014; SALDANA *et al.*, 2015; WALECKX *et al.*, 2015; KLOTZ *et al.*, 2016), não somente na zona rural, mas também em zona urbana de grandes centros por toda a América Latina (DIAS *et al.*, 2008; MEDRANO-MERCADO *et al.*, 2008; PINTO *et al.*, 2009; ALARCON DE NOYA *et al.*, 2010; BENITEZ *et al.*, 2013; RAMIREZ *et al.*, 2013; DIAZ-BELLO *et al.*, 2014; RIBEIRO *et al.*, 2015; SANGENIS *et al.*, 2015).

Sobre o processo de domiciliação, algumas espécies de triatomíneos tiveram demarcado sucesso no processo de ocupação do território domiciliar e conseguiram expandir sua distribuição além das ecorregiões e biomas originais (FORATTINI *et al.*, 1971) por processos ativos e passivos de dispersão (RIBEIRO JR *et al.*, 2006; STEVENS *et al.*, 2015). Como consequência, em muitas destas novas áreas, a espécie alóctone torna-se incapaz de sobreviver no ambiente silvestre diferente do seu e dependente do ambiente doméstico para sua sobrevivência, sendo considerada então, uma espécie não nativa, a exemplo de *T. infestans* na Bahia (SILVEIRA, 2011; SILVEIRA; DIAS, 2011), espécie cuja origem filogenética remonta áreas mais ao sul da América Latina (Figura 10), dispersando por todo Brasil em ambiente doméstico, durante esse processo de expansão territorial (BARGUES *et al.*, 2006).

Triatoma infestans é um vetor que apresenta algumas características que favorecem a transmissão de *T. cruzi*: a) grande capacidade hematofágica, b) defecação durante ou logo

após alimentação, c) boa capacidade de dispersão e domiciliação e d) ampla distribuição geográfica até a década de 1980 (POJO DE REGO *et al.*, 2006).

Apesar disso, *T. infestans*, tem origem em áreas mais ao sul da América Latina (BARGUES *et al.*, 2006), porém, a espécie ampliou sua distribuição geográfica durante a segunda metade do século XX e foi considerada, a principal espécie envolvida na transmissão de *T. cruzi* do Brasil (SILVEIRA, 2011). Apesar disso, outras espécies de triatomíneos foram de importância primordial em amplas áreas do País, especialmente *T. brasiliensis* e *P. megistus* na região nordeste (DIAS, 2007).

No Brasil, as ações contra os principais vetores da doença de Chagas iniciaram de forma organizada com o *Programa Nacional de Controle da Doença de Chagas* (PNCDCh) a partir de 1975 (SILVEIRA, 2011), neste período, a espécie *T. infestans* possuía ampla distribuição pelo País (Figura 10) e alta infestação no ambiente intradomiciliar em Unidades Domiciliares (UDs), além de alta sinantropia e capacidade de proliferação com altas densidades em ambiente domiciliar (GUARNERI *et al.*, 2000; PEREIRA *et al.*, 2006). Tais características fizeram com que o *T. infestans* fosse considerado o principal vetor da DCH no País (PASSOS; SILVEIRA, 2011; SILVEIRA, 2011; SILVEIRA; DIAS, 2011). Entretanto, por se tratar de uma espécie exótica na Bahia, natural da Bolívia (GARCIA *et al.*, 2013; PANZERA *et al.*, 2014), demonstra pouca competência em se adaptar as características naturais dos Biomas nacionais, sendo restrito aos ambientes antrópicos (SILVEIRA; DIAS, 2011).



Figura 10 – Área endêmica para a doença de Chagas, com risco de transmissão vetorial domiciliar no Brasil, 1983.
 Fonte: Passos e Silveira, 2011.

As ações massivas e sistemáticas de tratamento químico contra triatomíneos reforçadas a partir de 1991 surtiram efeito e, em abril de 2006 (DIAS, 2007), o Brasil recebeu a certificação da OPAS que foi amplamente discutida e negativamente criticada entre pesquisadores nesta área de conhecimento (DIAS, 2006; ABAD-FRANCH *et al.*, 2013; COURA, 2013; SALVATELLA *et al.*, 2014). Ademais, recentemente, foram evidenciados focos residuais desta espécie na Bahia (ARAUJO *et al.*, 2014; BRANDÃO *et al.*, 2015) e Rio Grande do Sul, além de populações resistentes ao tratamento químico utilizado no combate aos vetores (PESSOA *et al.*, 2015). Entretanto, apesar de considerarmos na década de XX *T. infestans* como principal espécie vetor de *T. cruzi* no Brasil, em se tratando da doença de Chagas, *T. cruzi* pode ser transmitido por cerca de 150 espécies, porém, a maioria não era considerada de relevância na transmissão vetorial domiciliar.

Na Bahia, por exemplo, as primeiras pesquisas realizadas para identificação dos triatomíneos catalogaram 18 espécies até a década de 1970 (NEIVA, 1914; SHETLOCK; SERAFIM, 1972; ALMEIDA, 2015). Recentemente, após diversas descrições e revisões, foram reconhecidas 26 espécies (DA SILVA *et al.*, 1968; COSTA *et al.*, 2015; GALVAO; JUSTI, 2015).

Adicionalmente, na Bahia, já foram descritas diversas áreas endêmicas para a transmissão de *T. cruzi* (GONÇALVES, 1912; COSTA, 1955; SILVA, 1966; BARRETT *et al.*, 1979; BARRETT *et al.*, 1980; MAGUIRE *et al.*, 1986; HADAMAK *et al.*, 2016; SILVEIRA *et al.*, 2016), associadas, principalmente, às espécies *T. infestans* e *P. megistus* (COSTA, 1955; LEAL *et al.*, 1965; SHETLOCK; SERAFIM, 1972; PIESMAN *et al.*, 1985; MAGUIRE *et al.*, 1986; ALMEIDA *et al.*, 2009). Mais recentemente, surtos de transmissão de *T. cruzi* no interior do estado da Bahia, associados a *T. sordida*, demonstraram o potencial papel de outros triatomíneos na transmissão de *T. cruzi* ao homem (DIAS *et al.*, 2008; BASTOS *et al.*, 2010).

Nos locais onde existem diversos vetores competentes, reconhecer a distribuição geográfica, o nível de domiciliação dos triatomíneos e, a sua classificação epidemiológica na transmissão da doença de Chagas é primordial para a vigilância dos triatomíneos sinantrópicos (SARKAR *et al.*, 2010; BERN *et al.*, 2011; CURTIS-ROBLES *et al.*, 2015). Neste cenário, observa-se que diversos fatores podem alterar a importância epidemiológica das espécies de triatomíneos em transmitir *T. cruzi* ao homem, ao longo do tempo e espaço.

As informações acima ilustram o fato de que os indicadores entomológicos podem variar de acordo com mudanças na ocorrência dos triatomíneos no espaço e tempo, havendo

a necessidade de avaliar essas informações para então, direcionar as ações de vigilância e determinar as áreas mais vulneráveis à transmissão vetorial de *T. cruzi* ao homem e seus animais domésticos. Desta forma, para o monitoramento dos vetores, os indicadores entomológicos são fundamentais, pois direcionam as atividades de vigilância e controle de doenças de transmissão vetorial, especialmente quando o agravo é transmitido por espécies com diferentes capacidades vetoriais (SILVEIRA, 2004). A utilização de matrizes para estratificação dos riscos de transmissão de *T. cruzi* é uma metodologia conhecida e recomendada (SVS-MS, 2006), entretanto, outros pesquisadores, defendem a utilização de características biológicas hierarquicamente definidas e que, se traduzam num esquema claro e algorítmico para a vigilância e controle de triatomíneos, útil em todas as escalas operacionalmente relevantes, desde iniciativas multinacionais até ações de vigilância e controle (ABAD-FRANCH, 2016).

Por fim, os indicadores entomológicos, quando associados aos dados socioeconômicos, demográficos, e índices do PNCDCh, num esquema algorítmico, compreensível e reprodutível, podem ajudar a revelar áreas de maior vulnerabilidade para doença de Chagas em uma determinada área geográfica (VINHAES *et al.*, 2014), sendo esta abordagem estratégica para a identificação de áreas prioritárias para realização de ações de vigilância e controle dos vetores e da transmissão do agravo.

3. JUSTIFICATIVA

A transmissão vetorial de *T. cruzi* foi muito frequente no Estado da Bahia em décadas passadas e seus principais vetores foram extensivamente combatidos. Apesar das ações realizadas, ainda existem várias espécies de triatomíneos competentes em transmitir *T. cruzi* na Bahia, um número relevante de pacientes crônicos, número desconhecido de pacientes agudos, dados negligenciados acerca da transmissão congênita, além de altas taxas de mortalidade devido ao agravo (Figura 11), indicando que a doença continua sendo de relevância em saúde pública e que há necessidade de manutenção da vigilância entomológica e epidemiológica.

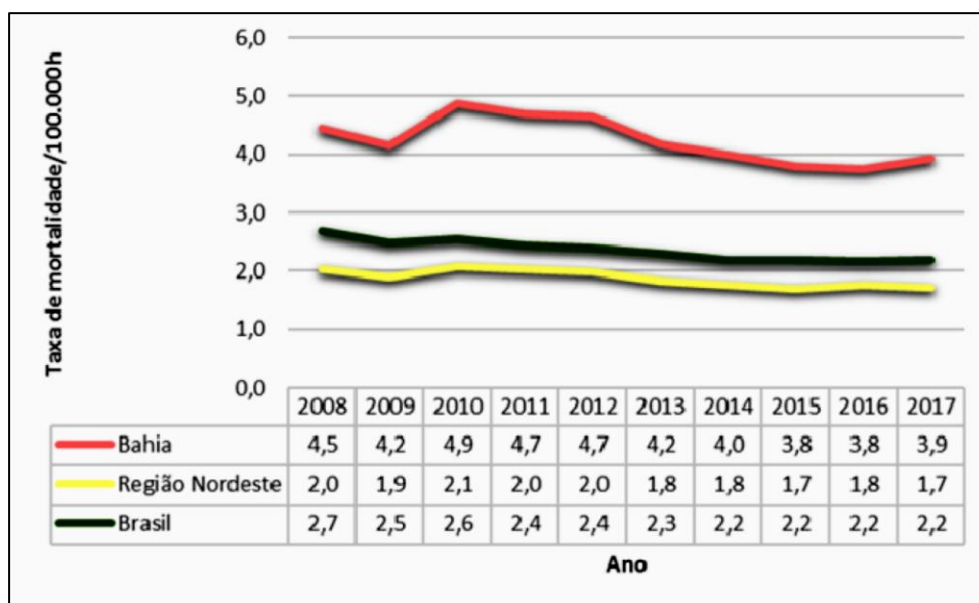


Figura 11 - Taxa de Mortalidade por Doença de Chagas, segundo local de residência, Bahia, Nordeste e Brasil, 2008 a 2017. Fonte: MS/SVS/CGIAE

Apesar da principal espécie vetor (*T. infestans*, exótica) estar controlada, outras espécies que podem transmitir *T. cruzi* para o homem e animais domésticos já foram registradas na Bahia. Para uma melhor avaliação dos cenários de transmissão, é fundamental obter indicadores entomológicos atualizados para as espécies em questão, bem como avaliar os níveis de infestação, infecção natural e, identificação de suas fontes alimentares em diferentes áreas da Bahia. Essas informações são importantes considerando o potencial adaptativo dos triatomíneos, incluindo a capacidade de colonizar o ambiente domiciliar, aproximando-se assim, da população humana e expondo a população rural e urbana ao risco de transmissão de *T. cruzi*. Nos últimos anos, muitos triatomíneos têm sido encontrados em áreas da cidade de Salvador; com altas taxas de infecção por *T. cruzi*, porém, sem evidências de alimentação em seres humanos.

O objetivo desta investigação foi identificar áreas vulneráveis à transmissão de *T. cruzi* na Bahia, com base na área de ocorrência, distribuição espacial, taxas de infecção dos triatomíneos e fontes alimentares dos vetores, dados socioeconômicos, demográficos, e informações do PNCDCh. Espera-se com esse tipo de abordagem integrativa, identificar com mais precisão, as principais áreas vulneráveis à transmissão vetorial de *T. cruzi* por triatomíneos sinantrópicos no estado da Bahia. Estas informações serão estratégicas para o delineamento das ações de vigilância e controle da doença de Chagas no estado da Bahia; e poderão ser utilizadas para determinar áreas para realização de inquéritos sorológicos em humanos e em animais domésticos das áreas mais vulneráveis.

4. OBJETIVOS

4.1. *Objetivo Geral*

Determinar os municípios mais vulneráveis para transmissão vetorial de *T. cruzi*, agente causador da doença de Chagas, no Estado da Bahia.

4.2. *Objetivos Específicos*

- I. **Manuscrito 1 – Wide distribution of *Trypanosoma cruzi*-infected triatomines in the State of Bahia, Brazil.**
 - a) Coletar triatomíneos no Estado em áreas com histórico prévio de infestação, entre 2013 e 2014;
 - b) Identificar as espécies de triatomíneos que infestaram as UDs;
 - c) Analisar a taxa de infecção por *T. cruzi*;
 - d) Determinar as fontes alimentares dos triatomíneos;
 - e) Georreferenciar as informações e, localizar áreas com vetores infectados por *T. cruzi* e alimentados com sangue humano e de animais domésticos.

- II. **Manuscrito 2 – TriatoScore: an entomological-risk score for Chagas disease vector control-surveillance.**
 - a) Calcular indicadores entomológicos das espécies de triatomíneos na Bahia;
 - b) Desenvolver um indicador composto alternativo para sumarizar a importância epidemiológica das espécies de triatomíneos na transmissão de *T. cruzi* ao homem e animais domésticos na Bahia;
 - c) Determinar as áreas da Bahia com valores mais elevados para o TriatoScore;

- III. **Manuscrito 3 – Triatomine fauna in the state of Bahia, Brazil: What changed after 40 years of the vector-control program?**
 - a) Determinar quais são os vetores de *T. cruzi* já registrados na Bahia.
 - b) Descrever quais municípios notificaram triatomíneos e analisar a distribuição geográfica das espécies em dois períodos: 1957-1971 e 2006-2019.
 - c) Analisar a abundância e distribuição espacial relativa das espécies de triatomíneos em ambiente intra e peridomiciliar nos municípios da Bahia nos períodos avaliados;
 - d) Identificar as espécies mais relevantes em ambos os períodos analisados.

- IV. **Manuscrito 4 – Out of sight, out of mind: Understanding Chagas' disease vulnerabilities in a regional level, Bahia, Brazil.**
 - a) Analisar os indicadores entomológicos, socioeconômicos, demográficos e epidemiológicos de vulnerabilidade para transmissão vetorial de *T. cruzi* na Bahia;
 - b) Identificar municípios e regiões de maior vulnerabilidade para o agravo doença de Chagas no Estado da Bahia.

5. RESULTADOS

5.1. ARTIGO 1 – *Wide distribution of Trypanosoma cruzi-infected triatomines in State of Bahia, Brazil.*

Neste capítulo são apresentados os resultados relativos ao primeiro objetivo específico apresentado: - Determinar a taxa de infecção por *T. cruzi* e fontes alimentares de triatomíneos da Bahia entre 2012 e 2014 e mapear a ocorrência de triatomíneos infectados alimentados em humanos e animais domésticos.

O artigo científico em questão está apresentado integralmente a seguir, compondo a presente seção, e foi publicado na revista *Parasites & Vectors* nº12(604) em dezembro de 2019, sob título: *Wide distribution of Trypanosoma cruzi-infected triatomines in State of Bahia, Brazil.* DOI: <https://doi.org/10.1186/s13071-019-3849-1>.

RESEARCH

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Wide distribution of *Trypanosoma cruzi*-infected triatomines in the State of Bahia, Brazil

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Abstract

Background: The identification of *Trypanosoma cruzi* and blood-meal sources in synanthropic triatomines is important to assess the potential risk of Chagas disease transmission. We identified *T. cruzi* infection and blood-meal sources of triatomines caught in and around houses in the state of Bahia, northeastern Brazil, and mapped the occurrence of infected triatomines that fed on humans and domestic animals.

Methods: Triatominae bugs were manually captured by trained agents from the Epidemiologic Surveillance team of Bahia State Health Service between 2013 and 2014. We applied conventional PCR to detect *T. cruzi* and blood-meal sources (dog, cat, human and bird) in a randomized sample of triatomines. We mapped triatomine distribution and analyzed vector hotspots with kernel density spatial analysis.

Results: In total, 5906 triatomines comprising 15 species were collected from 127 out of 417 municipalities in Bahia. The molecular analyses of 695 triatomines revealed a ~10% *T. cruzi* infection rate, which was highest in the *T. brasiliensis* species complex. Most bugs were found to have fed on birds (74.2%), and other blood-meal sources included dogs (6%), cats (0.6%) and humans (1%). *Trypanosoma cruzi*-infected triatomines that fed on humans were detected inside houses. Spatial analysis showed a wide distribution of *T. cruzi*-infected triatomines throughout Bahia; triatomines that fed on dogs, humans, and cats were observed mainly in the northeast region.

Conclusions: Synanthropic triatomines have a wide distribution and maintain the potential risk of *T. cruzi* transmission to humans and domestic animals in Bahia. Ten species were recorded inside houses, mainly *Triatoma sordida*, *T. pseudomaculata*, and the *T. brasiliensis* species complex. Molecular and spatial analysis are useful to reveal *T. cruzi* infection and blood-meal sources in synanthropic triatomines, identifying areas with ongoing threat for parasite transmission and improving entomological surveillance strategies.

Keywords: Chagas disease, Entomological surveillance, *Trypanosoma cruzi*, Blood meal, PCR

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Background

Chagas disease is the most frequent cause of heart failure in rural populations in vector-endemic countries in Latin America [1, 2]. It is an infection caused by *Trypanosoma cruzi* (Chagas, 1909), a protozoan transmitted by



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blood-feeding bugs [3]. No vaccines or effective antiparasitic treatments are available to cure Chagas cardiomyopathy, so vector surveillance and control are the main strategies to prevent human infection in areas with vectorial transmission [4].

In Brazil, the control of Chagas disease vectors was implemented systematically between 1975 and 1983 when the main vector, *Triatoma infestans* (Klug, 1834), infested domiciles in 12 states. In 1991, Brazil integrated an international consortium to reduce vectorial transmission through insecticide spraying [4, 5]. The systematic actions of chemical treatment were effective; in 2006, the World Health Organization (WHO) certified Brazil as free of *T. cruzi* transmission by *T. infestans*. However, some recent outbreaks have been associated with the oral transmission, mainly due to açai palm juice consumption and other *T. cruzi*-contaminated food in the Brazilian Amazon, where *Rhodnius* species are frequent [6]. Moreover, new cases of vector-borne Chagas disease transmitted by either sylvatic vectors invading houses or domestic/peridomestic populations [e.g. *T. brasiliensis*, *T. pseudomaculata*, *T. sordida*, *Panstrongylus megistus* (Burmeister, 1835)] are being recorded in Brazil [7, 8]. Epidemiological data show 2.2 deaths per 100,000 inhabitants in 2017 in Brazil and the highest value was recorded at Goiás State (22.4 deaths per 100,000 inhabitants). Between 2007 and 2016, 35 Brazilian municipalities accounted for 85% of confirmed cases in the Notification Disease Information System (SINAN). Of these 35 municipalities, 99.5% are located in the Amazon region and 87% in the State of Pará (Additional file 1: Figure S1). Most of the new confirmed acute Chagas disease cases notified to the Brazilian Ministry of Health were classified as oral transmission [8]. The presence of ~60 species of native vectors in Brazil [9] in a wide endemic area of Chagas disease with different transmission scenarios [10] and a progressive reduction of the human and financial resources needed to sustain the continuity of the control actions, highlight the need for updated studies about surveillance of triatomines in Brazilian states.

Endemic areas for *T. cruzi* transmission in the state of Bahia were described a few years after the discovery of Chagas disease and were mainly associated with *P. megistus* [11] and *T. infestans* [12]. More recently, outbreaks of *T. cruzi* transmission associated with *T. sordida* showed the potential role of this species to transmit *T. cruzi* to humans [13, 14]. In addition, residual foci of *T. infestans* were found in Bahia [15] and one acute case of Chagas disease was confirmed in 2018 (Additional file 1: Table S1).

In Bahia, 26 triatomine species have been registered [9, 16]; most are strictly associated with the wild environment or peridomestic, but others are detected

inside houses where they feed on domestic animals and humans [15, 17–21]. The identification of *T. cruzi* infection and blood-meal sources in synanthropic triatomines is important to assess the potential risk of Chagas disease transmission in human dwellings. Here, we identified *T. cruzi* infection and blood-meal sources of triatomines caught at different environments in Bahia, northeast Brazil, and mapped the occurrence of infected triatomines that fed on humans and domestic animals between 2013 and 2014.

Methods

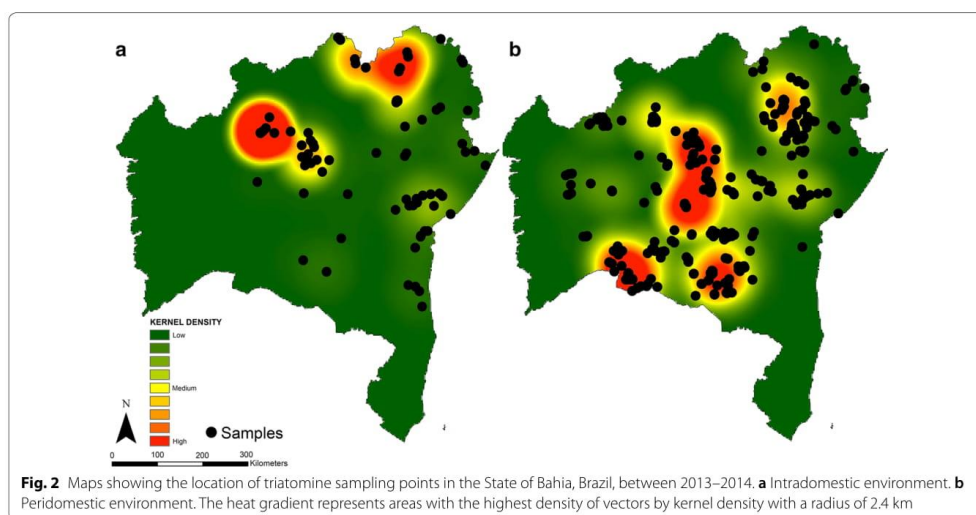
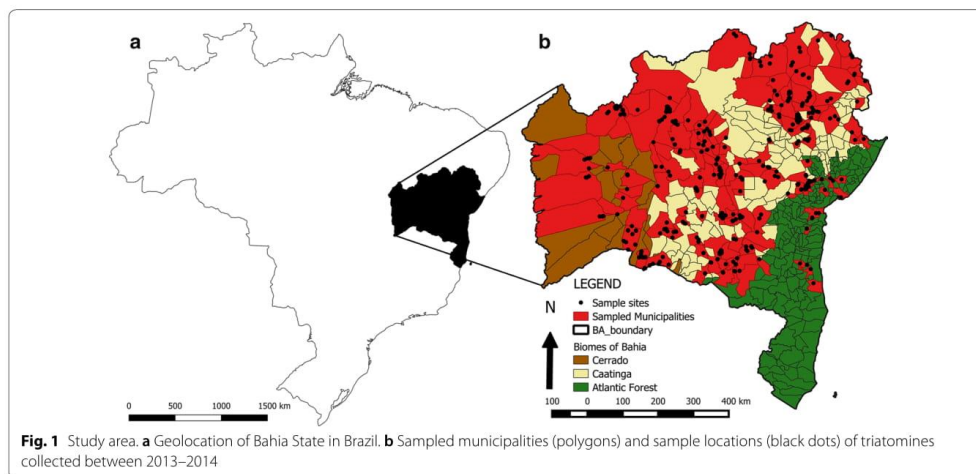
Study area

The state of Bahia has 417 municipalities, and it is situated in the northeast region of Brazil (Fig. 1). In the western region of Bahia, the Cerrado is the main biome, with relatively high precipitation between 300 and 800 mm and a tropical climate. A tropical climate of altitude is present in the region of Chapada Diamantina; however, in the semiarid region, where the Caatinga biome predominates, rainfall indices are very low, between 100 and 300 mm, and there are long dry periods. On the marine coast, annual rainfall can exceed 1500 mm, and the main biome is Atlantic Forest.

Insect collection

Geographical information system (GIS) data and triatomine bugs were obtained by trained agents from the Epidemiologic Surveillance team of Bahia State Health Service (SESAB) and IGM/FIOCRUZ-BA. Triatomines were captured monthly between 2013–2014. The collections were carried out as established by the National Programme for the Control of Chagas disease [22] in the localities with a prior history of infestation by triatomines (Fig. 2) and were part of the regular activity of the technicians of the health programs of Bahia health surveillance system. The inspections of the internal and external walls of the residence and inside rooms of the house unit and its annexes were carried out following the standardized inspection protocol of Brazil ministry of health [23].

Health agents performed exhaustive sampling and captured all the specimens found inside houses and at the peridomestic environment (kennel, cattery, corral, etc.). Additionally, triatomines were collected from the wild environment (away from human settlements) to serve as negative controls in the analyses of blood-meal sources, as DNA of humans and domestic animals (cats, dogs, and humans) was not expected in wild bugs. Metal forceps and flashlights were used to survey crevices and nonluminous sites. We selected the sampled municipalities according to epidemiological priority criteria and the capacity of SESAB at each municipality to carry out the program activities. The teams carried out collections



based on the surveillance strategy of the Chagas Disease Control Programme of SESAB.

Triatomines were identified using specific identification keys [24]. Then, we photographed dorsally and ventrally, and dissected the bugs, separating head and wings, legs, and abdomen. Samples were stored in 70% alcohol + 5% glycerin at 5 °C and labeled with a QR code. We carried out a blind identification process at LACEN-BA

and FIOCRUZ-BA, independently, and the teams discussed conflicting identifications individually. In addition, voucher specimens of triatomines were deposited into the SESAB entomological collection, as a reference of triatomine vectors, and all images taken of the insects are available for consultation.

Molecular procedures

Triatomines that were dead/dry or stored incorrectly and first- and second-stage nymphs were not included in the molecular analyses to increase the efficacy of DNA extraction with a DNAzol commercial kit (DNAzol; Gibco BRL/Life Technologies, Gaithersburg, MD, EUA). We dissected triatomines in a biological safety cabinet to avoid contamination of the sample with human DNA. We macerated abdomen samples with TissueLyser L-Beader (Loccus, São Paulo, Brazil), plastic disposable maceration pistils and metal beads. Each sample was kept in a 2 ml autoclaved conical tube with a screw cap, and 1 ml of DNAzol and five autoclaved stainless-steel metal beads were added following the DNAzol standard protocol.

After purification, we quantified the DNA with a NanoDrop™ Spectrophotometer and the samples were set to a concentration of ~100 ng/μl. Conventional [25] and multiplex [26, 27] PCRs were performed with specific primers to detect *T. cruzi* based on mini-exon genes and the cytochrome *c* oxidase subunit 2 (*cox2*) gene.

To confirm that good-quality DNA was present in the samples, we amplified a 127-bp fragment of the ITS2 nuclear rDNA marker [28]. For the amplification of molecular targets, a commercial kit with Qiagen PCR Master Mix (QIAamp, Qiagen, Hilden, Germany) was used in a Mastercycler Gradient thermocycler (Eppendorf, Foster City, California, USA). The PCR conditions and primers are described in Additional file 1: Tables S2–S4.

Samples of *T. cruzi* cultures were obtained from the Experimental Chagas Disease Laboratory (LACEI/CPqGM) and used for positive controls. The DNA samples of dogs (*Canis lupus familiaris* L.), birds (*Gallus gallus* L.) and cats (*Felis catus* L.) were obtained from the blood of healthy animals from the laboratory. The human blood sample was obtained from researchers of the team (GR and CGSS). All the samples used as controls had the DNA purity evaluated with a Nanodrop™ spectrophotometer and adjusted to a concentration of ~100 ng/μl. Then, the samples were aliquoted and kept at -70 °C until use.

During the standardization of the PCR, amplified products of the PCR (10 μl) were separated by electrophoresis in an agarose gel, stained with SYBR Safe (Invitrogen, CA, USA), visualized with blue light and photographed with a Photo-documenter MultiDoc-it (UVP, Imaging Systems, Upland, CA, USA). Images were analyzed with UVP GelStudio™ (VisionWorks, CA, USA) software. Before standardization, we analyzed PCR results by capillary electrophoresis in an Applied Biosystems ABI-3500 DNA sequencer [29].

The generated electropherograms were analyzed with GeneScan analysis software version 3.1.

Statistical analysis and mapping procedures

Trypanosoma cruzi infection and blood-meal frequencies were compared between triatomine species and habitats (intra-, peridomestic, wild environment) by the Chi-square or Fisher's exact tests using the StartCalc tool in EpiInfo™. For statistical analyses, 95% confidence intervals (CI) and *P*-values (<0.05) were evaluated. Records of triatomines in Bahia were referenced to geographical coordinates using a GPS. When there was no information on the specific GIS coordinates, we calculated the municipality centroid using ArcGIS/ArcMap 10.5 software which was also used to map triatomine spatial distribution.

We analyzed vector hotspots with kernel density spatial analysis. In order to determine if the spatial pattern of the data is either clustered, dispersed or random, the spatial autocorrelation was evaluated by global Moran's index (I), *z*-score and *P*-value statistics interpretation in the Spatial Autocorrelation tool [58]. To determine the appropriate distance threshold or radius to elaborate kernel density analysis, we used the Incremental Spatial Autocorrelation tool [58]. We used the vector measures spatial autocorrelation for a series of distance increments and reports, for each distance increment, the associated Moran's index, expected index, variance, *z*-score, and *P*-value. Peaks in *z*-scores reflect distances where the spatial processes promoting clustering are most pronounced. Hotspots were represented by the Kernel Density tool [58]. The layers (.shp) used during this study were obtained from the IBGE website (<https://download.sibge.gov.br/>).

Results

In total, we collected 5906 triatomines belonging to 15 species from 127 of 417 municipalities in Bahia. Most of them (*n*=4640) were collected in 823 household units (intra- and peridomestic environments), especially in peridomestic areas (90%). The distributions of sampled triatomines by species, collection environments and municipalities are shown in Table 1.

We collected 610 specimens of 10 species inside domiciles in 55 municipalities. We detected colonies of *T. sordida*, *T. pseudomaculata* and *T. juazeirensis* in houses mainly in municipalities in the Caatinga biome. We collected 4030 specimens of 13 species in peridomiciles of 97 municipalities. *Triatoma sordida* was the most captured and widely distributed species in Bahia State, followed by *T. pseudomaculata* (Table 1). We detected a colony of *T. infestans* with more than 400 specimens

Table 1 Triatomines (*Panstrongylus* spp., *Psammolestes* spp. and *Triatoma* spp.) collected in different environments in Bahia State, Brazil, between 2013–2014

Species	Intradomestic			Peridomestic			Wild environment			Not determined			Total	Distribution		
	AM	AF	N	AM	AF	N	AM	AF	N	AM	AF	N		n	%	H
<i>P. geniculatus</i>	3	8	0	3	0	0	0	0	0	0	0	0	14	9	7.32	10
<i>P. lutzi</i>	2	4	0	4	4	0	0	0	0	1	1	0	16 (3)	4	3.25	10
<i>P. megistus</i>	0	3	0	6	7	2	0	0	0	3	2	0	23 (6)	5	4.07	7
<i>Ps. tertius</i>	0	0	0	6	8	0	0	0	0	0	0	0	14 (8)	1	1.63	1
<i>T. brasiliensis</i>	23	16	0	19	24	44	10	14	27	1	0	0	178 (57)	20	16.26	41
<i>T. juazeirensis</i>	32	20	89	12	17	13	1	2	0	8	13	20	227 (96)	7	5.69	4
<i>T. melanica</i>	0	0	0	4	3	12	0	0	0	0	0	0	19 (7)	1	0.81	1
<i>T. infestans</i>	0	0	0	45	102	294	0	0	0	0	0	0	441 (80)	1	0.81	1
<i>T. lenti</i>	0	0	0	0	0	1	1	0	5	0	0	0	7 (2)	1	0.81	12
<i>T. melanocephala</i>	6	6	0	1	2	0	0	0	0	1	1	0	17 (1)	12	9.76	12
<i>T. petrocchiai</i>	0	1	0	0	0	0	0	0	0	0	0	0	1 (1)	1	0.81	1
<i>T. pseudomaculata</i>	23	16	25	117	166	250	0	0	33	40	244		914 (81)	45	36.59	89
<i>T. sordida</i>	89	103	122	712	773	1370	0	0	51	60	296		3576 (293)	70	56.91	267
<i>T. sherlocki</i>	0	0	0	0	0	0	70	199	154	0	0	0	423 (58)	1	0.70	^a
<i>T. tibiamaculata</i>	9	10	0	3	6	0	0	0	0	2	6	0	36 (3)	7	5.69	26
Total	187	187	236	932	1112	1986	82	215	186	100	123	560	5906 (696)	127	100	482

^a From sylvatic environment

Abbreviations: n, number of municipalities with triatomine occurrence; AM, adult male; AF, adult female; N, Nymph; (), selected samples for molecular biology experiments; H, number of houses with triatomine occurrence

inside a chicken coop, five meters from a household. We also captured 484 triatomines of four species in the wild environment in four municipalities.

We selected 696 triatomines for molecular evaluation of *T. cruzi* infection and blood-meal analysis (Table 1). A total of 99.85% ($n=695$) showed specific amplification for triatomine DNA with an ITS2 nuclear rDNA marker, indicating the DNA integrity of the samples. All molecular targets evaluated have shown a spatial pattern expressed as clustered and showed an appropriate radius of 1.86 km (*T. cruzi*), 2.11 km (human), 1.98 km (dog), 1.77 km (cat), 1.98 km (bird) (Fig. 3). Almost 10% ($n=68$) of the triatomines were infected with *T. cruzi* (95% CI: 7.5–12.1%), and the infection rate was highest in the *T. brasiliensis* species complex (Table 2). The proportion of *T. cruzi*-infected triatomines was higher in the wild environment ($\chi^2=134$, $df=1$, $P<0.001$). Infected triatomines were detected in 25 municipalities, mainly in the Caatinga biome. The kernel spatial analysis showed higher density areas of *T. cruzi*-infected triatomines in the northeast and central Bahia (Fig. 3b).

We found most bugs fed on birds (74.2%), other blood-meal sources were dogs (6%), cats (0.6%) and humans (1%) (Table 3). Triatomines that fed on birds were detected widely in Bahia State (Fig. 3), while those that fed on humans, dogs and cats were mainly detected in the northeast region, near the State of Pernambuco (Fig. 3).

There was no significant difference in the frequencies of triatomines fed on cats and humans between intra and peridomestic environments ($P>0.05$) but we detected a higher frequency of bugs that fed on dogs inside houses ($\chi^2=4.07$, $df=1$, $P=0.04$). The frequency of triatomines that fed on birds was practically the same as in the wild (86.30%) and peridomestic (86.57%) environments ($\chi^2=0.013$, $df=1$, $P=0.9076$) and statistically higher in the peridomestic environment than in the domestic environment ($\chi^2=8.0$, $df=1$, $P=0.004$).

We detected *T. cruzi*-infected triatomines fed on humans, dogs, and cats inside houses and triatomines fed on dogs and humans in peridomestic habitats. All infected triatomines detected in the wild environment contained bird DNA.

Discussion

The most salient findings about *T. cruzi* infection and blood-meal sources in synanthropic triatomines in Bahia were: (i) *T. cruzi*-infected triatomine bugs fed on human blood; (ii) *T. cruzi*-infected triatomines were widespread, but bugs that fed on dogs, humans, and cats were observed mainly in the northeast region; and (iii) most bugs fed on birds. These results show that triatomine bugs maintain the presence of *T. cruzi* in wild and domestic environments in the State of Bahia, Brazil.

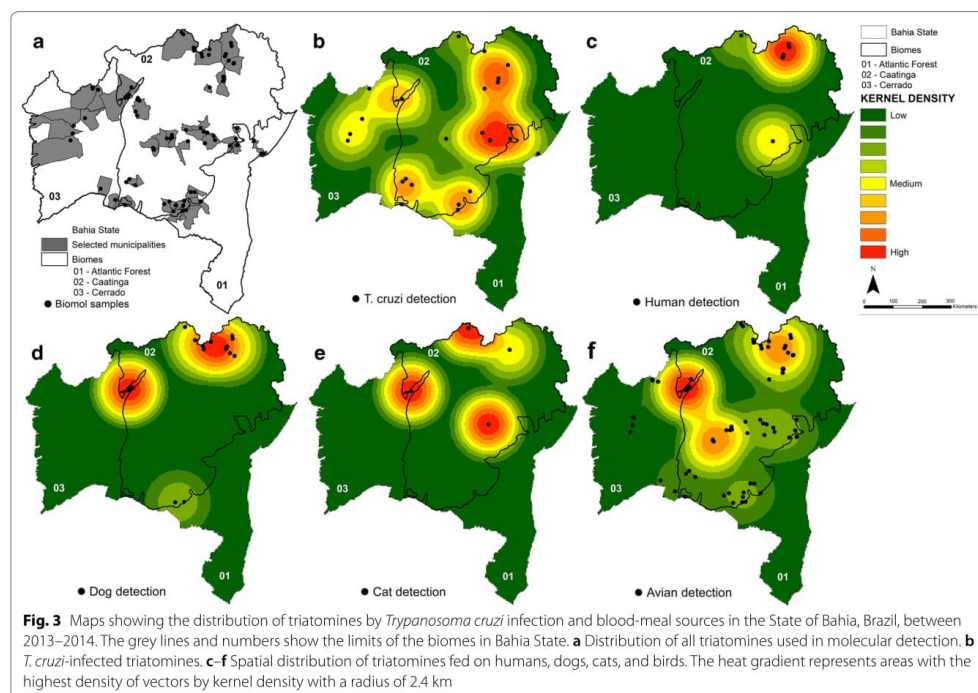


Table 2 *Trypanosoma cruzi*-infected triatomines collected in Bahia State, Brazil, between 2013 and 2014 broken down by species

Species	<i>n</i>	+	%	χ^2 /Fisher's exact test ^a	<i>P</i> -value
<i>P. lutzi</i>	3	0	0	–	–
<i>P. megistus</i>	6	0	0	–	–
<i>P. tertius</i>	8	2	25.0	^a	0.17
<i>T. brasiliensis</i>	57	9	15.8	2.1	0.14
<i>T. infestans</i>	80	0	0	–	–
<i>T. juazeirensis</i>	96	6	6.2	1.24	0.26
<i>T. lenti</i>	2	0	0	–	–
<i>T. melanica</i>	7	2	28.6	2.0	0.15
<i>T. melanocephala</i>	1	0	0	–	–
<i>T. pseudomaculata</i>	81	10	12.3	0.77	0.38
<i>T. sherlocki</i>	58	25	43.1	42.9	< 0.001
<i>T. sordida</i>	293	11	3.75	20.0	< 0.001
<i>T. tibiamaulata</i>	3	2	66.7	^a	0.26

^a Fisher's exact test

Note: *P*-values < 0.05 are indicated in bold

Abbreviations: *n*, number of triatomines; +, number of *T. cruzi*-infected triatomines; %, percent of positive samples; –, data not suitable for statistical analysis

We found 15 of the 26 recorded triatomine species in the State of Bahia during our two-year study. This result highlights the diversity of triatomines in this region [9] referring to Bahia as the Brazilian state with the highest number of triatomine species in Brazil. Ten species were recorded inside houses in sampled municipalities, mainly *Triatoma sordida*, *T. pseudomaculata*, and the *T. brasiliensis* species complex. The results differ from those observed before systematic Chagas disease vector control was carried out between 1975 and 1983 when *P. megistus* and *T. infestans* were the most captured species inside houses [5].

We observed house-invading *P. megistus* in few residences, and *T. infestans* occurred in one municipality in our study (Novo Horizonte); *T. infestans* were also recorded in other two municipalities in Bahia in the last years (Ibipeba and Tremedal) [15, 30, 31]. These results show the success in controlling domestic triatomines with the virtual elimination of *T. infestans* in Bahia municipalities [32, 33].

Our data show that *T. sordida* and *T. pseudomaculata* are the most frequently captured species in the State of Bahia, as already reported [9, 21, 34] which demonstrates

Table 3 Blood-meal sources detected in triatomines collected in different environments in Bahia State, Brazil, between 2013 and 2014

Species	No. of samples			Avian			Human			Dog			Cat		
	I	P	W	I	P	W	I	P	W	I	P	W	I	P	W
<i>P. lutzi</i>	2	1	0	1	1	0	0	0	0	1	0	0	0	0	0
<i>P. megistus</i>	0	6	0	0	4	0	0	0	0	0	0	0	0	0	0
<i>Ps. tertius</i>	0	8	0	0	5	0	0	0	0	0	0	0	0	0	0
<i>T. brasiliensis</i>	33	13	11	23	11	9	3	0	0	1	1	0	0	0	0
<i>T. infestans</i>	0	80	0	0	80	0	0	0	0	0	0	0	0	0	0
<i>T. juazeirensis</i>	58	35	3	38	26	3	2	0	0	9	5	0	2	2	0
<i>T. lenti</i>	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0
<i>T. melanica</i>	0	7	0	0	5	0	0	0	0	0	0	0	0	0	0
<i>T. melanocephala</i>	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>T. pseudomaculata</i>	26	55	0	8	47	3	0	2	0	0	2	0	0	0	0
<i>T. sherlocki</i>	0	0	58	0	0	50	0	0	0	0	0	0	0	0	0
<i>T. sordida</i>	201	92	0	120	77	0	0	0	0	16	5	0	0	0	0
<i>T. tibiamaculata</i>	3	0	0	2	1	0	0	0	0	0	0	0	0	0	0

Abbreviations: I, intradomestic environment; P, peridomestic environment; W, wild environment

their ability to colonize synanthropic environments. Other studies showed that domestic infestation with *T. pseudomaculata* increases when houses are located near preserved forests with natural ecotopes such as bird nests, tree hollows and palms [34]. Our results showed a wide distribution of *T. sordida* in the State of Bahia. A study of synanthropic triatomines in the southwest of Bahia between 2008 and 2013 [21] also showed that *T. sordida* was the most frequently captured species and presented the highest percentage of infection with *T. cruzi*. Although most specimens were captured in peridomestic habitats, as expected [9], we detected the presence of colonies and infected specimens inside houses revealing a potential risk for vectorial transmission. The other possible scenario is the risk of *T. cruzi* oral outbreaks mediated by *T. sordida* specimens inside houses, as already recorded in Bahia State [27].

Our results also revealed the high frequency of the *T. brasiliensis* species complex in Bahia. Costa et al. [35] showed high domestic infestation and infection rates for *T. brasiliensis* in Bahia between 1993 and 1999 when compared with other states of the northeast region. We detected *T. brasiliensis brasiliensis* in 20 municipalities, mainly in the northeast region of the state. To the best of our knowledge, our results show the first record of *T. brasiliensis brasiliensis* in the Bahia State. The most recent data recorded only *T. juazeirensis*, *T. melanica*, *T. lenti*, *T. bahiensis*, *T. sherlocki* and *T. petrocchiae* in Bahia [36]. Therefore, our results expand the knowledge of the geographical distribution of *T. brasiliensis brasiliensis* in northeastern Brazil. *Triatoma juazeirensis* is a recently described species [37] that was commonly misidentified as *T. brasiliensis*. Our results add new information about

the behavior of this species revealing a higher number of triatomines in houses than in peridomestic habitats. We also revealed a high infection rate of *T. sherlocki* in a domestic environment in Bahia. Colonies of *T. sherlocki* were already found in houses of Bahia with *T. cruzi* infection of ~11% [38], revealing a domiciliation process and the potential risk for vectorial transmission to humans.

Trypanosoma cruzi has been detected in vectors in all regions of Bahia. We found high infection rates for *T. sherlocki* and *T. tibiamaculata* as already observed by Almeida et al. [38] and Ribeiro et al. [20], respectively. *Trypanosoma cruzi* infections in triatomines based on parasite morphology after optical microscopy are underestimated [39]. Consequently, the risk of *T. cruzi* transmission should be higher than the entomological-parasitological routine surveillance suggests [40, 41]. For example, test-specific naïve indices of *T. cruzi* infection in triatomines varied from 17.8%, considering only optical microscopy results, to 41.5%, considering PCR results (23.1% positive by conventional PCR and 41.3–41.4% by qPCR) [41]. Our results revealed a triatomine infection rate of approximately 10% by conventional PCR, suggesting that the triatomine infection in Bahia may be even higher than that observed in our study. These infection rates also vary according to the species and development stage of the sampled specimens. For example, *T. cruzi* infection rates observed for *T. tibiamaculata* ranged between 50–65% [20].

The infection rates of *T. cruzi* observed in our study were similar to those obtained in recent studies carried out in Pernambuco [42], Mato Grosso do Sul [43, 44] Ceará [45, 46], the Rio Grande do Norte [47, 48] and Bahia [19, 20]. Infection rates were high for *T. brasiliensis*

species complex, especially for *T. sherlocki* (43.1%). Almeida et al. [47] detected *T. cruzi* in 52–71% of *T. brasiliensis* captured in Rio Grande do Norte, a higher percentage than that observed in our study in Bahia (15.8%) that could be explained by different blood-feeding habitats. The *T. sherlocki* infection rate observed in our study was four times higher than that reported by Almeida et al. [38]. Most of *T. sherlocki* specimens fed on avian blood, but the high level of infection rates of *T. cruzi* indicate an eclectic feeding behavior of *T. sherlocki*. The infection with *T. cruzi* detected in *Ps. tertius*, a species commonly associated with furnariid birds could also be explained by opportunistic feeding on mammals that eventually are found in furnariid nests. The results suggest a previous feeding of *Ps. tertius* with infected mammal blood.

Trypanosoma cruzi infection rates in *T. sordida* were generally less than 5% based on parasitological methods; however, Minuzzi-Souza et al. [41] estimated rates of 35% based on qPCR, a more sensitive evaluation method, which reinforces the relevance of this species as a potential *T. cruzi* vector. *Triatoma infestans* was not found to be infected by *T. cruzi*, as all the specimens were collected from a single colony into a chicken coop near the household unit; this is an unusual situation for this species, as it is considered to be exotic and domestic in Bahia.

The most frequent blood-meal source detected in triatomines in Bahia was birds (74%), similar to other studies [20, 49]. Birds are an important link in the domiciliation process of triatomines because they are common blood-meal sources in the peridomestic habitat due to be an important source of human food, through the raising of chickens, usually in the peridomicile of households [50–52]. Birds were also the main food source for *T. brasiliensis* species complex, contrasting with other studies highlighting the importance of rodents as feeding sources for *T. brasiliensis* in the Rio Grande do Norte [47, 48] and Ceará [53]. Human, dog and cat DNA, at 1%, 6%, and 0.6%, respectively, were observed less frequently. It is important to point out that previous studies have shown the key role of domestic animals in maintaining *T. cruzi* circulation within human dwellings [54, 55]. We found a higher frequency of triatomines fed on dogs inside houses highlighting the role of dogs as a potential source of *T. cruzi* in domestic transmission cycles, as already discussed [56], showing that dogs can sleep in places that are more accessible to the bugs, increasing the probability of infecting an initially uninfected bug.

The species found with human DNA were *T. brasiliensis*, *T. juazeirensis*, and *T. pseudomaculata*, and several other studies have revealed that these species are capable of transmitting *T. cruzi* to humans in the domestic

environment [34, 35, 37, 48, 53, 57]. Regarding the spatial distribution of *T. cruzi* and blood-meal sources, we observed clusters of infected triatomines that fed on humans and domestic animals in the municipalities of Curaçá and Irecê, located in the northeast region of Bahia. Simulations of vulnerability to *T. cruzi* vector-borne transmission in Brazil based on the most prevalent species also have indicated the northeast region of Bahia as having high vulnerability to the vector-borne transmission of *T. cruzi*; vulnerable municipalities exhibited a higher occurrence of synanthropic triatomines, lower socioeconomic levels (high percentage of properties in rural areas with individuals living in extreme poverty), and more extensive anthropized areas (percentage of deforested area in the municipality) [10].

Conclusions

Triatomines remain widely distributed in Bahia, with relevant *T. cruzi* infections and feeding on human and domestic animals inside houses, mainly in the northeast region of Bahia, thus maintaining the potential risk of *T. cruzi* transmission to humans. Ten species were recorded inside houses, mainly *Triatoma sordida*, *T. pseudomaculata*, and the *T. brasiliensis* species complex. Molecular and spatial analysis are useful to reveal *T. cruzi* infection and blood-meal sources in synanthropic triatomines, identifying areas with an ongoing threat for parasite transmission and improving entomological surveillance strategies.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13071-019-3849-1>.

Additional file 1: Figure S1. Notified and confirmed acute cases of Chagas disease in Brazil. **Table S1.** Confirmed cases of acute Chagas disease in Brazil between 2016 and 2019. **Table S2.** Reagents used for the amplification of molecular targets. **Table S3.** Thermocycling conditions used for amplification of molecular targets. **Table S4.** Sequences of the primers used.

Abbreviations

PCR: polymerase chain reaction; SESAB: Epidemiologic Surveillance team of Bahia State Health Service; DNA: deoxyribonucleic acid; ITS2: internal transcribed spacer 2; GPS: Global Position System; GIS: Geographical Information System; qPCR: quantitative polymerase chain reaction; SINAN: Notification Disease Information System.

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Authors' contributions

GRJ, MGR and RGG outlined the research project and performed gathering of data, writing, technical editing, statistical analysis calculation, Triatominae DNA purification, processing of triatomine samples, Triatominae identification,

molecular evaluation, statistical analysis, georeferencing, and language editing. CGSS, GMC, CMMC, RFS, OMF5 and EOLF acquired data from SESAB, gathered data, conducted proofreading and Triatominae identification and proofread the manuscript. JR, FV, FL and CD processed triatomine samples, conducted Triatominae DNA purification and molecular evaluation of samples. RFA and RBR proofread and edited the manuscript and performed georeferencing. DLPm, WNA and RBR proofread the manuscript, performed language editing, technical editing. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional file. The datasets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The adopted procedures were in accordance with the ethical standards of the Research Ethics Committee of the Gonçalo Moniz Institute (FIOCRUZ, Bahia, Brazil, no. 2.552.284) and with the Helsinki Declaration of 1964, revised in 1975, 1983, 1989, 1996 and 2000. The consent form was waived because the analysis was based on a state surveillance service of Bahia, Brazil. However, no personal identification data were used to ensure the complete anonymity of the participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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5.2. ARTIGO 2 – *TriatoScore: an entomological-risk score for Chagas' disease vector control-surveillance.*

Neste capítulo são apresentados os resultados relativos aos objetivos específicos:

- a) Calcular os principais indicadores entomológicos das espécies de triatomíneos na Bahia;
- b) Desenvolver um indicador composto alternativo para sumarizar a importância epidemiológica das espécies de triatomíneos na transmissão de *T. cruzi* ao homem e animais domésticos na Bahia;
- c) Determinar as áreas da Bahia com valores mais elevados para o TriatoScore;

O artigo científico em questão está apresentado integralmente a seguir, compondo a presente seção, e foi publicado na revista *Parasites & Vectors* nº14(492) em setembro de 2021, sob título: ***TriatoScore: an entomological-risk score for Chagas disease vector control-surveillance.*** DOI: <https://doi.org/10.1186/s13071-021-04954-5>.

RESEARCH

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TriatoScore: an entomological-risk score for Chagas disease vector control-surveillance

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Abstract

Background: Triatomine bugs transmit Chagas disease across Latin America, where vector control-surveillance is increasingly decentralized. Locally run systems often deal with highly diverse native-vector faunas—plus, in some areas, domestic populations of non-native species. Flexible entomological-risk indicators that cover native and non-native vectors and can support local decision-making are therefore needed.

Methods: We present a local-scale entomological-risk score (“TriatoScore”) that leverages and builds upon information on the ecology-behavior and distribution-biogeography of individual triatomine bug species. We illustrate our approach by calculating TriatoScores for the 417 municipalities of Bahia state, Brazil. For this, we (i) listed all triatomine bug species recorded statewide; (ii) derived a “species relevance score” reflecting whether each species is native/non-native and, if native, whether/how often it invades/colonizes dwellings; (iii) mapped each species’ presence by municipality; (iv) for native vectors, weighted presence by the proportion of municipal territory within ecoregions occupied by each species; (v) multiplied “species relevance score” × “weighted presence” to get species-specific “weighted scores”; and (vi) summed “weighted scores” across species to get municipal TriatoScores. Using standardized TriatoScores, we then grouped municipalities into high/moderate/low entomological-risk strata.

Results: TriatoScores were higher in municipalities dominated by dry-to-semiarid ecoregions than in those dominated by savanna-grassland or, especially, moist-forest ecoregions. Bahia’s native triatomines can maintain high to moderate risk of vector-borne Chagas disease in 318 (76.3%) municipalities. Historical elimination of *Triatoma infestans* from 125 municipalities reduced TriatoScores by ~27% (range, 20–44%); eight municipalities reported *T. infestans* since Bahia was certified free of *Trypanosoma cruzi* transmission by this non-native species. Entomological-risk strata based on TriatoScores agreed well with Bahia’s official disease-risk strata, but TriatoScores suggest that the official classification likely underestimates risk in 42 municipalities. Of 152 municipalities failing to report triatomines in 2006–2019, two and 71 had TriatoScores corresponding to, respectively, high and moderate entomological risk.

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Conclusions: TriatoScore can help control-surveillance managers to flexibly assess and stratify the entomological risk of Chagas disease at operationally relevant scales. Integrating eco-epidemiological, demographic, socioeconomic, or operational data (on, e.g., local-scale dwelling-infestation or vector-infection frequencies, land-use change and urbanization, housing conditions, poverty, or the functioning of control-surveillance systems) is also straightforward. TriatoScore may thus become a useful addition to the triatomine bug control-surveillance toolbox.

Keywords: Chagas disease, Triatominae, Vector control, Vector surveillance, Risk stratification

Background

Public health researchers, managers, policy-makers, and international agency officials make regular use of indices or indicators of population health and well-being [1, 2]. Such indices range from simple counts or rates of disease cases or events to more complex measurements of human development or socioeconomic deprivation [1–3]. Health-related indices aim primarily at summarizing population-level information on health and disease, on disease risk or exposure, or on the performance of health systems, policies or interventions in a given place (from local to global) and over a given time period (from weeks to centuries) [1, 2]. In the case of insect-borne diseases, entomological indices are essential to guide control-surveillance efforts [4–8].

Chagas disease is among the most important vector-borne diseases in the Americas; estimates for 2019 suggest that about 6.4 million people (95% uncertainty interval [UI], 5.5–7.3 million) are infected with its causal agent, *Trypanosoma cruzi*, in Latin America, leading to annual losses of ~274,000 disability-adjusted life years (UI, 170,000–490,000) [9–11]. *Trypanosoma cruzi* is transmitted by 140+ species of blood-sucking triatomine bugs, and vector control-surveillance is, together with blood- and organ-donor screening, the cornerstone of disease prevention [7, 8, 11, 12]. Entomological indices are critical for the planning, running, and evaluation of Chagas disease vector control-surveillance systems [7, 8]. In particular, for a given vector species, place, and time period, the World Health Organization advocates the use of indices measuring (i) vector presence, breeding, and abundance inside and/or around houses (indexed by, respectively, infestation, colonization, and density); (ii) vector infection with *T. cruzi*; and (iii) “dispersion” across localities within a given territory—an index that helps measure progress towards local elimination of non-native species (see definitions in [7, 8]).

Each of these entomological-risk indices, however, has important practical limitations. The most general setback is that, to calculate them, control managers need to regularly organize expensive area-wide, door-to-door surveys that are difficult to maintain in resource-limited settings [11, 12]. Even in the hands of highly trained staff, moreover, routine vector and parasite detection methods

have imperfect sensitivity, and indices that rely on those methods will almost always be biased down [12, 13]. In particular, there is growing evidence that (i) not all vectors will be detected during routine dwelling inspections, especially after insecticide spraying reduces bug densities [13–15], and (ii) not all *T. cruzi* infections will be detected during routine vector testing [16]. To further complicate things, local control-surveillance systems frequently deal with a wide variety of native triatomine bug species—plus, at times, introduced populations of non-native species [11, 12, 17–20]. Because different triatomine bug species or populations can play very different roles in disease transmission, control-surveillance tactics must be tailored to the local vector fauna [11, 12, 19, 20]. Although working classifications of Chagas disease vectors are available for this purpose [7, 19], they often (i) lack the flexibility needed to track key time-varying events (such as, say, the local elimination of non-native species and their replacement by less-known native vectors) and (ii) tend to conflate biological and operational considerations, which makes them confusing and potentially ineffective [11, 12, 20, 21].

In the current context of decentralized health systems across Latin America, Chagas disease vector control-surveillance decisions are increasingly made at the local level [22, 23]. Flexible entomological-risk indicators that work well for both native and non-native vector species and that can support local, timely decision-making are therefore needed [20]. Here, we present an entomological-risk score (“TriatoScore”) that leverages and builds upon information on the ecology, behavior, distribution, and biogeography of individual triatomine bug species and can help control-surveillance managers to assess, stratify, map, and monitor Chagas disease entomological risk at operationally relevant scales. To illustrate the use of TriatoScore in practice, we applied our approach to the 417 municipalities of Bahia state, Brazil, where native vectors are highly diverse and Chagas disease remains a major public health concern.

Methods

We aimed at deriving an entomological-risk score based on vector species potentially involved in Chagas disease transmission at the relatively fine scale matching the

needs of decentralized control-surveillance [23]. This raised two general issues. First, we needed to allow for the sharp differences in epidemiological relevance across vector species [7, 19, 24]. To do this, we combined the hierarchical working classification of triatomine bug species, populations, foci, and individuals proposed by one of us (see [20] and Table 1) with current knowledge about the ecological-behavioral features that drive such interspecies differences [7, 11, 12, 18, 19, 24–26]. Second, we needed to derive a reliable picture of each species' geographic distribution. Because local-scale bug presence data are almost inevitably incomplete, we complemented actual records (see below and [27–33]) with the best available knowledge about the ecoregional biogeography of each species—following [17, 34] and hence based on Dinerstein et al.'s proposal [35, 36]. We then applied this approach to the 400+ municipalities of the northeastern Brazilian state of Bahia; below we (i) briefly describe the setting and data used for this illustrative study; (ii) provide a step-by-step guide to calculating

municipality-specific entomological-risk scores (“TriatoScores”); and (iii) show how TriatoScores can then be used to flexibly stratify and map Chagas disease entomological risk at the municipality scale.

Study setting and triatomine bug data

The state of Bahia, Brazil, has 417 municipalities in ~565,000 km² and nearly 15 million residents, of which ~30% (or ~4.5 million people) live in rural areas [37]. By the time the last demographic census was conducted by the Brazilian Instituto Nacional de Geografia e Estatística (IBGE; 2010), about 60% of houses in the state were considered to be in poor (57%) or very poor condition (3%) [37]. Bahia's natural vegetation cover corresponds to nine ecoregions [35, 36] that can be grouped into three major habitat classes—dry-semiarid (Caatinga and Atlantic dry forest), savanna-grassland (Cerrado and Campos Rupestres), and moist forests (Bahia coastal and interior forests, Pernambuco interior forest, and coastal Restingas and

Table 1 Triatomine bug species in Bahia state, Brazil: “species relevance score” based on the approach of Abad-Franch [20]

Species or species pair	Hierarchical level				Species relevance score
	Species	Populations	Foci	Individuals	
<i>Triatoma infestans</i> ^a	Non-native	Non-wild	Domestic/peridomestic	In foci	10
<i>Triatoma rubrofasciata</i>	Non-native	Non-wild	Domestic/peridomestic	In foci	5
<i>Triatoma juazeirensis/brasiliensis</i> ^b	Native	Wild/non-wild	Domestic/peridomestic	In foci/invaders	4
<i>Triatoma sordida</i>	Native	Wild/non-wild	Peridomestic/domestic	In foci/invaders	4
<i>Triatoma pseudomaculata</i>	Native	Wild/non-wild	Peridomestic/domestic	In foci/invaders	4
<i>Panstrongylus megistus</i>	Native	Wild/non-wild	Peridomestic/domestic	In foci/invaders	4
<i>Triatoma lenti/bahiensis</i> ^b	Native	Wild/non-wild	Peridomestic/domestic	Invaders/in foci	4
<i>Triatoma vitticeps</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Triatoma costalimai</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Panstrongylus lutzi</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Rhodnius neglectus</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Rhodnius nasutus</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Triatoma melanica</i>	Native	Wild/non-wild	Peridomestic/rarely domestic	Invaders/in foci	3
<i>Triatoma tibiamaculata</i>	Native	Wild	Natural	Frequent invaders	3
<i>Panstrongylus geniculatus</i>	Native	Wild	Natural	Frequent invaders	3
<i>Triatoma petrocchiai</i>	Native	Wild	Natural	Invaders	2
<i>Triatoma sherlocki</i>	Native	Wild	Natural	Invaders	2
<i>Triatoma melanocephala</i>	Native	Wild	Natural	Invaders	2
<i>Panstrongylus diasi</i>	Native	Wild	Natural	Invaders	2
<i>Panstrongylus lenti</i>	Native	Wild	Natural	Invaders	2
<i>Cavernicola pilosa</i>	Native	Wild	Natural	Rare invaders	1
<i>Rhodnius domesticus</i>	Native	Wild	Natural	Rare invaders	1
<i>Psammolestes tertius</i>	Native	Wild	Natural	Rare invaders	1
<i>Parabelminus yurupucu</i>	Native	Wild	Natural	Rare invaders	1

^a The most dangerous domestic vector of *Trypanosoma cruzi*

^b Merged because most records available do not distinguish the species within each pair; we note that (i) most, if not all, “*T. brasiliensis*” records from Bahia most likely refer to *T. juazeirensis*, and (ii) genetic similarity between *T. lenti* and *T. bahiensis* suggest they might be conspecific [17]

Table 2 Triatomine bug species in Bahia state, Brazil: ecoregional biogeography and municipality-level occurrence frequency

Species or species pair	Habitat class and ecoregion ^a										Notes ^b	Municipalities	
	Dry-semiarid		Savanna-grassland		Moist forest								
	Ca	ADF	Ce	CRS	BIF	BCF	PIF	Re	Ma				
<i>Triatoma infestans</i>	-	-	-	-	-	-	-	-	-	-	-	As reported after 2006/since 2000/at any time	8/31/133
<i>Triatoma rubrofasciata</i>	-	-	-	-	-	-	-	-	-	-	-	As reported	4
<i>Triatoma juazeirensis/brazilensis</i> ^c	1	1	0	0	0	0	0	0	0	0	0	Across the state	269
<i>Triatoma sordida</i>	1	1	1	0	1	0	1	0	0	0	0	Across the state	342
<i>Triatoma pseudomaculata</i>	1	1	1	0	1	0	1	0	1	0	0	Across the state	342
<i>Panstrongylus megistus</i>	1	1	1	0	1	1	1	1	1	1	0	Across the state	417
<i>Triatoma lenti/bahiensis</i> ^c	1	1	0	1	0	0	0	0	0	0	0	Chapada Diamantina and southern São Francisco River valley	69
<i>Triatoma vitticeps</i>	0	1	1	0	1	0	0	0	0	0	0	Southern municipalities	51
<i>Triatoma costalimai</i>	0	1	0	0	0	0	0	0	0	0	0	On the Serra Geral de Goiás	9
<i>Panstrongylus lutzi</i>	1	1	0 ^d	0	0	0	0	0	0	0	0	Across the state	277
<i>Rhodnius neglectus</i>	1 ^e	1	1	0	0	0	0	0	0	0	0	Western and northern Bahia; some Caatinga populations	152
<i>Rhodnius nasutus</i>	1	1	0	0	0	0	0	0	0	0	0	North of the lower São Francisco River	15
<i>Triatoma melanica</i>	0	1	1	0	0	0	0	0	0	0	0	Southern São Francisco River valley	22
<i>Triatoma itiamaculata</i>	0 ^d	0 ^d	0	0 ^d	1	1	1	1	1	1	0	Across the state	267
<i>Panstrongylus geniculatus</i>	1	1	1	0	1	1	1	1	1	1	0	Across the state	417
<i>Triatoma petracchiæ</i>	1	0	0	0	0	0	0	0	0	0	0	North of the lower São Francisco River	15
<i>Triatoma sherlocki</i>	1	1	0	1	0	0	0	0	0	0	0	Serra da Mangabeira-Serra do Angelim (Jacaré-Verde basin)	24
<i>Triatoma melanocephala</i>	1	1	0	1	1	1	1	1	1	1	0	South and east of the São Francisco River	305
<i>Panstrongylus diasi</i>	0	1	1	0	0	0	0	0	0	0	0	Across the state	102
<i>Panstrongylus lenti</i>	1	1	0	0	0	0	0	0	0	0	0	Southern São Francisco River valley and adjacent areas	48
<i>Cavernicola pilosa</i>	1	1	1	0	1	1	1	1	1	1	0	Across the state	417
<i>Rhodnius domesticus</i>	0 ^d	0	0	0	1	1	1	1	1	1	0	Across the state	206
<i>Psamolestes tertius</i>	1	1	1	0	0	0	0	0	0	0	0	Across the state	276
<i>Parabulminus yurupucu</i>	0 ^d	0	0	0	0	1	1	1	1	1	0	Across the state	206

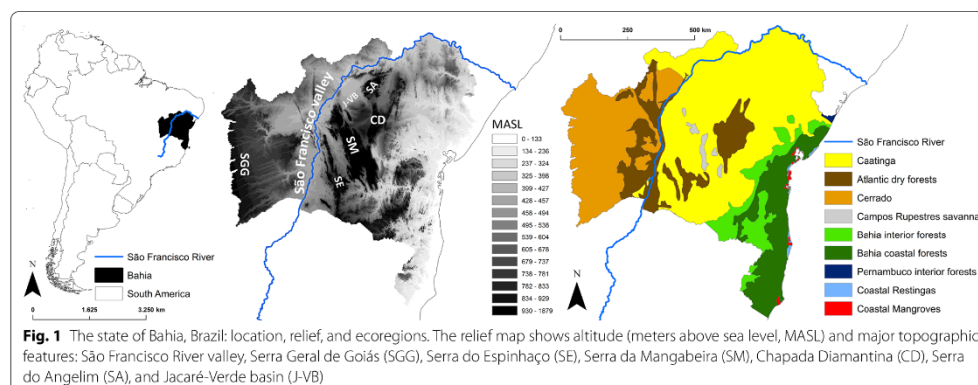
^a A presence/absence (1/0) matrix ("–", non-native species that only occur in human-made environments); Ca, Caatinga; ADF, Atlantic dry forest; Ce, Cerrado; CRS, Campos Rupestres savanna; BIF, Bahia interior forest; BCF, Bahia coastal forest; PIF, Pernambuco interior forest; Re, coastal Restingas; Ma, coastal Mangroves

^b See Additional file 2: Figures S1–S24 for municipality-level species-specific occurrence maps

^c Merged because most records available did not distinguish the species within each pair; we note that (i) most, if not all, "*T. brazilensis*" records from Bahia most likely refer to *T. juazeirensis*, and (ii) genetic similarity between *T. lenti* and *T. bahiensis* suggest they might be conspecific [17]

^d Presence records from these ecoregions most likely reflect lack of ecoregional map resolution, with suitable habitat present in some municipalities but not captured by the rough ecoregion-classification map we used (see Fig. 1 and [17]); we assigned a 0.001 value to the "weighted presence" of the species in those municipalities

^e Local *R. neglectus* populations of northern-central Bahia seem to have adapted to Caatinga environments



Mangroves) (see Table 2, Fig. 1 and [36]). Twenty-six triatomine bug species, of which 24 are known to be native to the state's territory, have so far been recorded in Bahia [27–33] (Tables 1, 2). Bahia's state health department (SESAB hereafter) uses entomological, epidemiological, demographic, socioeconomic and environmental indicators to group municipalities into three Chagas disease risk strata [32, 38, 39]; currently, 101 municipalities are considered at “low” disease risk, 214 at “moderate” risk, and 102 at “high” disease risk [38]. Recent estimates by the Global Burden of Disease (GBD) study suggest that ~220,000 people (UI, ~190,000–259,000) may carry *T. cruzi* in Bahia, with ~5.9–7.5 thousand new infections and ~300–1000 deaths attributable to Chagas disease occurring annually since 2010 [10].

We used two main sources of information to map triatomine bug species occurrence across Bahia's municipalities: (i) routine control-surveillance records (1999–2019) from SESAB, and (ii) published reports providing either (a) occurrence records at the ecoregion or locality/municipality level [17, 27–34] or (b) species distribution maps based on ecological niche models [29, 31, 40–45]. Because many of the records available do not distinguish either *Triatoma juazeirensis* from *T. brasiliensis* or *T. lenti* from *T. bahiensis*, we treated the former pair as “*T. juazeirensis/brasiliensis*” and the latter pair as “*T. lenti/bahiensis*”. Thus, our analyses cover 24 taxa—22 individual species and two species-pairs (Tables 1, 2). For simplicity, in what follows we will use “species” to refer to these taxa. We note that (i) current knowledge suggests that “*T. brasiliensis*” records from Bahia most likely refer to *T. juazeirensis* [17, 29, 40] and (ii) genetic similarity between *T. lenti* and *T. bahiensis* suggest they might be conspecific [17].

Calculating municipal TriatoScores: step-by-step procedure

Calculation of TriatoScores for the 417 municipalities of Bahia state involved the following six steps:

- i. List all triatomine bug species recorded statewide (see above and Table 1);
- ii. Derive a “species relevance score”, based on [20], reflecting whether each species (a) is native or non-native to the region under consideration (here, the state of Bahia), and, if native, (b) whether and how often it invades or colonizes human habitats. This “species relevance score”, hence, roughly measures the known epidemiological relevance of each taxon (see Table 1 and [20]);
- iii. Tabulate each species' presence/absence (coded 1/0, respectively) by municipality (see above and Additional file 1: Table S1). Our main analyses were done with the dataset including *T. infestans* records since 2006, which is when Brazil was certified free of *T. cruzi* transmission by this non-native species [12]; complementary analyses were done (a) with data on native vector species only and (b) with all historical records of *T. infestans* (Additional file 1: Table S1);
- iv. For native vectors (Table 1), weight presence/absence by the extent of municipal territory within ecoregions occupied by each species (see above and Table 2). To compute these “weighted presence” values, we first used municipal [46] and ecoregion shapefiles [36] to calculate, for each municipality, the proportion of territory within each ecoregion (see Table 2 and Additional file 1: Table S2). Then, we multiplied each species' presence (1) or absence (0) by the sum of municipality-specific ecoregion proportions. For each species, therefore, municipi-

- pal “weighted presence” values range from 0 to 1 (Additional file 1: Table S3). We used ArcMap[®] 10.5 [47] both to calculate ecoregion territorial shares and to map “weighted presence” values (see Additional file 2: Figures. S1–S24);
- v. For each species and municipality, multiply “species relevance score” by “weighted presence” to get “weighted scores” (Additional file 1: Table S4); and
 - vi. Sum “weighted scores” across species to get municipal TriatoScores (Additional file 1: Tables S4, S5).

Entomological-risk assessment: stratification, mapping, and spatial analysis

We standardized TriatoScores (to mean 0 and SD 1) and grouped municipalities into three strata of entomological risk: “high” risk (TriatoScores above 1 SD from the overall mean), “moderate” risk (TriatoScores within ± 1 SD from the overall mean), and “low” risk (TriatoScores below 1 SD from the overall mean) (Additional file 1: Table S5). Note that, with this standardization-based procedure, entomological risk is evaluated, for each municipality, relative to the overall (average) risk across the state. Thus, if the TriatoScore of any municipality changes over time (because, say, one non-native species is eliminated—or newly recorded), then the statewide TriatoScore mean and SD will change also, potentially leading to some municipalities moving from one entomological-risk stratum to another. In other words, our approach to entomological-risk classification is fundamentally dynamic—it will highlight municipalities that are substantially above, substantially below, or close to the average risk at the time of the assessment, using updated statewide summary risk statistics (mean, SD) as the benchmark. To illustrate this, we calculated TriatoScores both including (Additional file 1: Table S5) and excluding (Additional file 1: Table S6) records of the two non-native species known to occur in Bahia—*T. infestans* and *T. rubrofasciata*. Importantly, our analyses of the latter dataset are in fact about the “baseline” entomological risk associated with native, and hence non-eliminable, vector species across the state’s municipalities (see [11, 20] and Table 1). Note also that these analyses are mathematically equivalent to assigning a zero “species relevance score” to *T. infestans* and *T. rubrofasciata*.

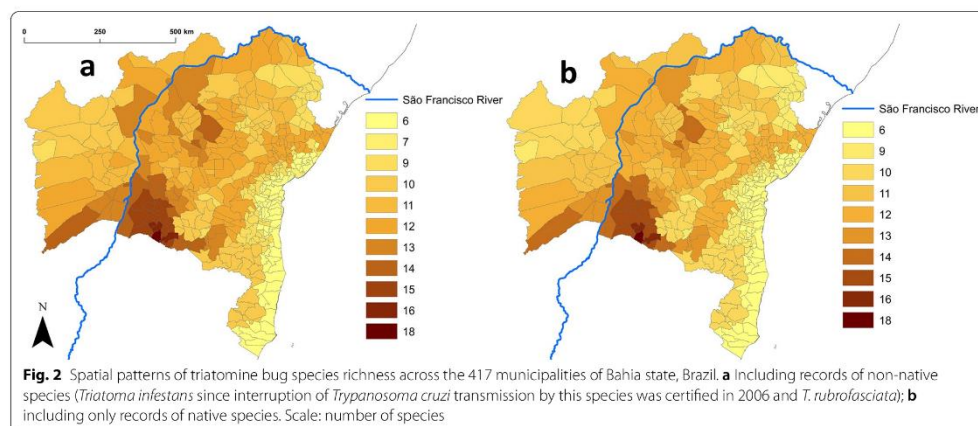
To assess the degree of agreement between our entomological-risk stratification based on TriatoScores and SESAB’s official disease-risk stratification [39], we used the *irrCAC* 1.0 package [48] in R 3.6.3 [49] to calculate Gwet’s AC_2 coefficients [50]. Gwet’s AC_2 suitably deals with ordinal data (via the *weights* = “ordinal” argument in *irrCAC* [48]) and is more robust to variation in rating frequency distributions than commonly used agreement

coefficients such as Cohen’s κ [50, 51]. We note that comparing risk ratings derived from TriatoScore and SESAB’s criteria is somewhat “unfair”—TriatoScore uses only vector data, while SESAB also takes epidemiological, demographic, socioeconomic and environmental information into account [33, 38, 39]. The comparison, however, can be useful if it helps pinpoint municipalities where SESAB’s stratification indicates a *lower* disease risk than the (just) entomological risk suggested by TriatoScores. We also note that integrating additional sources of information into a TriatoScore-based assessment is straightforward—one just needs to add a new weight for each extra metric. To illustrate how this might work, we used housing quality data from the last demographic census (IBGE, 2010; [37]) to calculate, for each municipality, the proportion of houses in poor or very poor condition—a rough measure of dwelling vulnerability to infestation by triatomines [24, 25, 39] (see Additional file 1: Table S2). We then weighted municipal TriatoScores by this housing quality metric, standardized the resulting “TriatoScore-plus” values (to mean 0 and SD 1 as above), and grouped our study municipalities into three risk strata (defined using the ± 1 SD criterion as above) (Additional file 1: Table S7).

We used ArcMap[®] 10.5 [47] and the data in Additional file 1 to build municipality-scale maps of vector species-specific “weighted presence”, vector species richness, TriatoScore and TriatoScore-plus values, and risk strata—both disease-risk strata as per the official SESAB classification and entomological-risk strata based on TriatoScores. Finally, we used the “Hot and Cold Spot Analysis” tool of ArcMap[®] 10.5 [47] to identify and map spatial clusters of municipalities with high (“hotspots”) and low (“coldspots”) TriatoScore values; these analyses were run with the Getis-Ord G_i^* statistic [52] and the “contiguity—edges and corners”, “row standardization” and “false discovery rate correction” options [47].

Results

Table 1 lists all triatomine bug species recorded so far in Bahia. Triatomine bugs are known or expected to occur in all of the state’s municipalities; the maps in Additional file 2: Figures S1–S24 show the municipality-level distribution of each species, with “weighted presence” values used for native species (see Additional file 1: Tables S1, S3). Our main dataset (including *T. infestans* records since 2006) reveals a mean richness of 10.2 ± 2.5 SD species (range, 6–18) per municipality (Fig. 2). Urandi is the municipality with the highest richness (18 species, all native), followed by Candiba, Jacaraci, and Pindaí (16 native species); these species-rich municipalities are located in southwestern Bahia (Fig. 2a). The geographical patterns of



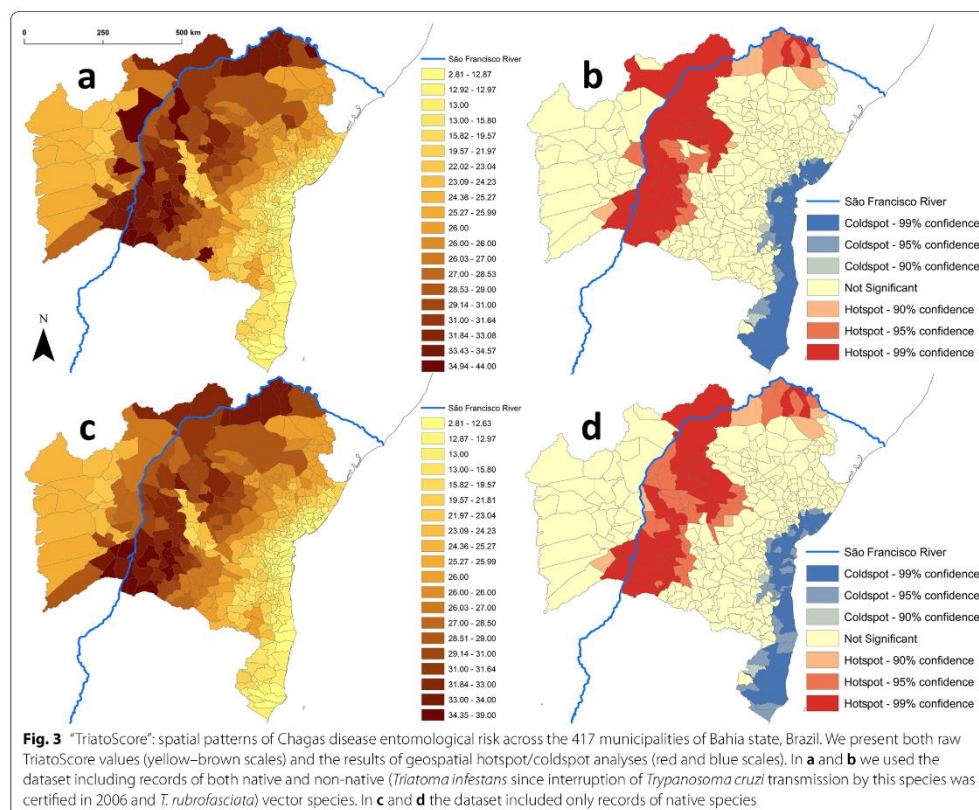
triatomine bug species richness do not vary substantially when only native species are considered (Fig. 2b). In Bahia, the Brazilian Atlantic dry forest is home to 17 native species, the semiarid Caatinga to 15, the Cerrado savanna to 11, and the moister Bahia interior forest to 10 species; in contrast, the coastal Mangroves (where triatomines most likely do not occur in Bahia) and Restingas (two species), as well as the higher-altitude Campos Rupestres montane savannas (three species), have the lowest richness values (Table 2). Municipality-level species richness hence seems to be somewhat higher in the ecoregion-transitional areas that roughly follow the dry San Francisco River valley and its fringes, and is clearly lower along the moister coastal region (Figs. 1, 2).

Vector species richness patterns were also reflected in municipal TriatoScore values (main dataset: mean, 23.7 ± 7.3 SD; range, 2.8–44.0; Fig. 3). TriatoScore mapping, however, revealed a clearer pattern of higher entomological risk along the São Francisco River valley and lower entomological risk along the coast; this was true both for the main dataset including non-native species (Fig. 3a) and for the native-species-only dataset (Fig. 3c). Geospatial analyses recovered a sharp hotspot cluster along the São Francisco River and the uplands that bound its valley (especially to the east and north) and a coldspot cluster along the moister coast; again, these results were largely independent of whether all vector species or only native species were considered (Fig. 3b, d). Overall, TriatoScores were higher in municipalities dominated by dry-to-semiarid ecoregions (Caatinga and Atlantic dry forest; mean TriatoScore, 28.8 ± 3.8 SD) than in those dominated

by savanna-grassland (Cerrado and Campos Rupestres; mean TriatoScore, 25.1 ± 3.3 SD) or, especially, by the moist-forest ecoregions along the coast and on coastal ranges (16.7 ± 5.1 SD; Fig. 4).

Bahia's SESAB official disease-risk stratification and our TriatoScore-based entomological-risk stratification agreed well (Gwet's $AC_2 = 0.81 \pm 0.02$ SE), with no instances of disagreement involving high/low or low/high risk strata (Table 3). However, TriatoScore values suggest that the official classification may be underestimating risk in 42 municipalities (Table 3). Importantly, out of 152 municipalities failing to report triatomines to SESAB in 2006–2019, two (Ibiassucé and Lajedinho) had TriatoScores corresponding to high entomological risk and 71 had TriatoScores corresponding to moderate entomological risk (Additional file 1: Table S8). Figure 5 shows entomological-risk strata when TriatoScores are computed using data on all vector species (main dataset) vs. data on native species only; as summarized in Table 3, agreement with Bahia's SESAB official disease-risk classification was again substantial (Gwet's $AC_2 = 0.79 \pm 0.02$ SE). These results suggest that Bahia's native triatomines (Tables 1, 2) can maintain high to moderate risk of vector-borne Chagas disease in 318 (76.3%) of the state's municipalities (Fig. 5b, Table 3; Additional file 1).

The straightforward derivation of "TriatoScore-plus" values illustrates the potential of our approach to integrate diverse data sources—in our example, data on municipality-level housing quality. This particular extra weight reduces TriatoScores by an amount that is proportional to the share of good-quality houses in each municipality. In our case-study, reductions ranged from nil for five municipalities where that share was zero to

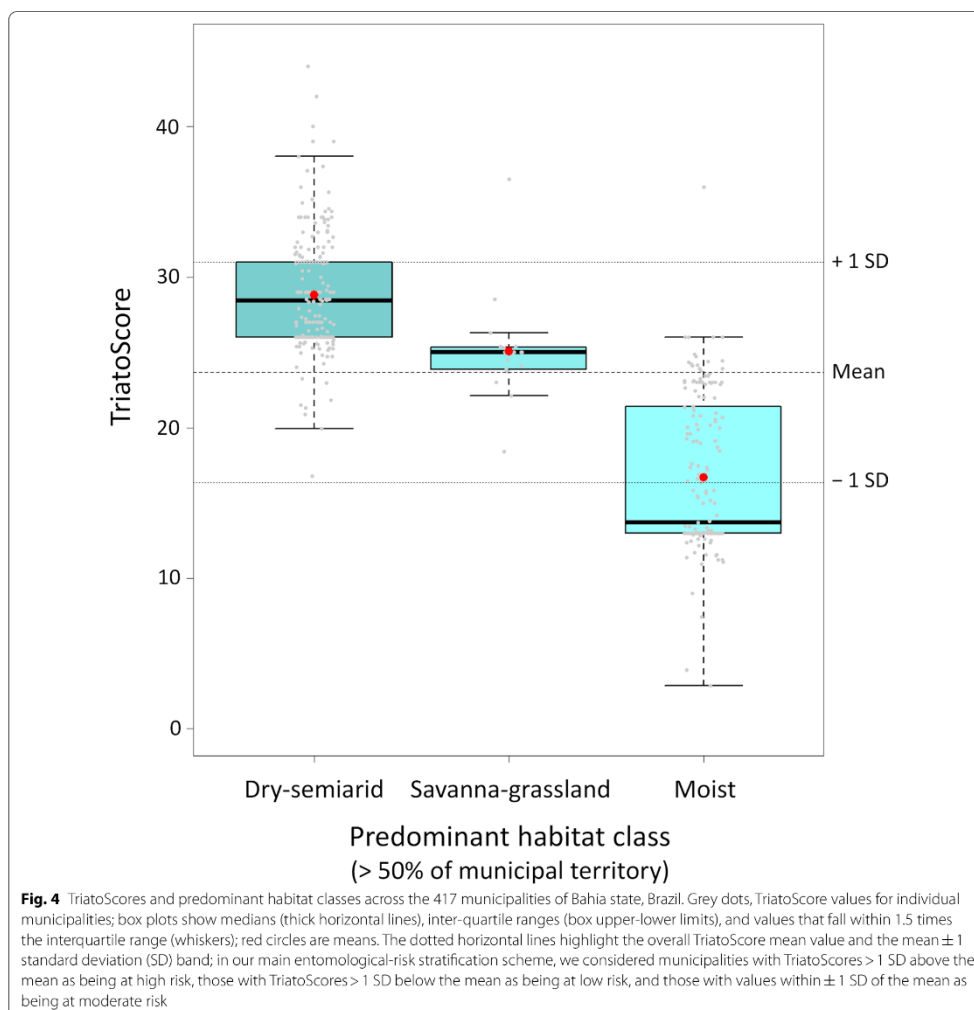


75.5% for two municipalities (including the state's capital) were most houses were in good condition (see Fig. 6 and Additional file 1: Table S7). Table 4 shows the cross-classification of municipalities into risk strata based on standardized TriatoScore and TriatoScore-plus values. Note how one municipality that was at high entomological risk after TriatoScore moved to the low-risk stratum when housing conditions were considered; this reflects the fact that just 33.7% of houses were in poor or very poor condition in this municipality—Paulo Afonso, which on the other hand has a dangerous vector fauna including *T. juazeirensis/brasiliensis*, *T. sordida*, *T. pseudomaculata*, *Panstrongylus megistus*, or *P. lutzii* (Additional file 1: Tables S1, S2, S5, S7). As with TriatoScore (Table 3), TriatoScore-plus suggests that the official SESAB stratification may in some cases underestimate risk—27 and 13 municipalities that SESAB suggests are at low and moderate disease risk are classified as being

at moderate and high risk, respectively, when standardized TriatoScore-plus values are used to define risk strata (Table 4). Overall, Figs. 5 and 6 show how entomological-risk patterns become less tightly associated with biogeographic-ecological features as socioeconomic information is taken into account.

Discussion

TriatoScore is a single-figure measure of Chagas disease entomological risk that (i) covers both native and non-native triatomine bug species, (ii) tackles the issue of locally incomplete vector-occurrence records, and (iii) is designed to support decision-making at the spatial scale most relevant to decentralized control-surveillance systems. Our approach leverages the best available knowledge on the ecology-behavior and distribution-biogeography of individual triatomine bug species to compute local (e.g., municipal) TriatoScores, which can



in turn be used to stratify and map entomological risk over larger spatial units. When based on standardized TriatoScores, risk stratification becomes a fundamentally dynamic exercise—changes in local vector faunas (or in our knowledge about them) are automatically accounted for as local spatial units are reassigned to risk strata relative to the recalculated average risk across all units in the region of interest. Although the most basic version of TriatoScore uses only vector data, integrating

epidemiological, demographic, environmental, or operational information is straightforward. TriatoScore may hence become a useful addition to the Chagas disease vector control-surveillance toolbox.

Our approach draws primarily on the hierarchical working classification of Chagas disease vectors put forward by one of us with the suggestion that it was not only simple and biologically sound, but also potentially useful [20] see also [39]. Here, we illustrate how this idea can be

Table 3 Agreement between the entomological-risk stratification based on TriatoScore and the official Chagas disease-risk stratification of the state's health department (SESAB) across the 417 municipalities of Bahia state, Brazil

	SESAB (disease risk)			Total	%
	Low	Moderate	High		
All species ^a					
TriatoScore (entomological risk)					
Low	76	21	0	97	23.2
Moderate	25	176	44	245	58.8
High	0	17	58	75	18.0
Total	101	214	102	417	100
%	24.2	51.3	24.5	100	
Native species ^b					
TriatoScore (entomological risk)					
Low	78	21	0	99	23.7
Moderate	23	171	50	244	58.5
High	0	22	52	74	17.8
Total	101	214	102	417	100
%	24.2	51.3	24.5	100	

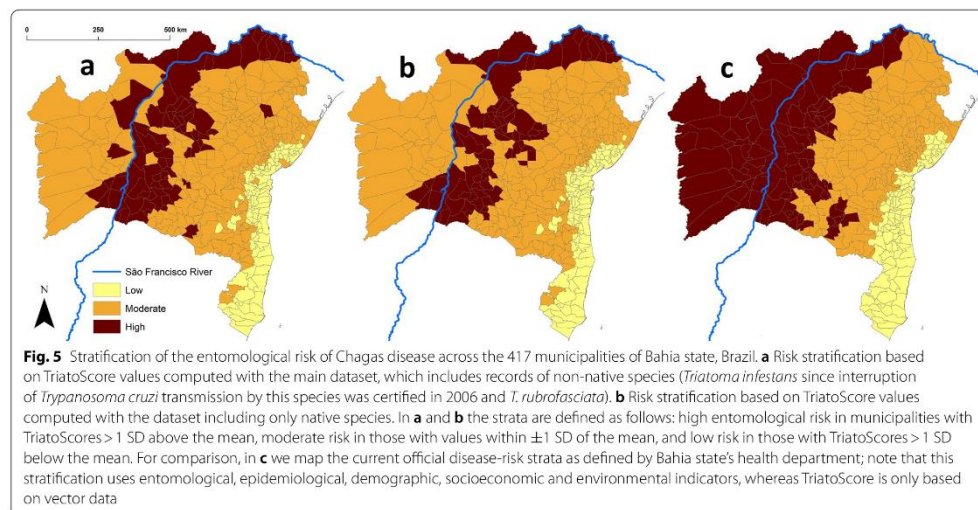
^a Estimate of agreement, TriatoScore—all species vs. Bahia state: Gwet's AC₂ = 0.81 ± 0.02 SE

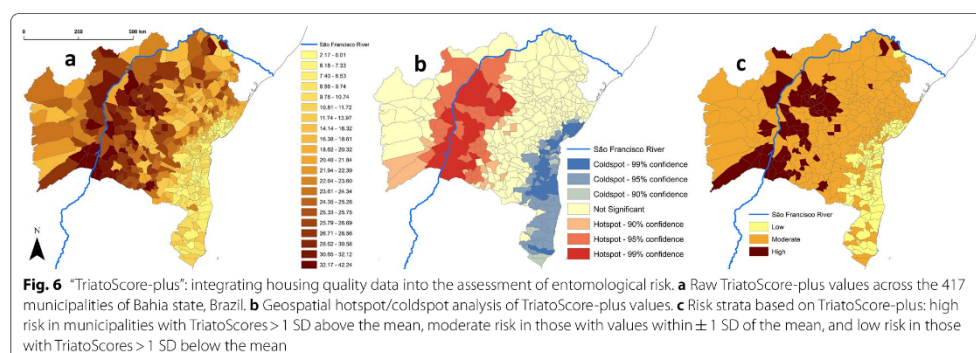
^b Estimate of agreement, TriatoScore—native species vs. SESAB: Gwet's AC₂ = 0.79 ± 0.02 SE

put to work in practice. At the highest level of the hierarchy [20], two triatomine bug species recorded in Bahia are non-native to the state. *Triatoma infestans* is the most dangerous domestic vector of Chagas disease [7, 11,

12, 19, 20, 24, 25], and was hence given the highest “species relevance score” (Table 1). *Triatoma rubrofasciata* is strongly associated with rats of the genus *Rattus*, among which it transmits *Trypanosoma conorhini*; although it can also support *T. cruzi* infections, this originally Asian species has limited relevance as a vector of Chagas disease [17, 24–26, 53], and its “species relevance score” is therefore much lower (Table 1). Importantly, these non-native species can and should be targeted for local elimination, and this critical operational consideration sets them apart from the species that are locally native [12, 20, 39]. Native species were given “species relevance scores” ranging from 4 for those known to often breed inside and/or around houses (*T. juazeirensis/brasiliensis*, *T. pseudomaculata*, *T. sordida*, *T. lenti/bahiensis* and *P. megistus*) to 1 for those that have only rarely been found invading human dwellings (e.g., *Rhodnius domesticus* or *Parabelminus yurupucu*) (see Table 1 and [7, 11, 12, 14–17, 19–21, 24–34, 39]). While the values we chose for scoring are admittedly arbitrary, they reflect our best knowledge about the epidemiological relevance of each species—and, most importantly, the scores' relative sizes reflect the species' relative relevance [20]. In practice, the initial step of our approach thus entails eliciting expert opinion on the relevance of each triatomine bug species known to be present in the region of interest, with special attention paid to *relative* relevance.

The second general requirement was to map each species' occurrence at the scale of municipalities. We used vector-presence records generated by local surveillance





systems as our main data source, yet as many as 152 municipalities (36.5%) did not produce any record over the period 2006–2019—and the tally remains at 125 (or 30%) when considering the two decades since 1999 (Additional file 1: Table S8). This, of course, could not be taken to mean that triatomines do not occur (and enter houses) in any of those municipalities; rather, the absence of records almost certainly springs from the imperfect functioning of entomological surveillance [13–15, 21]. To fill in the spurious blanks in the species-by-municipality matrices and maps, we complemented this dataset with

Table 4 Agreement between risk stratification schemes based on TriatoScore-plus (which integrates housing quality data) and TriatoScore (only entomological data), and the official Chagas disease-risk classification of the state's health department (SESAB) across the 417 municipalities of Bahia state, Brazil

	TriatoScore-plus			Total	%
	Low	Moderate	High		
TriatoScore ^a					
Low	76	21	0	97	23.3
Moderate	21	211	13	245	58.8
High	1	25	49	75	18.0
Total	98	257	62	417	100
%	23.5	61.6	14.9	100	
SESAB ^b					
Low	74	27	0	101	24.2
Moderate	24	177	13	214	51.3
High	0	53	49	102	24.5
Total	98	257	62	417	100
%	23.5	61.6	14.9	100	

^a Estimate of agreement, TriatoScore-plus vs. TriatoScore: Gwet's $AC_2 = 0.86 \pm 0.01$ SE

^b Estimate of agreement, TriatoScore-plus vs. SESAB: Gwet's $AC_2 = 0.79 \pm 0.02$ SE

records from the literature, including (i) actual occurrence records that we could map to a municipality, (ii) species distribution maps derived from ecological niche models, and (iii) the best available knowledge about the ecoregional biogeography of each species (Table 2) [17, 27–34, 40–45].

This mapping procedure was straightforward for most species, but somewhat challenging for a few (see Table 2 and its footnotes). First, some species are local endemics restricted to a specific subarea within an ecoregion. For example, *T. sherlocki* seems to be endemic to the Jacaré-Verde basin and the ranges that bound it [17, 30–33, 40] (Fig. 1), and *T. melanica* does not seem to extend into the Cerrado to the northwest of Bahia [17, 30–33, 40, 45] (see Table 2 and Additional file 2: Figures S13, S17). Second, we found that some species occur in municipalities with supposedly unsuitable ecoregional ecologies. For example, *T. tibiamaculata*, *R. neglectus*, *R. domesticus* and *Pa. yurupucu* have been recorded in municipalities with 100% of the land classified as Caatinga (Table 2). Most such cases are readily explained by the relatively low spatial resolution of available ecoregional classifications—suitable habitat is likely present in small patches not captured by our coarse-scale ecoregion map (Fig. 1). To reflect this “marginal occurrence”, we assigned a 0.001 value to the “weighted presence” of *T. tibiamaculata*, *P. lutzi*, *R. domesticus* and *Pa. yurupucu* in municipalities with this kind of mismatch between records and ecoregions (see Table 2 and [17, 34]). The case of *R. neglectus* appears to be different—local populations of this species, which is primarily from the Cerrado [17, 24, 25, 34, 42, 43], seem to have adapted to drier Caatinga environments in northern-central Bahia [32, 33, 42] (see Table 2 and Additional file 2: Figure S11). Finally, although *P. megistus* is primarily a moist-forest species, wild populations are also common in drier ecoregions including the

Cerrado and Caatinga, where they occupy gallery forests and other moister-habitat patches [17, 29]; again, our ecoregional assessment does not capture such fine-scale environmental heterogeneity. The full set of species-specific “weighted presence” maps is presented in Additional file 2: Figures S3–S24, and the data used to build them are available in Additional file 1: Tables S1–S7.

TriatoScore values were overall higher in municipalities dominated by dry-to-semiarid ecoregions, lower in municipalities where moister forests dominate, and intermediate in municipalities dominated by seasonally dry savanna-grassland (Fig. 4). The higher entomological risk in the Caatinga and Atlantic dry forest reflects both a particularly high triatomine species richness (Table 2) and the fact that many of those species are often found infesting or invading houses (Table 1) [7, 11, 12, 15, 17, 19, 24–34, 40–45]. While the eastern portion of the Cerrado that covers western Bahia is also fairly species-rich (Table 2), at least three of the 11 triatomine bug species occurring there (*Panstrongylus diasi*, *Cavernicola pilosa* and *Psammolestes tertius*) are seldom found in or around houses (Table 1) [7, 11, 12, 17, 24–34, 40–45]. In Bahia, the species-rich Caatinga, Atlantic dry forest and Cerrado meet along the São Francisco River valley (Fig. 1), and TriatoScore mapping revealed a pattern of higher entomological risk in that region (Fig. 3). In contrast, the Campos Rupestres montane savannas of the Serra da Mangabeira (Fig. 1) are home to just three triatomine bug species (Table 2) [17, 27–33], and TriatoScore values were accordingly low in ten municipalities (with ~10% to ~50% of territory corresponding to Campos Rupestres) located along a southeast-northwest diagonal, narrow strip in the center of the state (Figs. 1, 3). Finally, 6 of the 10 triatomine bug species known to occur in the moister coastal ecoregions do not seem able to stably infest houses (Tables 1, 2) [7, 11, 12, 17, 24–34]. Therefore, TriatoScore values are particularly low along the central and southern coast of Bahia (Fig. 3). Geospatial analyses confirmed these patterns by showing (i) a clear-cut hotspot of higher entomological risk in municipalities along the São Francisco valley and on the ranges that bound it (particularly to the east and north), (ii) a clear-cut coldspot of lower entomological risk in municipalities along the central-southern coast, and (iii) two separate areas of nonsignificant clustering of TriatoScore values: (a) the Cerrado-dominated western region and (b) the Caatinga-dominated region east of the central uplands (the Serra do Angelim-Chapada Diamantina-Serra da Mangabeira-Serra do Espinhaço complex) plus the overall drier northern coast (Figs. 1, 3).

One particularly attractive feature of the TriatoScore approach is that it is fundamentally dynamic. This can be illustrated with a hypothetical example. Suppose that

in some year in the near future (i) *T. infestans* infestation foci are discovered in two municipalities where the species was historically present but from where it was not reported since 2006 (say, Abaré and Anagé), and (ii) intensive but negative searches strongly suggest that *T. rubrofasciata* is no longer present in two municipalities where it was recorded in the past (say, Glória and Jus-siape). In Additional file 1: Table S9 we show how these changes can swiftly be incorporated into an exercise of entomological-risk assessment and stratification—by typing the new “species relevance score” values into the appropriate cells (here, “10.0” in cells B4 and B15, and “0.0” in cells C141 and C224; highlighted in red font in Additional file 1: Table S9), TriatoScores are automatically updated and standardized and risk strata automatically recalculated (see columns Z to AC in Additional file 1: Table S9). To provide a real-life (if retrospective) example of TriatoScore’s flexibility, we examined how the historical elimination of non-native *T. infestans* from 125 municipalities [27–33] changed entomological-risk patterns across Bahia. We found that TriatoScores were reduced by an average of 26.5% (range, 20.4–43.5%) in those municipalities; declines were steeper in municipalities where the “baseline” entomological risk brought about by native vectors is lower (see Additional file 1: Table S10). A further example (this time prospective) is our evaluation of “baseline” risk—what would be the patterns of Chagas disease entomological risk if the two non-native vector species still found in the state were finally eliminated (Figs. 2, 3; Additional file 1: Tables S3, S6, S10). In general, thus, our approach can swiftly incorporate new data on local-scale vector-species distribution. Note also that to quickly identify municipalities where risk is *much* higher or *much* lower than average one just needs to tinker with the threshold used to define risk strata. For example, a ± 1.5 SD criterion highlights 14 municipalities with very high, and 20 with very low, entomological risk (Additional file 1: Table S11).

Finally, we again draw attention to the fact that the basic version of TriatoScore we have presented uses only vector data, yet Chagas disease transmission risk depends on a constellation of social, cultural, economic, demographic, ecological, environmental, political, and operational determinants [7, 23, 24, 39, 54–56]. For example, and as Carlos Chagas vividly described in his 1909 paper [57], poor-quality housing sets the stage for frequent contact between vectors and humans [58]. It would therefore be of interest to develop a more elaborate version of TriatoScore in which metrics describing further potential determinants of transmission could be incorporated (see, e.g., [39]). As we have shown with our computation of municipal “TriatoScore-plus” values, this is straightforward enough—all that is needed is a set of weights, one

for each putative determinant, derived from measurements taken at the scale of interest (Fig. 6). We chose to illustrate this procedure with a municipality-level measure of housing conditions, but many other possibilities could merit consideration [39]. A non-exhaustive list of examples might include local-scale measures of (i) the frequencies of dwelling infestation with triatomines or of *T. cruzi* infection in humans or vectors; (ii) the levels of poverty or human development; (iii) the patterns and dynamics of land-use change and deforestation; (iv) the demographics of urban/rural populations; or (v) whether control-surveillance systems are adequately funded, staffed, and operated in each municipality.

Conclusions

Chagas disease is caused by a multi-host zoonotic pathogen transmitted by over 100 locally native vector species, many of which persistently invade and infest houses from the USA to Argentina [7, 11, 12, 17, 19]. Completely interrupting *T. cruzi* transmission is therefore unfeasible [11, 12]. Instead, decades of hard-won experience clearly indicate that disease control, defined as the “reduction of [...] incidence, prevalence, morbidity and/or mortality to a locally acceptable level” [59], is the most that we can aim for in the Americas [11]. Long-term vector control-surveillance has a major role to play in disease control [11, 12, 14], and entomological-risk stratification and mapping is and will remain a key tool for local program managers [11, 12, 20]. TriatoScore provides a sound, dynamic, and flexible means to this end—and one that, moreover, can be easily extended to integrate operationally relevant information beyond vector data.

Abbreviation

SESAB: Bahia's state health department.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-021-04954-5>.

Additional file 1: **Table S1.** Vector presence and species richness (columns AB, AC). **Table S2.** Ecoregions and housing quality. **Table S3.** “Weighted presence” (native vectors). **Table S4.** “Weighted scores” and “TriatoScores” (column Z). **Table S5.** “TriatoScores” and risk strata (with *Triatoma infestans* since 2006). **Table S6.** “TriatoScores” and risk strata (native species only). **Table S7.** “TriatoScore-plus” values and risk strata. **Table S8.** Municipalities with no vector records from surveillance (red font, 1999–2019; black font, 2006–2019). **Table S9.** Hypothetical scenario: “weighted scores” and “TriatoScores” (column Z). **Table S10.** “TriatoScore” changes with the elimination of *Triatoma infestans*. **Table S11.** “TriatoScores” and extreme-risk strata (± 1.5 SD criterion) (with *T. infestans* since 2006). Triatomine bug species occurrence based on routine control-surveillance records (SESAB, 1999–2019) and published reports [17, 27–29, 29–31, 31–34, 40–45].

Additional file 2: **Figure S1.** *Triatoma infestans* in Bahia, Brazil (since 2006). **Figure S2.** *Triatoma rubrofasciata* in Bahia, Brazil (as reported).

Figure S3. *Triatoma juazeirensis/brasiliensis* in Bahia, Brazil (“weighted presence”). **Figure S4.** *Triatoma sordida* in Bahia, Brazil (“weighted presence”). **Figure S5.** *Triatoma pseudomaculata* in Bahia, Brazil (“weighted presence”). **Figure S6.** *Panstrongylus megistus* in Bahia, Brazil (“weighted presence”). **Figure S7.** *Triatoma lenti/bahiensis* in Bahia, Brazil (“weighted presence”). **Figure S8.** *Triatoma vitticeps* in Bahia, Brazil (“weighted presence”). **Figure S9.** *Triatoma costalimai* in Bahia, Brazil (“weighted presence”). **Figure S10.** *Panstrongylus lutzi* in Bahia, Brazil (“weighted presence”). **Figure S11.** *Rhodnius neglectus* in Bahia, Brazil (“weighted presence”). **Figure S12.** *Rhodnius nasutus* in Bahia, Brazil (“weighted presence”). **Figure S13.** *Triatoma melanica* in Bahia, Brazil (“weighted presence”). **Figure S14.** *Triatoma tibi-amaculata* in Bahia, Brazil (“weighted presence”). **Figure S15.** *Panstrongylus geniculatus* in Bahia, Brazil (“weighted presence”). **Figure S16.** *Triatoma petrocchiai* in Bahia, Brazil (“weighted presence”). **Figure S17.** *Triatoma sherlockii* in Bahia, Brazil (“weighted presence”). **Figure S18.** *Triatoma melanocephala* in Bahia, Brazil (“weighted presence”). **Figure S19.** *Panstrongylus diasi* in Bahia, Brazil (“weighted presence”). **Figure S20.** *Panstrongylus lenti* in Bahia, Brazil (“weighted presence”). **Figure S21.** *Cavernicola pilosa* in Bahia, Brazil (“weighted presence”). **Figure S22.** *Rhodnius domesticus* in Bahia, Brazil (“weighted presence”). **Figure S23.** *Psammolestes tertius* in Bahia, Brazil (“weighted presence”). **Figure S24.** *Parabulminius yurupucu* in Bahia, Brazil (“weighted presence”). Triatomine bug species occurrence based on routine control-surveillance records (SESAB, 1999–2019) and published reports [17, 27–29, 29–31, 31–34, 40–45].

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Authors' contributions

GR-Jr, FA-F, RG-G and MGR conceived the study. MGR raised funds and administered the project. GR-Jr, FA-F, RBR, RG-G and MGR contributed to the design of the methods. GR-Jr, FA-F, CMMC, GMC, OMFS, CGSS, EOLF and RFS performed research. GR-Jr, RBR, CMMC, GMC, OMFS, CGSS, and RFS obtained and organized databases. GR-Jr, FAF and RG-G curated the dataset and analyzed the data. GR-Jr, FA-F, RG-G and MGR drafted the first version of the manuscript. All authors contributed to the interpretation of results, read and commented on manuscript drafts, and approved the final version.

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Availability of data and materials

All data are available in the article and its Additional files.

Declarations

Ethics approval and consent to participate

The project was reviewed and approved by the Research Ethics Committee of the Gonçalo Moniz Institute—Fiocruz Bahia, Ministry of Health, Brazil (# 2.552.284).

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest associated with this study.

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5.3. ARTIGO 3 – *Triatomine fauna in the state of Bahia, Brazil: What changed after 40 years of the vector-control program?*

Neste capítulo são apresentados os resultados relativos aos objetivos específicos:

- a) Determinar os vetores de *T. cruzi* já registrados na Bahia.
- b) Discriminar quais municípios registraram triatomíneos e analisar a distribuição geográfica das espécies em dois períodos: 1957-1971 e 2006-2019.
- c) Analisar a abundância relativa das espécies de triatomíneos em ambiente intra e peridomiciliar nos municípios da Bahia entre 2006 e 2019;
- d) Identificar as espécies mais relevantes em ambos os períodos analisados.

O artigo científico em questão está apresentado integralmente a seguir, compondo a presente seção, e foi publicado na Revista da Sociedade Brasileira de Medicina Tropical nº55(e0732-2021) em abril de 2022, sob título: ***Triatomine fauna in the state of Bahia, Brazil: What changed after 40 years of the vector-control program?*** DOI: <https://doi.org/10.1590/0037-8682-0732-2021>.

Major Article

Triatomine fauna in the state of Bahia, Brazil: What changed after 40 years of the vector-control program?

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ABSTRACT

Background: Neglected tropical diseases are a growing threat to global health, and endemic Chagas disease has emerged as one of the most important health problems in America. The main strategy to prevent *Trypanosoma cruzi* transmission is chemical control of vectors. This study presents a descriptive analysis of synanthropic triatomines before and after the implementation of a vector-control program in Bahia, Brazil.

Methods: Descriptive analysis and geospatial statistics were performed on triatomine data, (1) the relative abundance and (2) proportional spatial distribution, from Bahia during two periods: (A) 1957 to 1971 and (B) 2006 to 2019.

Results: We observed a decrease in the relative abundance of *Panstrongylus megistus* (A: n=22.032, 61.9%; B: n=1.842, 1.0%) and *Triatoma infestans* (A: n=1.310, 3.7%; B: n=763, 0.43%), as well as an increase in the relative abundance of *T. sordida* (A: n=8.314, 23.4%; B: n=146.901, 81.6%) and *T. pseudomaculata* (A: n=894, 2.5%; B: n=16.717, 9.3%).

Conclusions: Our results indicate a clear reduction in the occurrence of *P. megistus* and *T. infestans* (last record in 2015) and an increase in the relative abundance and geographical distribution of *T. sordida* and *T. pseudomaculata* after 40 years of the vector-control program. The high frequency of other triatomine species in the municipalities of the state of Bahia and their abundance in recent years highlight the need to reinforce permanent entomological surveillance actions to prevent Chagas disease.

Keywords: Triatominae. Northeastern Brazil. *Trypanosoma cruzi*. Chagas disease. Public health.

*Mitermayer Galvão dos Reis and Rodrigo Gurgel-Gonçalves are joint senior authors.

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Authors' contribution: GR-Jr, RG-G, and MGR conceived the study. MGR raised funds and administered the project. GR-Jr, RG-G, MGR, contributed to the design of trial methods. RFA, CMMC, GMC, FCL, DLPM, OMFS, CGSS, EOLF, RFS, and RBR performed research. GR-Jr and MGR supervised students involved in field and laboratory research. CMMC, RFA, GMC, OMFS, CGSS, RFS obtain the databases, GR-Jr curated the dataset and analyzed the data. GR-Jr, RG-G, and MGR drafted the first version of the manuscript. All authors contributed to the interpretation of results, read and commented on manuscript drafts, and approved the final version.

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INTRODUCTION

Triatomines are blood-sucking insects (Hemiptera: Reduviidae) that are vectors of the parasite *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas disease, also known as American trypanosomiasis. Some synanthropic triatomine species adapt to anthropic changes within their natural landscape, colonizing the household environment (animal breeding sites) and occasionally inside houses, increasing the risk of *T. cruzi* transmission^{1,2}. Many triatomines invade houses, but few can initiate the processes of colonization and domiciliation, which depend on the characteristics of the invasive species^{3,4}, the invaded dwelling^{5,6} and the environment around the household⁷.

In Brazil, some triatomine species have succeeded in occupying the domestic environment and expanding their spatial distribution beyond their original biomes⁸ through active and passive dispersal^{9,10}. One of these species is *Triatoma infestans* (Klug, 1835), which occupied household units among 12 states of Brazil and was considered the main vector involved in *T. cruzi* transmission in Brazil^{11,12}. After the first standardized chemical-based control actions were implemented by the National Chagas Disease Control Program in 1975 and by the integrated initiatives of the Southern Cone of Latin America to eliminate *T. infestans* in 1991, a sustained decrease in domestic populations of vectors was observed¹³⁻¹⁵. In 2006, Brazil received certification of interruption of Chagas disease transmission by *T. infestans* from the Pan American Health Organization (PAHO) and the World Health Organization (WHO)¹⁶. However, many regions of Brazil, such as the state of Bahia, still have several species of native vectors that can transmit *T. cruzi* to humans and domestic animals.

Pirajá da Silva initially described the existence of triatomines in the state of Bahia in 1911, shortly after *T. cruzi* was described by Carlos Chagas¹⁷. Pirajá da Silva identified *Conorhinus megistus* triatomines - syn. *Panstrongylus megistus* (Burmeister, 1835) - in the city of Mata de São João, near Salvador, the capital city of Bahia. Pirajá da Silva also identified triatomines among the cities of Feira de Santana, Candeias, São Francisco do Conde, and Salvador¹⁸. Almost 40 years after Pirajá's initial research description, Chagas disease was recognized as a serious health problem in the state of Bahia, where triatomines were captured simultaneously to the record of autochthonous cases of Chagas heart disease in Salvador¹⁹. *P. megistus* and *Triatoma rubrofasciata* (De Geer, 1773) naturally infected by *T. cruzi* were found at the historic center of Salvador city, associated with human cases, which motivated a chemical control campaign²⁰. Nevertheless, some foci of *P. megistus* maintained *T. cruzi* transmission to families in the neighborhoods of Salvador²⁰. In the early 1970s, more than 600 specimens of *P. megistus* and *T. rubrofasciata* were examined in Salvador, of which 16% were infected with *T. cruzi*²¹. During this period, efforts were made to identify and better understand the ecology of triatomines in Bahia, and 18 triatomine species were cataloged²².

National campaigns focused on active vector surveillance for the identification of household infestation areas in Brazil and strategies of triatomine vector-control using chemical insecticides (BHC and pyrethroids). The 1983 Brazilian campaign was executed by the Superintendence of Public Health Campaigns (SUCAM) of Brazil's Health Ministry. During this period, new triatomines were recorded, and new species were described²².

Recent epidemiological studies indicate that Bahia had a high prevalence of the human *T. cruzi* infection (0.77% to 2.22%)

compared to the Northeast region of Brazil (0.69% to 0.88%) between 1987 and 1994¹⁵. Data between 2008 and 2017 indicate that Chagas disease mortality rates in the state of Bahia (3.8 to 4.8 deaths/100,000 inhabitants) are the highest among the northeastern states and the fourth highest among all Brazilian states. Moreover, two deaths were registered in children younger than one year old, indicating acute cases and risk of domestic *T. cruzi* transmission²⁷.

Currently, 26 triatomine species have been recognized in Bahia²⁵⁻²⁶. Several factors can modify the spatial distribution and abundance of synanthropic triatomines. To understand the changes in *T. cruzi* vector occurrence, the use of retrospective epidemiological and entomological data has become a relevant strategy. In this study, we describe the relative abundance and proportional spatial distribution of synanthropic triatomines at the municipal level before (1957 to 1971) and after (2006 to 2019) the vector-control program implementation in the state of Bahia, which is one of the states with the greatest diversity of triatomines and highest rates of epidemiological information related to Chagas disease in northeastern Brazil.

METHODS

The state of Bahia has 417 municipalities and is located in the southern region of northeastern Brazil, bordering eight other Brazilian states and the Atlantic Ocean in the east (**Figure 1**). Descriptive analyses of both the spatial and temporal distribution of the synanthropic triatomine species data from Bahia was performed during two periods: (A) from 1957 to 1971, and (B) from 2006 to 2019. Data regarding period A present information on the Chagas disease vector surveillance program of Bahia between 1957 and 1971²², which represents the period before the standardized implementation of systematic vector surveillance programs in Brazil²⁷.

Information regarding period B was obtained from the following state government databases after the certification of interruption of Chagas disease transmission by *T. infestans*: Epidemiologic Surveillance team of the State of Bahia's Health Service (SESAB), Epidemiological Surveillance Office (DIVEP), and Central Public Health Laboratory of Bahia (LACEN/BA)²⁹.

Entomological data were obtained in three different ways: (a) notification of triatomines (surveillance and community participation) performed by the population itself by taking suspicious insects to health agents; (b) notification attendance (active surveillance) performed by the health agents of each municipality, dependent on (a); and (c) active search (active surveillance), in which vectors were searched among household units around the area, regardless of any notification performed by the population.

To compare *T. cruzi* vector species information from both periods of the study, we evaluated: (1) the relative abundance, calculated as the proportion of each triatomine species divided by the total number of triatomines for each period; and (2) proportional spatial distribution, calculated as the proportion of municipalities that recorded a triatomine species divided by the number of sampled municipalities.

Spreadsheets were used to collect the following data: species, municipality, date, geographic information system (GIS) coordinates, sampling environment (intradomestic, peridomestic), and data reference. Graphs and descriptive statistics

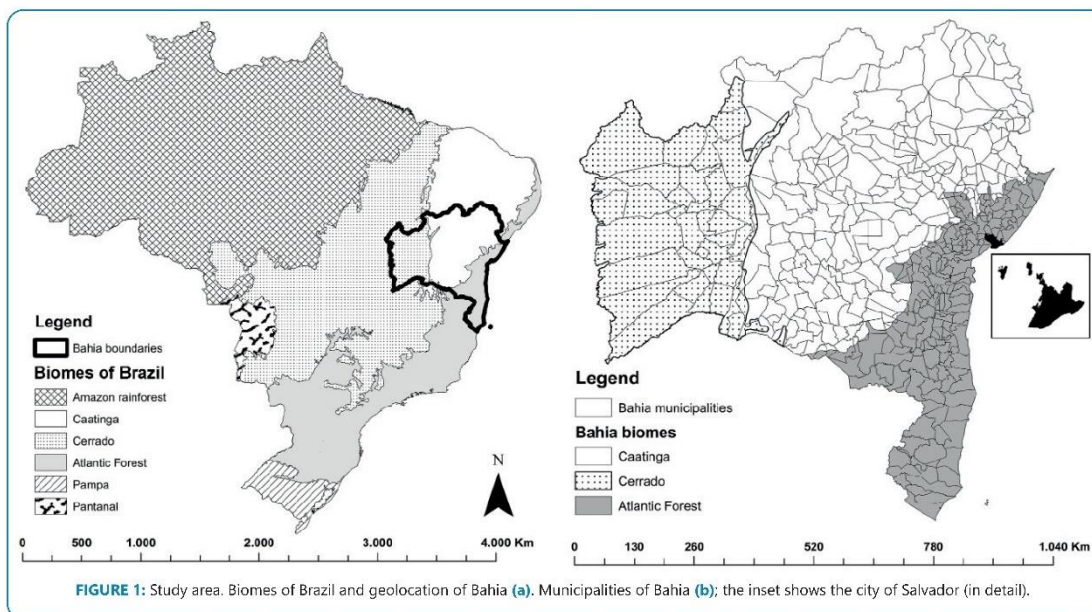


FIGURE 1: Study area. Biomes of Brazil and geolocation of Bahia (a). Municipalities of Bahia (b); the inset shows the city of Salvador (in detail).

were computed using SPSS 24. In the absence of specific GIS information, the coordinates of the city's headquarters from the Brazilian Institute of Geography and Statistics (IBGE) were used³⁰. Data processing was performed using the ArcGis® Software 10.5³¹.

RESULTS

In this study, information regarding 315 municipalities and 215597 triatomines collected during the two periods was evaluated, among which 35588 were from period A (1957-1971) and 180020 were from period B (2006-2019). During period A, data were gathered from 202/290 (71%) municipalities²², and in period B, 258/417 (61.8%) municipalities were evaluated. Regarding the number of triatomine species, 18 and 21 species were sampled during periods A and B, respectively, and 16 of them were sampled during both periods (Table 1).

Figure 2 shows the spatial distribution of triatomines in the municipalities during periods A and B. During period A, *P. megistus* was present in 122 (60.4%) of the sampled municipalities, followed by *T. sordida* (37.1%), *T. pseudomaculata* (19.8%), and *T. infestans* (12.8%). During period B, *T. pseudomaculata* was widely distributed, being recorded in 176 of 258 (68.2%) sampled municipalities, followed by *T. sordida* (61.6%), and *T. brasiliensis* (36.4%) (Figure 3).

In period A, *P. megistus* was the most abundant species (n=22032), representing 61.9% of triatomines, followed by *T. sordida* (23.3%), *T. brasiliensis* species complex (4.3%), and *T. infestans* (3.6%). Conversely, in period B, *T. sordida* represented 81.6% of all collected triatomines, followed by *T. pseudomaculata* (9.2%) and *T. brasiliensis* species complex (6.3%).

By analyzing the spatial distribution of the main triatomine species before and after *T. cruzi* vector-control actions, we

observed a reduction in the municipal occurrence of synanthropic populations of *T. infestans* and *P. megistus*. *T. infestans* was no longer detected in some municipalities in the west (e.g., Santa Maria Vitória, Barreiras) and north (e.g., Juazeiro, Curaçá) of Bahia (Figure 3) and had been first detected among other municipalities in the Caatinga (e.g., Itaguaçu da Bahia, Novo Horizonte) and Atlantic Forest biomes (e.g., Tremedal and Presidente Tancredo Neves). During period B, *T. infestans* was found only at residual foci and was last recorded in 2015 in the municipality of Novo Horizonte (Figure 3).

During period B, we observed a higher occurrence of *T. sordida* in western Bahia, where the Cerrado biome predominates, in south-central Bahia, and in some municipalities of Recôncavo Baiano, eastern Bahia. However, we observed a remarkable change in the spatial distribution of *T. pseudomaculata*, which expanded its area of occurrence among western municipalities (Cerrado biome) and the central region (Caatinga biome) of Bahia (Figure 3).

A comparison of the total number of individuals (n) and the relative abundance (%) of species in periods A and B showed a reduction for *P. megistus* (A: n=22032, 61.9%; B: n=1804, 1.0%) and *T. infestans* (A: n=1310, 3.6%; B: n=763, 0.4%), as well as an increase in the relative abundance of *T. sordida* (A: n=8314, 23.3%; B: n=146901, 81.6%) and *T. pseudomaculata* (A: n=894, 2.5%; B: n=16717, 9.2%). During period B, most triatomines were captured in peridomestic habitats, with a predominance of *T. sordida* (Table 2).

DISCUSSION

This study indicated changes in the spatial distribution and relative abundance of synanthropic triatomines in the state of Bahia, Northeast Brazil, before and after 44 years of structured

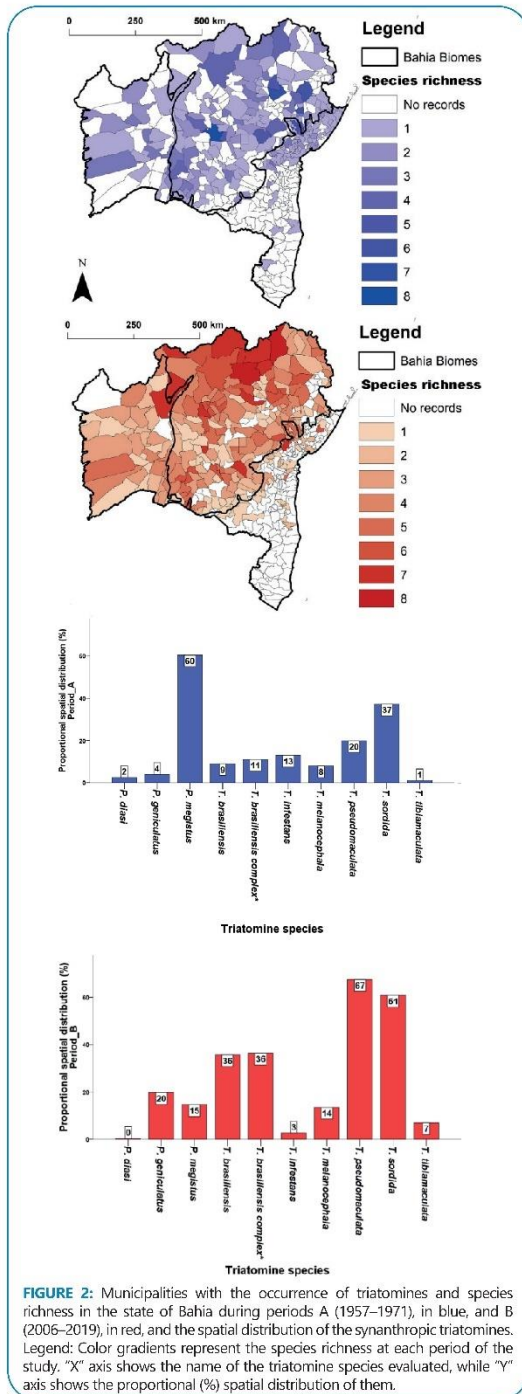


FIGURE 2: Municipalities with the occurrence of triatomines and species richness in the state of Bahia during periods A (1957–1971), in blue, and B (2006–2019), in red, and the spatial distribution of the synanthropic triatomines. Legend: Color gradients represent the species richness at each period of the study. "X" axis shows the name of the triatomine species evaluated, while "Y" axis shows the proportional (%) spatial distribution of them.

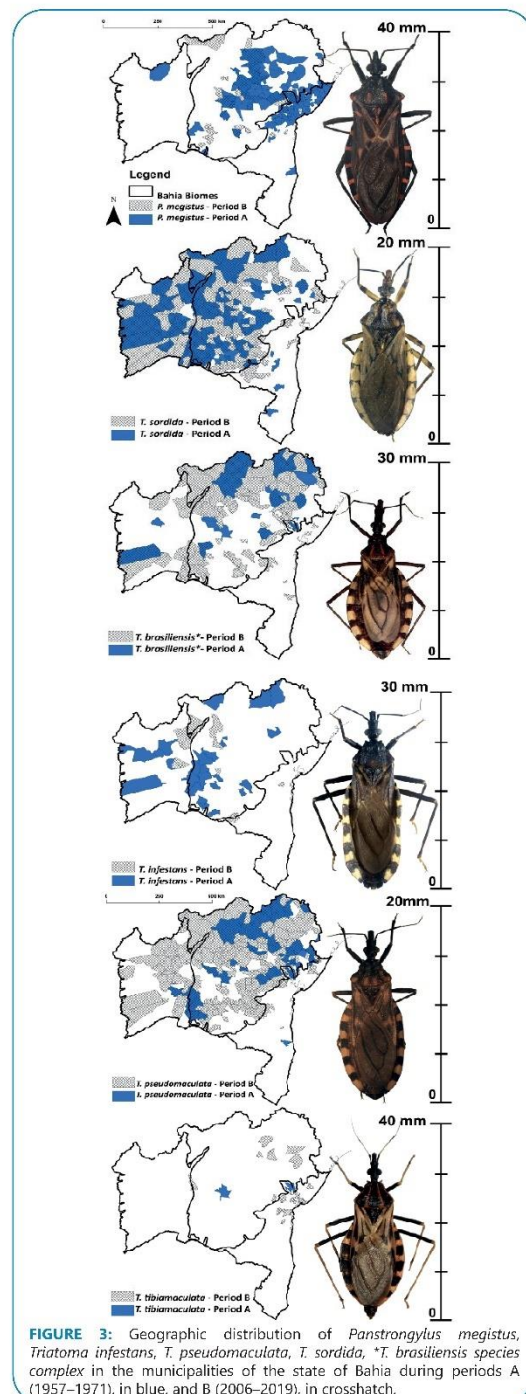


FIGURE 3: Geographic distribution of *Panstrongylus megalus*, *Triatoma infestans*, *T. pseudomaculata*, *T. sordida*, *T. brasiliensis* species complex in the municipalities of the state of Bahia during periods A (1957–1971), in blue, and B (2006–2019), in crosshatch.

TABLE 1: Synanthropic triatomine species from the state of Bahia, Brazil, recorded between 1957-1971 and between 2006-2019.

Triatominae species	Presence		Specimens				Municipalities			
	A (1957-1971)	B (2006-2019)	A (n)	A (%)	B (n)	B (%)	A (n)	A (%)	B (n)	B (%)
<i>Cavernicola pilosa</i> Barber, 1937	*	*	0	-	0	-	0	-	0	-
<i>Panstrongylus lenti</i> Galvão & Palma, 1968;	*	*	0	-	0	-	0	-	0	-
<i>Panstrongylus lutzi</i> (Neiva & Pinto, 1923);	X	X	62	0.17	369	0.20	11	5.45	66	25.58
<i>Panstrongylus megistus</i> (Burmeister, 1835);	X	X	22032	61.93	1842	1.02	122	60.40	38	14.73
<i>Panstrongylus diasi</i> Pinto & Lent, 1946;	X	X	17	0.05	6	0.00	5	2.48	1	0.39
<i>Panstrongylus geniculatus</i> (Latreille, 1811);	X	X	29	0.08	155	0.09	8	3.96	51	19.77
<i>Parabelminus yurupucu</i> Lent & Wygodzinsky, 1979	*	*	0	-	0	-	0	-	0	-
<i>Psammolestes tertius</i> Lent & Jurberg, 1965;	X	X	836	2.35	39	0.02	8	3.96	2	0.78
<i>Rhodnius domesticus</i> Neiva & Pinto, 1923;	*	*	0	-	0	-	0	-	0	-
<i>Rhodnius nasutus</i> Stål, 1859		*	0	-	16	0.01	0	-	0	-
<i>Rhodnius neglectus</i> Lent, 1954;	X	X	1	0.00	100	0.06	1	0.50	21	8.14
<i>Triatoma bahiensis</i> Sherlock & Serafim, 1967;	X		5	0.01	0	-	1	0.50	0	-
<i>Triatoma brasiliensis</i> Neiva, 1911;	X	X	1405	3.95	11054	6.14	18	8.91	92	35.66
<i>Triatoma costalimai</i> Verano & Galvão, 1958;	X	X	4	0.01	2	0.00	1	0.50	1	0.39
<i>Triatoma infestans</i> (Klug, 1834);	X	X	1310	3.68	763	0.42	26	12.87	7	2.71
<i>Triatoma juazeirensis</i> Costa & Felix, 2007;		X	0	-	225	0.12	0	-	8	3.10
<i>Triatoma lenti</i> Sherlock & Serafim, 1967;	X	X	56	0.16	226	0.13	3	1.49	2	0.78
<i>Triatoma melanica</i> Costa, Argolo & Felix, 2006;		X	0	-	19	0.01	0	-	1	0.39
<i>Triatoma melanocephala</i> Neiva & Pinto, 1923;	X	X	68	0.19	233	0.13	16	7.92	35	13.57
<i>Triatoma pessoai</i> Sherlock & Serafim, 1967;	X**		52	0.15	0	-	2	0.99	0	-
<i>Triatoma petrocchiai</i> Pinto & Barreto, 1925;	X	X	16	0.04	1	0.00	1	0.50	1	0.39
<i>Triatoma pseudomaculata</i> Corrêa & Espínola, 1964;	X	X	894	2.51	16717	9.29	40	19.80	174	67.44
<i>Triatoma rubrofasciata</i> (De Geer, 1773);	X	X	474	1.33	6	0.00	2	0.99	2	0.78
<i>Triatoma sherlocki</i> Papa, Jurberg, Carcavallo, Cerqueira & Barata, 2002		X***	0	-	323	0.18	0	-	1	0.39
<i>Triatoma sordida</i> (Stål, 1859);	X	X	8314	23.37	146901	81.60	75	37.13	157	60.85
<i>Triatoma tibiamaculata</i> (Pinto, 1926);	X	X	2	0.01	985	0.55	2	0.99	18	6.98
<i>Triatoma vitticeps</i> (Stål, 1859);		X	0	-	38	0.02	0	-	2	0.78
<i>Triatoma brasiliensis</i> spp#	X	X	1534	4.31	11848	6.58	22	10.89	94	36.43
TOTAL	-	-	35577	100.00	180020	100.00	202	100.00	258	100.00

*Species have already been recorded in Bahia during another period. **Species today is considered synonymous with *T. lenti*. ***Captured by health agents in a wild environment. #*Triatoma brasiliensis* complex.

TABLE 2: Synanthropic triatomines collected in the state of Bahia, Brazil, by species and collection environment between 2006 and 2019.

Species	Intradomestic		Peridomestic		Not informed		Total	
	n	%	n	%	N	%	N	%
<i>Panstrongylus diasi</i>	6	0.04	0	0	0	0	6	0
<i>P. geniculatus</i>	64	0.41	43	0.03	48	1.01	155	0.09
<i>P. lutzii</i>	186	1.18	103	0.06	80	1.68	369	0.21
<i>P. megistus</i>	292	1.86	1435	0.9	115	2.41	1842	1.03
<i>Psammolestes tertius</i>	0	0	14	0.01	25	0.52	39	0.02
<i>Rhodnius nasutus</i>	4	0.03	0	0	12	0.25	16	0.01
<i>R. neglectus</i>	22	0.14	66	0.04	12	0.25	100	0.06
<i>Triatoma brasiliensis</i>	4281	27.23	6639	4.17	134	2.81	11054	6.15
<i>T. costalimai</i>	2	0.01	0	0	0	0	2	0
<i>T. infestans</i>	104	0.66	642	0.4	17	0.36	763	0.42
<i>T. juazeirensis</i>	141	0.9	42	0.03	42	0.88	225	0.13
<i>T. lenti</i>	19	0.12	197	0.12	10	0.21	226	0.13
<i>T. melanica</i>	0	0	19	0.01	0	0	19	0.01
<i>T. melanocephala</i>	32	0.2	4	0	197	4.13	233	0.13
<i>T. petrocchiai</i>	1	0.01	0	0	0	0	1	0
<i>T. pseudomaculata</i>	2348	14.94	13632	8.56	737	15.45	16717	9.3
<i>T. rubrofasciata</i>	4	0.03	0	0	2	0.04	6	0
<i>T. sordida</i>	7755	49.34	135865	85.34	3281	68.8	146901	81.75
<i>T. tibiamaculata</i>	456	2.9	500	0.31	29	0.61	985	0.55
<i>T. vitticeps</i>	2	0.01	8	0.01	28	0.59	38	0.02
TOTAL	15719	100%	159209	100%	4769	100%	179697	100%

Source: Health department of the state of Bahia (SESAB). Legend: N: absolute number of samples; Mun: Number of municipalities. %: percentage per column.

control actions of *T. cruzi* vectors, initiated in 1975. Our results indicate a clear reduction in the occurrence of *P. megistus* and *T. infestans* (last record in 2015) and an increase in the relative abundance and geographical distribution of *T. sordida* and *T. pseudomaculata* after the Brazilian vector-control program.

Panstrongylus megistus was the predominant species between 1957 and 1971; it was found in 60% of municipalities, with a relative abundance of 62% of collected triatomines²². Its proportional spatial distribution has been greatly reduced in the state of Bahia, especially in the areas of Recôncavo Baiano and the metropolitan region of Salvador. *P. megistus* spatial distribution has also been reduced in other Brazilian states^{13,36-38}. Three hypotheses could explain this reduction in the metropolitan region of Salvador: (a) chemical control was successfully performed over four decades, resulting in the elimination of domestic populations of *P. megistus*; (b) intense urbanization in these municipalities resulted in deforestation and fragmentation of the Atlantic Forest biome areas, which is the natural habitat of this species²²; and (c) housing improvement, with progressive depletion of adobe houses^{39,40}, a favorable environment for *P. megistus* colonization. *P. megistus* is a native Brazilian species with a wide spatial distribution and high epidemiological and entomological relevance due to the high rates of *T. cruzi* infection and its proximity to human dwellings, as it can colonize intra-and/or peridomicile areas. In 2021, *P. megistus* foci were described in the metropolitan region of São Paulo, with

T. cruzi-infected triatomines associated with marsupials, revealing the importance of continued surveillance of synanthropic *P. megistus*⁴⁶.

During period A, *T. infestans* were found in 12.8% of the sampled municipalities and represented 3.7% of the sampled triatomines. During period B, *T. infestans* was identified in only seven municipalities with residual colonies³⁴⁻³⁵. Bahia was the last Brazilian state to receive PAHO certification, possibly because of specific *T. infestans* identification errors and the appearance of new records of this species in the study area³². The elimination of *T. infestans* in several municipalities in western and central Bahia can be explained by the *T. infestans* elimination plan. This plan was intensified in 2004 and included spraying households with insecticides, followed by research and capture of triatomines, surveying approximately 500000 households²⁶. However, residual colonies of this species have been detected in a few municipalities^{28,29}, requiring constant monitoring to eliminate residual foci from the state of Bahia. In addition to the chemical control performed in Bahia since 1975, intensified in 1991, which aimed to eliminate *T. infestans*^{11,15}, other social actions were implemented by the federal government. These included the growth acceleration program, intended to improve housing quality, with the replacement of mud houses with brick houses and health education actions on Chagas disease. This may also have influenced the reduction in household colonization by *T. infestans* and other household species^{39,40}.

Although successful in controlling *T. infestans*, several native species have been recorded in a large number of municipalities. They were captured in households and frequently colonized peri domestic areas. Among them, some were infected by *T. cruzi* and many of them fed on domestic animals and human blood⁴². In period A, *T. sordida* was recorded in 37% of the municipalities, representing 23% of the collected triatomines, while *T. pseudomaculata* was recorded in 19% of the municipalities, with a relative abundance of 2.5% of the gathered triatomines²³. In period B, *T. sordida* was recorded in 60% of the municipalities, representing approximately 81% of the triatomines. *T. sordida* is the most common species in different regions of Bahia. Similarly, *T. pseudomaculata* was identified in the largest number of municipalities during period B (67.4%). Systematic vector-control actions had a low impact on the spatial distribution of *T. sordida* and *T. pseudomaculata* in Bahia. There was a higher occurrence of *T. sordida* in western and south-central Bahia, where there are areas of Cerrado, which is the original biome of the species' natural populations^{1,2,25}. The highest occurrence of *T. pseudomaculata* in western and central Bahia followed the occurrence predictions of the species based on environmental variables⁴². The number of specimens of *T. sordida* and *T. pseudomaculata* exceeded 90% of all triatomines collected in the state between 2006 and 2019.

We observed a higher occurrence of *T. brasiliensis* and similar species in Bahia, thus expanding the observations of Ribeiro-Jr *et. al.*⁴¹. Before systematized control actions, the *T. brasiliensis* species complex was registered in 10% of the municipalities of Bahia, and between 2006 and 2019, at least 94 municipalities (36%) registered this species. In the last few years, other species of the *T. brasiliensis* complex have been described in the state of Bahia, including *T. juazeirensis*, *T. melanica*, *T. sherlocki*, and *T. petrocchia*⁴³. Among these species, *T. juazeirensis* is noteworthy because it was predominantly collected inside household units. Other species, such as *T. tibiamaculata*, *P. geniculatus*, *T. melanocephala*, and *P. lutzi*, were intrusively detected inside the houses, mainly adult specimens.

There was a significant difference in the occurrence of *T. tibiamaculata* between the two periods. In period A, it was recorded in approximately 1% of the municipalities with a relative abundance of 0.01%²³. In period B, it was recorded in 7% of the municipalities with a relative abundance of 0.55%. In recent decades, even though *T. tibiamaculata* has been naturally found inside nests of marsupials, rodents, and epiphytes in forests^{1,22}, it has been recorded in peri domestic palms and inside households. Therefore, *T. cruzi*-infected *T. tibiamaculata* generates a transmission risk in Salvador, probably in the entire metropolitan region, and in the Atlantic Forest areas⁴⁴.

Triatomine vectors of *T. cruzi* can be classified as native/non native, wild/non wild, domestic/peridomestic to enable the definition of effective vector-control strategies⁴⁵. While domestic and peridomestic populations of species of triatomines are subject to spraying of chemical pesticides, which is the main strategy of vector control, native-wild species can persistently invade or colonize the household (peri-and intradomicile). Thus, this may represent a challenge for controlling *T. cruzi* transmission in the domestic environment.

This study has several limitations. Since the use of reference databases does not allow a broad analysis of data, it was impossible to obtain data on triatomines within habitats

(intra-and peridomicile) in period A (1957-1971). The effects of passive dispersion and seasonal changes on vector behavior were impossible to measure. In addition, the database does not allow descriptive evaluations of nymph occurrence. Moreover, not all municipalities collected data regularly during the evaluated periods, and the health surveillance service classifies municipalities among the high, medium, and low risk of transmission, emphasizing that there is no obligation to conduct regular entomological research among low-risk municipalities based on the classification presented by Brazil's Health Ministry²⁶. Future studies reassessing risk classification at the municipal level are urgently needed for the redefinition of areas and risk of transmission. Further studies should analyze the situation of these areas to explain whether the absence of triatomines is due to the functioning of the service or biogeographic issues related to triatomines. In periods A and B, *T. brasiliensis* complex species were considered as one, and to avoid misinterpretations, data obtained in this study were evaluated at *stricto sensu* level⁴⁴. In Bahia, nine species that are unlikely to be found within households^{25,33} were recorded by the surveillance services. These included *Belminus laportei*, *Eratyrus mucronatus*, *Microtriatoma trinidadensis*, *Panstrongylus tupynambai*, *P. lignarius*, *Rhodnius prolixus*, *Triatoma maculata*, *T. circummaculata*, and *T. rubrovaria*. To reduce taxonomic identification errors, a guide was developed³³ and, more recently, a guidance manual for the surveillance of triatomines in Bahia, with identification keys, diagnosis, and distribution of vector species²⁶.

This study describes changes in the triatomine fauna of Bahia between the analyzed periods more than 40 years after the implementation of the vector-control program in the state. During this period, we observed changes in the Chagas disease surveillance policies in Brazil due to decentralization. The centralized Brazilian control program was transferred to municipalities without corresponding training. The decentralization policy of the Chagas disease vector-control program aimed to bring the actions of the government closer to the communities, but true engagement by local communities has not been achieved. After decentralization, the efficacy of some municipalities in detecting triatomines has been reduced. We observed a reduction in the relative abundance and proportional spatial distribution of domestic and domesticated species of *T. cruzi* vectors *P. megistus* and *T. infestans*, respectively. In addition, we described an increase in the native species *T. sordida*, *T. pseudomaculata*, and *T. brasiliensis* species subcomplex and highlighted the important role of native triatomine species in *T. cruzi* transmission in the domiciliary environment. The observed changes in *T. cruzi* main vectors between the evaluated periods demonstrate the importance of reinforcing entomological surveillance actions. Furthermore, promoting and disseminating community-based scientific knowledge and health education actions on Chagas disease at a local scale will help mitigate surveillance challenges and control native triatomines.

Ethics

The procedures followed the ethical standards of the Research Ethics Committee of the Gonçalo Moniz Institute (IGM – FIOCRUZ, Bahia, Brazil). The consent form was waived since the analysis was performed using a database. However, to provide full anonymity to participants, no personal identification data were used. The research did not cause any physical, psychological, moral, intellectual, social, cultural, or religious risks to the residents or animals in the study areas. In addition, this study did not include endangered or protected species.

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5.4. ARTIGO 4 (MANUSCRITO) – Out of sight, out of mind: Understanding Chagas' disease vulnerabilities in a regional level, Bahia, Brazil.

Neste capítulo são apresentados os resultados preliminares relativos aos objetivos específicos:

- a) Analisar os indicadores entomológicos, socioeconômicos, demográficos e epidemiológicos de vulnerabilidade para transmissão vetorial de *T. cruzi* na Bahia;
- b) Identificar municípios e regiões de maior vulnerabilidade para o agravo doença de Chagas no Estado da Bahia.

As informações referentes a este manuscrito serão apresentadas sob a forma de artigo científico ainda não publicado – resultados preliminares.

**Out of sight, out of mind: Understanding Chagas' disease vulnerabilities
in a regional level, Bahia, Brazil.**

- Preliminary results -

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Resumo

Background: Indicadores em saúde, quando associados à geoestatística, podem ajudar a identificar áreas e populações mais vulneráveis a um determinado agravo, além do padrão espaço-temporal dos seus determinantes em um determinado território, nesse sentido, a Bahia, em relação ao Brasil e toda América Latina, é considerada como área endêmica para doença de Chagas, e apesar disso, existem limitadas informações sobre as áreas mais vulneráveis em escala regional no Estado. **Objective:** Determinar as regiões mais vulneráveis à doença de Chagas no Estado da Bahia, Brasil. **Methodology/Principal Findings:** Utilizamos a Análise Multicritério de Decisão (AMD) para ranquear os municípios da Bahia a partir de indicadores socioeconômicos, demográficos, entomológicos, epidemiológicos e ambientais, e o geoprocessamento destes dados para determinar regiões cujos municípios apresentaram valor elevado na AMD. Diferentes modelos e simulações foram realizadas e comparadas. **Results:** Através da AMD identificamos 80/415 (19%) municípios com alta vulnerabilidade em todo o território do Bahia, com exceção do Sul do Estado, classificado como de baixa vulnerabilidade. Os municípios de alta vulnerabilidade estão agrupados em quatro clusters diferentes, localizados nas regiões Centro-Sul, Vale do São Francisco, Metropolitana de Salvador e em especial a região Centro-Norte, que apresentou maior número de municípios considerados como de alta vulnerabilidade, além de ser uma área recorrente, independente das simulações realizadas. Os municípios classificados como alta vulnerabilidade apresentaram maior pobreza na zona rural, menor densidade demográfica e maior frequência de espécies sinantrópicas e epidemiologicamente relevantes de vetores de *T. cruzi*. A principal limitação deste estudo está associada ao uso de dados secundários, e neste caso, à ausência de notificações e subnotificações de algumas das variáveis analisadas em diversos municípios, além da utilização de dados do Censo 2010, sendo recomendada a reanálises destes dados utilizando as informações do Censo 2022, ainda não disponibilizado pelo IBGE. **Conclusions/Significance:** A associação da AMD e geoestatística auxiliou a identificamos 80 municípios (19%) classificados como de alta vulnerabilidade para a doença de Chagas, apesar disso, o risco de transmissão de *T. cruzi*, em suas diferentes formas, continua presente em todo Estado.

Palavras-chave: Doença de Chagas, doença negligenciada, *T. cruzi*, vulnerabilidades, AMD.

Author summary

A transmissão de *T. cruzi* ao homem e seus animais domésticos pode ser influenciada por diversos fatores, por exemplo: o comportamento da espécie dos triatomíneos da região; condições socioeconômicas da população; fatores ambientais, como o uso da terra, o desmatamento e a fragmentação dos biomas locais; e até mesmo a questões culturais, como o consumo de animais silvestres e a partir de alimentos contaminados, dentre outros fatores. Neste estudo utilizamos dados entomológicos, socioeconômicos, ambientais e epidemiológicos para identificar as regiões mais vulneráveis à doença de Chagas, numa perspectiva regional, mediante utilização de uma metodologia racional e reprodutível. Identificamos 80 municípios (19%) classificados como de alta vulnerabilidade para a doença de Chagas, e agrupamento destes municípios nas regiões Centro-Sul, Vale do São Francisco, Metropolitana de Salvador e em especial a região Centro-Norte da Bahia, apesar disso, o risco de transmissão de *T. cruzi*, em suas diferentes formas, continua presente em todo Estado.

Introdução

A doença de Chagas ainda representa um problema de saúde pública com forte impacto social e econômico em países endêmicos na América Latina (Lee, Bacon et al. 2013, da Nobrega, de Araujo et al. 2014, Lidani, Andrade et al. 2019) e em regiões até então indenes, como América do Norte (Perez-Zetune, Bialek et al. 2020) e Europa (Imaz-Iglesia, Miguel et al. 2015). A Organização Mundial de Saúde (OMS) estimou cerca de 70 milhões de pessoas sob risco de infecção por *T. cruzi*, o que acarreta em custos sociais diretos e indiretos como, por exemplo, o ônus decorrente do tratamento para a pessoa acometida e o sistema público de saúde (Castillo-Riquelme, Guhl et al. 2008, Olivera and Buitrago 2020) e a incapacidade para o trabalho entre pessoas jovens.

A Bahia é o Estado na região Nordeste do Brasil com mais elevado coeficiente de mortalidade por doença de Chagas, sendo então necessárias ações eficazes de vigilância, compreendendo o controle vetorial, diagnóstico e tratamento dos casos agudos (Castillo-Riquelme, Guhl et al. 2008, Bartsch, Avelis et al. 2018) e crônicos, de acordo com a indicação terapêutica e monitoramento das mulheres infectadas ainda em período reprodutível fértil para mitigar o risco e consequências da transmissão congênita. Além disso, a Bahia possui a maior riqueza de espécies de triatomíneos (Dias, Machado et al. 2000), que possuem área de ocorrência específica e capacidade vetorial diferenciada na transmissão de *T. cruzi* ao homem e seus animais domésticos (Shetlock and Serafim 1972, Silveira, Ribeiro et al. 2016, Ribeiro, Dos Santos et al. 2019).

Nesse sentido, a abordagem multicritério de decisão tem sido amplamente utilizada em pesquisa, para entender os determinantes de um agravo, em especialmente para ajudar gestores em saúde na tomada de decisão baseada em evidências (Hongoh, Hoen et al. 2011, Silveira, Ribeiro et al. 2016). Thokala and Duenas (2012) descreveram que a AMD é um processo de quatro estágios: (1) identificação das alternativas que serão consideradas, (2)

estabelecimento dos critérios, (3) determinação de escores para cada critério; e (4) cálculo dos pesos de importância para cada critério, na tentativa de estabelecer, desta maneira, uma relação mensurável entre os critérios estabelecidos.

Desta forma, esse estudo tem como objetivo principal classificar os municípios com base no grau de vulnerabilidade para a doença de Chagas no Estado da Bahia.

Métodos

Área de estudo

O Estado da Bahia é composto por 417 municípios e está situado na região Nordeste do Brasil (Fig. 1), com população estimada de 14.016.906 de pessoas no ano de 2010, 27,9% dela residente em área rural. Seu território tem 567.295 km² e possui densidade demográfica de 24,82 hab./km².

Em 2018 ocupou a sétima posição entre os Estados Brasileiros com a maior proporção de pessoas com rendimento domiciliar per capita inferior a US\$ 1,90 e inferior a US\$ 5,50 PPC diários e uma das maiores taxas de desocupação do País.

Indicadores de risco para a transmissão vetorial do *T. cruzi*

Analisamos indicadores entomológicos, socioeconômicos, demográficos, epidemiológicos e de atenção à saúde.

(i) Indicador entomológico utilizado foi o TriatoScore: Os triatomíneos foram listados, as distribuições geográficas estimadas foram estabelecidas e os vetores foram classificados de acordo com seu papel potencial na transmissão de *T. cruzi* ao homem e animais domésticos. A partir desta classificação, os municípios da Bahia foram então ranqueados e agrupados com base nos scores obtidos (Ribeiro-Jr, Abad-Franch et al. 2021).

(ii) Indicadores socioeconômico, demográficos e ambientais: foram empregados: (a) o percentual de pobreza rural para 2010, (b) densidade demográfica a nível municipal em 2008, e (c) área municipal antropizada para 2010, seguindo observações de pesquisas prévias

(Vinhaes, de Oliveira et al. 2014). Esses dados foram obtidos das bases do censo demográfico 2010, do Instituto Brasileiro de Geografia e Estatística (IBGE) <http://www.ibge.gov.br> (Censo 2010) e do Atlas Brasileiro de Vulnerabilidades Socioambientais, organizado pelo Centro Brasileiro de Análise e Planejamento - CEBRAP e Departamento de Vigilância em Saúde Ambiental e Saúde do Trabalhador da Secretaria de Vigilância em Saúde do Ministério da Saúde (PISAST) <http://189.28.128.179/atlasvulnerabilidade/index.php>.

(iii) Indicadores epidemiológicos e de atenção à saúde: avaliamos os seguintes indicadores: (d) prevalência de dispensação de benzonidazol a nível municipal entre 2008 e 2018 (DIVEP – 2008-2018); (e) prevalência de internações hospitalares por doença de Chagas (2007-2018); (f) coeficiente de mortalidade específico por Doença de Chagas municipal (2008-2018); (g) prevalência de doença de Chagas autorreferida de 1 a 14 anos e em pessoas com 15 anos ou mais (2006-2015); (h) naturalidade de pessoas reagentes para *T. cruzi* detectados na triagem sorológica entre 2008 e 2018 da Fundação de Hematologia e Hemoterapia do Estado da Bahia (HEMOBA-SESAB).

Os dados foram obtidos juntamente com a Diretoria de Vigilância Epidemiológica da Secretaria de Saúde do Estado da Bahia (DIVEP-SESAB) e na HEMOBA.

Análise de dados e geoprocessamento

Após a checagem e revisão das informações, os indicadores foram descritos por meio das suas médias, desvios-padrões e valores mínimo e máximo, empregando o *Statistical Package for the Social Science* (IBM SPSS®) versão 24 (Março 2018).

As variáveis analisadas das classes (ii e iii) foram padronizadas por meio da razão entre os valores do indicador analisado e a população estimada para cada município, multiplicados por 100.000 habitantes (hab.).

O conjunto de etapas para classificação dos municípios foi operacionalizado pelo método *Prométhée* II (Athawale, Chatterjee et al. 2012) e implementado

computacionalmente no Programa de Apoio à Tomada de Decisão baseado em Indicadores (PRADIN), disponível em www.anipes.org.br (Almeida and Produção 2002, Cavalcante and Almeida 2005). Para a AMD, foram estabelecidas sete simulações, utilizando pesos arbitrados pelos autores, levando em consideração os diferentes agrupamentos de indicadores (Tabela 1). Foram desenvolvidos sete diferentes modelos, onde os pesos foram atribuídos de forma a evidenciar a relevância de uma determinada classe de indicador, a cada simulação realizada.

Na simulação S1, todos indicadores tiveram os mesmos pesos e foram considerados com a mesma relevância. Na simulação S2, ao indicador entomológico foi atribuído maior peso; na simulação S3, ao indicador socioeconômico foi atribuído o maior peso, e assim por diante (Tabela 1).

Geoprocessamento e geoestatística

A unidade espacial utilizada para o georreferenciamento foi o nome do município ou o geocódigo do IBGE e as camadas vetoriais (.shp) base para os mapas foram obtidas diretamente do IBGE. Os dados foram apresentados em cinco classes, obtidas por meio dos quantis da distribuição dos dados, em cinco classes.

Para demonstrar as áreas com *hotspots* de ocorrência dos dados da AMD foi utilizado o teste Hot Spot Analysis (Getis-Ord Gi Statistic) ao nível de significância de 5% ($\alpha=0,05$). Para a visualização, análise e determinação dos padrões de agrupamento dos dados foi utilizado o sistema de informação geográfica do software ArcMap® versão 10.5.

Resultados

A primeira análise realizada nesta pesquisa foi a análise descritiva dos indicadores. Cada indicador apresentou um padrão específico, de acordo com a natureza da informação analisada, entretanto, os dados epidemiológicos analisados individualmente demonstram

áreas de *hotspots* similares e recorrentes nas regiões: extremo oeste, centro norte baiano e vale do São Francisco, Fig. 2 (F-J) e 3 (F-J).

O resultado detalhado das análises da estatística descritiva (média, desvio padrão, valor máximo, valor mínimo) dos indicadores analisados pode ser observado na Tabela 02. Os mapas resultantes da análise individual de cada um dos indicadores estão disponíveis nas Fig.2 (A-E) e 3 (A-E), e os respectivos *hotspots* e *coldspots* podem ser observados na Fig.2 (F-J) e 3 (F-J).

As simulações da Análise Multicritério de Decisão, bem como os pesos atribuídos às variáveis em cada simulação podem ser observados na Tabela 1. Na primeira simulação atribuímos o mesmo peso para todos os indicadores, sendo testadas diferentes combinações de pesos nas simulações seguintes (Tabela 1). Os mapas das simulações S1-S7 resultantes, bem como as áreas de *hotspots* e *coldspots* podem ser observados na Fig 3 (A-N). As simulações da AMD foram espacialmente compatíveis e apresentaram áreas de *hotspots* e *coldspots* semelhantes para nas simulações S1-S3, S6 e S7, Fig. 3 (H-N). As principais áreas salientadas foram a região do extremo oeste, vale do São Francisco, a parte norte da região centro sul, e em especial a da região centro-norte baiano, esta última sendo observada de forma recorrente nas diferentes simulações avaliadas.

As simulações em que atribuímos maior peso para os indicadores demográfico e ambiental (S4 e S5) demonstram que as áreas com alta densidade demográfica e com o maior percentual de área antropizada no município são áreas distintas das simulações realizadas com os dados entomológicos, socioeconômicos e epidemiológicos. As simulações S6 e S7 diferenciam-se, pois nessa última foram considerados apenas os indicadores epidemiológicos, apesar disso, as regiões vulneráveis foram essencialmente as mesmas.

Discussão

Com base na AMD identificamos 19% de municípios do Estado da Bahia com maior grau de vulnerabilidade para o risco da transmissão da doença de Chagas. Essa análise utiliza um conjunto de técnicas para auxiliar um agente decisor – indivíduo, grupo de pessoas ou comitê de técnicos ou dirigentes a tomar decisões acerca de um problema complexo, avaliando e escolhendo alternativas para solucioná-lo segundo diferentes cenários (Jannuzzi and Miranda 2009, Longaray, Munhoz et al. 2016). Permite que a decisão seja pautada com base nos critérios técnicos considerados relevantes para o problema em questão pelos agentes decisor, cuja importância é definida por especialistas, em um processo interativo com outros atores técnico-políticos. Desta forma, os gestores podem orientar sua agenda de prioridades, conferindo maior importância a questões sociais e estratégias de intervenção (Jannuzzi, Miranda et al. 2009).

Os mapas resultantes da AMD indicaram a existência de municípios com alta vulnerabilidade em praticamente todo o território do Bahia, com exceção da região Sul, onde a grande maioria dos municípios foi classificada como de baixa vulnerabilidade em todas as simulações realizadas destacando-se por apresentar menor pobreza, em relação às outras mesorregiões avaliadas (Fig. 4).

As análise com base na utilização de indicadores entomológicos (Ribeiro-Jr, Abad-Franch et al. 2021), demonstrou padrões semelhantes com municípios com valores menores na região Sul. Tal fato pode estar associado a questões biológicas dos vetores, que naturalmente são delimitados por biomas e o nível de equilíbrio nesses ecossistemas. Na região sul da Bahia o bioma predominante é a Mata Atlântica, que apresenta características muito distintas dos outros biomas (Caatinga e Cerrado) e nestas áreas ainda existem grandes remanescentes de mata e biodiversidade local. Das 26 espécies de triatomíneos que ocorrem na Bahia, a menor diversidade de espécies foi observada em áreas do bioma Mata Atlântica. Outro fator que pode ter contribuído para a baixa representatividade de vetores nessa área é

o fato da classificação de risco atual (Fig. 5) considerar essa região como de baixo risco, desta forma, os municípios não têm a obrigatoriedade de montar o sistema de vigilância para os vetores da doença de Chagas, e se não há vigilância vetorial, não haverá registros de vetores. Entretanto, vale ressaltar que já foi demonstrada a aproximação de vetores infectados no entrono de moradias humanas assim como na forma intrusiva demonstrando a possibilidade de risco de transmissão vetorial nessas regiões classificadas como de baixo risco (Ribeiro, Gurgel-Goncalves et al. 2015).

A existência de áreas vulneráveis à transmissão vetorial de *T. cruzi* por triatomíneos sinantrópicos, através da AMD, já havia sido descrita, chamando atenção para o grande número de municípios vulneráveis em contraste com o baixo número de casos agudos e crônicos notificados no Brasil (Vinhaes, de Oliveira et al. 2014). De forma a aprimorar o proposto por Vinhaes, de Oliveira et al. (2014) e dar aplicação regional à metodologia proposta, detalhamos nesta pesquisa as classes de indicadores, aprimoramos a classificação ponderada dos vetores (TriatoScore), levando em considerações importantes aspectos regionais da fauna dos vetores do *T. cruzi* (Ribeiro-Jr, Abad-Franch et al. 2021). Ademais, consideramos os indicadores epidemiológicos disponíveis no sistema de vigilância estadual para a avaliação de áreas vulneráveis à transmissão de *T. cruzi* através da AMD, bem como utilizamos ferramentas de geoprocessamento específicas para delimitação de áreas de *hotspots* e *coldspots* (*Getis-Ord Gi Statistic*), facilitando o processo de localização e agrupamento de municípios vizinhos e altamente vulneráveis.

Acerca dos indicadores utilizados para gerar as diferentes simulações, duas delas apresentaram padrão distinto das demais – densidade demográfica e área antropizada municipal. Sobre o indicador densidade demográfica, observamos áreas de *hotspots* apenas na região metropolitana de Salvador, capital do estado, dessa forma, esse padrão pode atribuir

viés à simulação caso seja atribuído peso elevado a essa variável, Fig. 2 (I), especialmente na região indicada, com predomínio de alguns poucos municípios densamente povoados.

O indicador ambiental analisado foi a área antropizada a nível municipal (Censo 2010), como um proxy de vegetação, entretanto a metodologia utilizada pelo IBGE para obtenção dessa informação não considera a diferença entre os três diferentes biomas da Bahia (Caatinga, Cerrado e Mata Atlântica), sendo este um fator limitante para a correta avaliação da influência desta informação nas simulações. Apesar disso, acreditamos que o desmatamento tem forte influência sobre o processo de domiciliação de vetores e ocorrência de vetores intrusivos nas residências, em especial em áreas de Mata Atlântica (Ribeiro, Gurgel-Goncalves et al. 2015). Fontes de informação mais detalhada sobre o processo de desmatamento no Brasil e o acesso a estes dados de forma ampla só foram disponibilizados a partir de 2015, através do projeto MapBiomas®, com séries históricas para o Mapeamento Anual da Cobertura e Uso do Solo do Brasil (que é a iniciativa MapBiomas).

O indicador entomológico apresentou resultados compatíveis aos indicadores epidemiológicos analisados. A metodologia utilizada para classificar os triatomíneos levou em consideração diversos aspectos biológicos da espécie, o que gera uma informação robusta e importante para a delimitação de vulnerabilidades entomológicas e relacionadas à transmissão vetorial do *T. cruzi* (Ribeiro-Jr, Abad-Franch et al. 2021).

A análise do indicador pobreza na zona rural demonstrou *hotspots* com compatibilidade geográfica às áreas de *hotspots* dos indicadores entomológicos. O fator pobreza é considerado um importante determinante em diferentes pesquisas em doença de Chagas (Grijalva, Villacis et al. 2015, Liu and Zhou 2015), pois reflete problemas sociais que agravam o nível de vulnerabilidade dessas populações, como as condições de moradia e acesso à educação em saúde (Vinhaes, de Oliveira et al. 2014, Valdez Tah, Huicochea Gomez

et al. 2015), sendo um importante indicador do nível de vulnerabilidade à transmissão do *T. cruzi* na Bahia.

Sobre os indicadores epidemiológicos, dispensação de benzonidazol; internações por doença de Chagas; óbitos por doença de Chagas; prevalência de detecção na triagem sorológica da HEMOBA (Naturalidade); doenças de Chagas autorreferida 0-14 anos mediante inquérito epidemiológico; doenças de Chagas autorreferido >15 anos mediante inquérito epidemiológico, ambos com entrevistas individuais, todos corroboram, demonstrando regiões de *hotspots* similares.

Indicadores como mortalidade (Martins-Melo, Ramos et al. 2012, da Nobrega, de Araujo et al. 2014), internação hospitalar (Benchimol-Barbosa 2010) além dos casos detectados em triagem sorológica de hemocentros (Miranda, Ribeiro et al. 2019) são dados importantes na tentativa de identificar regiões historicamente prioritárias pois revelam diferentes vulnerabilidades relacionadas à doença de Chagas e atribuem maior relevância ao modelo gerado (Vinhaes, de Oliveira et al. 2014, Valdez Tah, Huicochea Gomez et al. 2015).

Das simulações analisadas, acreditamos que possuem maior relevância as simulações S1, S2, S3 e S6, pois evitam respectivamente as limitações observadas para os indicadores: densidade demográfica e área antropizada; ou as limitações de considerar apenas uma classe de indicador. E dentre estas, acreditamos que a simulação S2 melhor represente a vulnerabilidade em doença de Chagas na Bahia.

Na simulação S2, por exemplo, que atribui maior peso ao indicador vetorial, Fig. 5B (B), a AMD classificou 151 (36%) municípios com algum nível de vulnerabilidade pela análise dos scores obtidos na AMD (Tab. 3), e com a ajuda a análise de *hotspots* e *coldspots* identificamos os agrupamentos de municípios vulneráveis e vizinhos, através da análise do GiZScore e número de vizinhos das feições analisadas, sendo 80 (19%) considerados

municípios de alta vulnerabilidade (apêndice 1) (Fig. 5), e agrupados em quatro diferentes *hotspots*.

Este estudo apresenta algumas limitações: Nossa proposta se baseia na utilização de dados dos sistemas de vigilância municipal e estadual, que dependem da qualidade de registro da informação. Nos últimos anos, foram registrados pontuais casos agudos de DC no estado da Bahia. Um caso em 2013 de residente do município de Tanhaçu e outro do município de Lapão. Ainda não há registro dos casos crônicos da doença de Chagas, a notificação desses casos está em vias de se iniciar. Essas informações são importantes para a avaliação dos modelos gerados. Não estão disponíveis informações sobre casos agudos e casos crônicos, informações importantes para a avaliação dos modelos gerados. Acreditamos ainda que a notificação compulsória de casos crônicos de doença de Chagas possa acrescentar importantes informações à avaliação de vulnerabilidade de transmissão de *T. cruzi* na Bahia. Adicionalmente, acreditamos na necessidade de realizar os dados utilizando as informações mais recentes do censo 2022, as informações contidas neste manuscrito utilizam os dados do censo 2010, anterior à maioria dos dados epidemiológicos analisados.

Por fim, a estratégia apresentada de associar indicadores entomológicos, socioeconômicos, demográficos e epidemiológicos à AMD em associação com a geoestatística para delimitação de *cold* e *hotspots* foi exitosa em estabelecer regiões específicas mais vulneráveis no estado da Bahia, tangíveis à ação dos sistemas de vigilância e controle do agravo, além de ser uma metodologia reprodutível e escalonável. Essas informações poderão ser utilizadas para determinar áreas para realização de inquéritos sorológicos em humanos e em animais domésticos das áreas mais vulneráveis.

Conclusões

As simulações da AMD, em combinação com a análise geoestatística de *cold* e *hotspots* (Getis-Ord G_i^* Statistic), indicam para a existência de agrupamentos de municípios vulneráveis à transmissão do *T. cruzi* em toda a Bahia, com exceção da região Sul. As áreas consideradas altamente vulneráveis apresentaram altos valores dos indicadores entomológicos e epidemiológicos analisados (Tabela 3).

Identificamos 80 municípios (15%) considerados como de alta vulnerabilidade para transmissão vetorial de *T. cruzi* ao homem e seus animais domésticos no estado da Bahia. Estes municípios estão agrupados em quatro clusters diferentes, localizados nas regiões centro sul, vale do São Francisco, metropolitana de Salvador e em especial a região centro-norte, que apresentou maior número de municípios considerados como de alta vulnerabilidade, além de ser uma área recorrente, independente das simulações realizadas.

A proposta de classificação apresentada leva em consideração importantes indicadores relacionados à transmissão vetorial da doença de Chagas e delimita as áreas mais vulneráveis no Estado da Bahia, e em consonância com o sistema de vigilância pode se tornar uma importante estratégia na delimitação de áreas prioritárias. A análise destas informações a nível municipal permite agregar os dados a diferentes níveis socioeconômicos ou de identidade regional, favorecendo a tomada de decisão por parte dos gestores para o enfrentamento do agravo. Tal estratégia de análise neste artigo pode servir para outros estudos em diferentes regiões do país e em parte do mundo com reconhecida prevalência de Doença de Chagas autóctone por transmissão vetorial, ajustando aos contextos dos biomas a serem analisados.

A combinação da AMD com a geoestatística facilitou no processo de localização de agrupamento de municípios vizinhos e altamente vulneráveis, delimitando áreas tangíveis para as ações de vigilância e controle da transmissão da doença de Chagas.

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Contribuição dos Autores

GR-Jr, RG-G e MGR conceberam o estudo. O MGR arrecadou fundos e administrou o projeto. GR-Jr, RG-G, MGR, contribuíram para a concepção dos métodos de ensaio. GR-Jr, FCL, DLM, RBR, WN e CMMC, realizaram pesquisas. GR-Jr e MGR supervisionaram alunos envolvidos em pesquisas de campo e de laboratório. GR-Jr, CMMC, GMC, obtêm os bancos de dados, GR-Jr selecionou o conjunto de dados e analisou os dados. GR-Jr, RG-G e MGR redigiram a primeira versão do manuscrito. Todos os autores contribuíram com a interpretação dos resultados, leram e comentaram as versões preliminares do manuscrito e aprovaram a versão final.

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Figure Legends

Fig. 1. A. Localização do Estado da Bahia e Biomas do Brasil. B e C. Municípios e agrupamentos de macrorregiões da Bahia. Legenda: 1. Centro norte baiano; 2. Centro Sul Baiano; 3. Extremo Oeste Baiano; 4. Metropolitana de Salvador; 5. Nordeste baiano; 6. Sul baiano; 7. Vale do São Francisco;

Fig. 2. Indicadores analisados: A. TriatoScore; B. Área municipal antropizada; C. Densidade demográfica; D. Pobreza na Zona Rural; Legenda: Na parte superior os dados estão exibidos por quantil; *Hot Spot Analysis* (Getis-Ord G_i^*) dos dados descritos acima;

Fig. 3. Indicadores analisados: E. Dispensação de Benzonidazol. F. Internações por doença de Chagas; G. Óbitos por doença de Chagas; H. Prevalência de detecção na triagem sorológica da Hemoba (Naturalidade); I. Chagas autorreferido 0-14 anos; J. Chagas autorreferido >15 anos. Legenda: Na parte superior os dados estão exibidos por quantil; *Hot Spot Analysis* (Getis-Ord G_i^*) dos dados descritos acima;

Fig. 4. Simulações da Análise Multicritério de Decisão: Simulações S1, S2, S3, S4, S5, S6 e S7; Legenda: Na parte superior os dados estão exibidos por quantil; na parte inferior são apresentadas as *Hot Spot Analysis* (Getis-Ord G_i^*), agrupados por mesorregião, para os dados descritos logo acima;

Fig. 5. Comparação entre a classificação atual de risco de transmissão da doença de Chagas e modelo de vulnerabilidade de transmissão vetorial do *T. cruzi* na Bahia.

Legenda: A. Distribuição dos municípios segundo grau de risco. B. Modelo de vulnerabilidade proposto.

Checklist S1: Strobe checklist

TABELAS

Tabela 02. Simulações realizadas com apoio da análise multicritério de decisão (AMD) para identificação das regiões mais vulneráveis à transmissão vetorial do *T. cruzi* na Bahia e pesos atribuídos aos indicadores em cada simulação realizada.

Indicadores	Simulações						
	S1	S2	S3	S4	S5	S6	S7
Entomológico							
Ocorrência ponderada dos vetores da Bahia	1,00	5,05	0,55	0,55	0,55	0,55	0
Socioeconômico							
Pobreza rural	1,00	0,55	5,05	0,55	0,55	0,55	0
Demográfico							
Densidade demográfica	1,00	0,55	0,55	5,05	0,55	0,55	0
Ambiental							
Área antropizada dos municípios da Bahia	1,00	0,55	0,55	0,55	5,05	0,55	0
Epidemiológico							
Prevalência de detecção na triagem sorológica da Hemoba por município de naturalidade	1,00	0,55	0,55	0,55	0,55	1,30	1,66
Prevalência de dispensação do benzonidazol pela SESAB para os municípios da Bahia	1,00	0,55	0,55	0,55	0,55	1,30	1,66
Prevalência de internação por Doença de Chagas nos municípios da Bahia	1,00	0,55	0,55	0,55	0,55	1,30	1,66
Prevalência de óbito por doença de Chagas nos municípios da Bahia	1,00	0,55	0,55	0,55	0,55	1,30	1,66
Doença de Chagas autor referido por menores de 15 anos - Sistema de informação da atenção básica	1,00	0,55	0,55	0,55	0,55	1,30	1,66
Doença de Chagas autor referido por maiores de 15 anos - Sistema de informação da atenção básica	1,00	0,55	0,55	0,55	0,55	1,30	1,66

Tabela 02. Estatística descritiva das variáveis utilizadas para realizar a análise multicritério de decisão para identificação de áreas vulneráveis à transmissão do *T. cruzi* no estado da Bahia.

Indicadores	Média	SD	Max	Min
TriatoScore	14,981	16,009	72,000	0,000
Densidade demográfica (hab./km ²)	61,525	254,167	4172,000	0,000
Pobreza Rural (%)	0,072	0,046	0,217	0,000
Área antropizada municipal (%)	0,735	0,340	1,000	0,001
Dispensação de Benzonidazol (med./10.000hab.)	1,768	7,074	89,908	0,000
Internações hospitalares (internações/10.000hab.)	0,310	0,727	8,783	0,000
Óbitos (óbitos/10.000hab.)	5,631	8,662	61,951	0,000
Doença de Chagas autorreferido 1-14 anos (casos/10.000hab.)	1,580	3,234	37,083	0,000
Doença de Chagas autorreferido >15 anos (casos/10.000hab.)	272,087	627,547	712,001	0,000
Hemoba_Naturalidade (casos/10.000hab.)	0,698	1,883	22,971	0,000

Tabela 3. Média por classe das variáveis utilizadas para realizar identificação de áreas vulneráveis à transmissão do *T. cruzi* no estado da Bahia.

Class.	Dados									
	Vuln.	Ento.	Pob. Rural	Dens. Dem.	Área antrop.	Hemoba	Benzonidazol	Internação	Óbito	SIAB (0-14)
1 (baixa)	-	0.05	32.70	0.81	0.08	0.04	0.05	0.85	0.80	21.97
2	3.44	0.07	93.79	0.77	0.73	0.95	0.33	3.40	1.72	225.87
3	12.74	0.08	39.82	0.74	0.47	1.15	0.24	5.81	1.01	176.47
4	20.81	0.08	49.97	0.68	1.02	3.07	0.35	9.18	2.15	494.33
5 (alta)	38.52	0.09	92.44	0.68	1.21	3.70	0.59	9.03	2.24	448.09
Total	14.93	0.07	61.52	0.73	0.70	1.77	0.31	5.63	1.58	272.09

FIGURAS

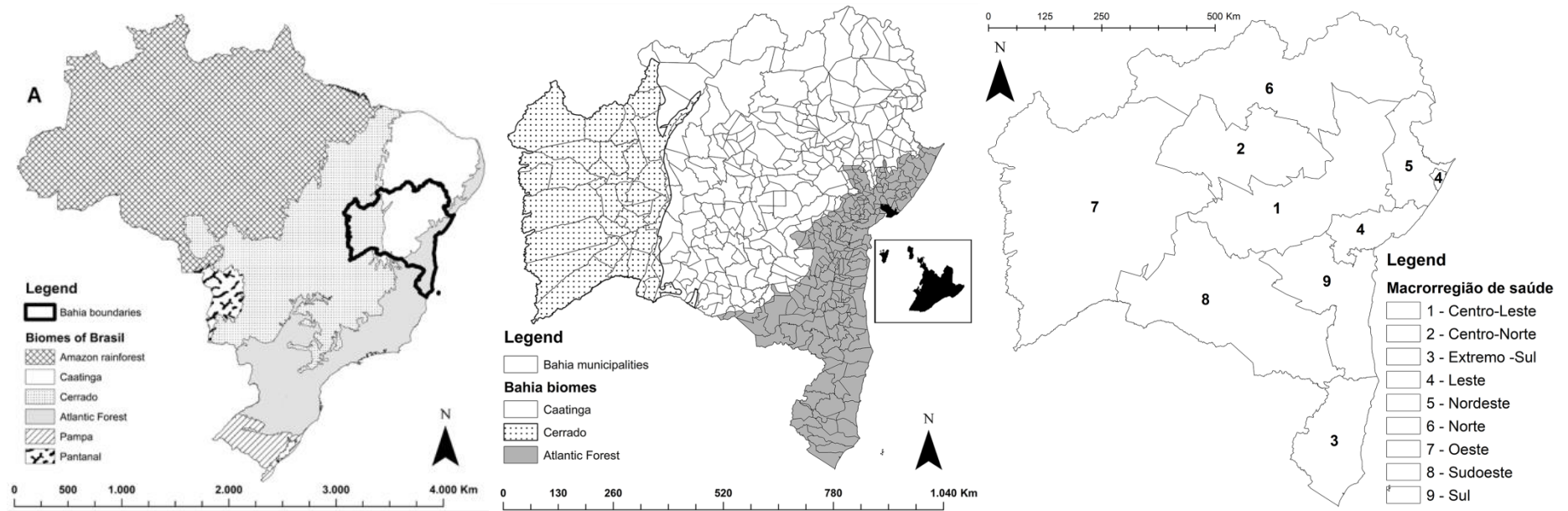


Fig. 1. (A.) Localização do Estado da Bahia e Biomas do Brasil. (B.) Municípios e (C.) agrupamentos de macrorregiões da Bahia.

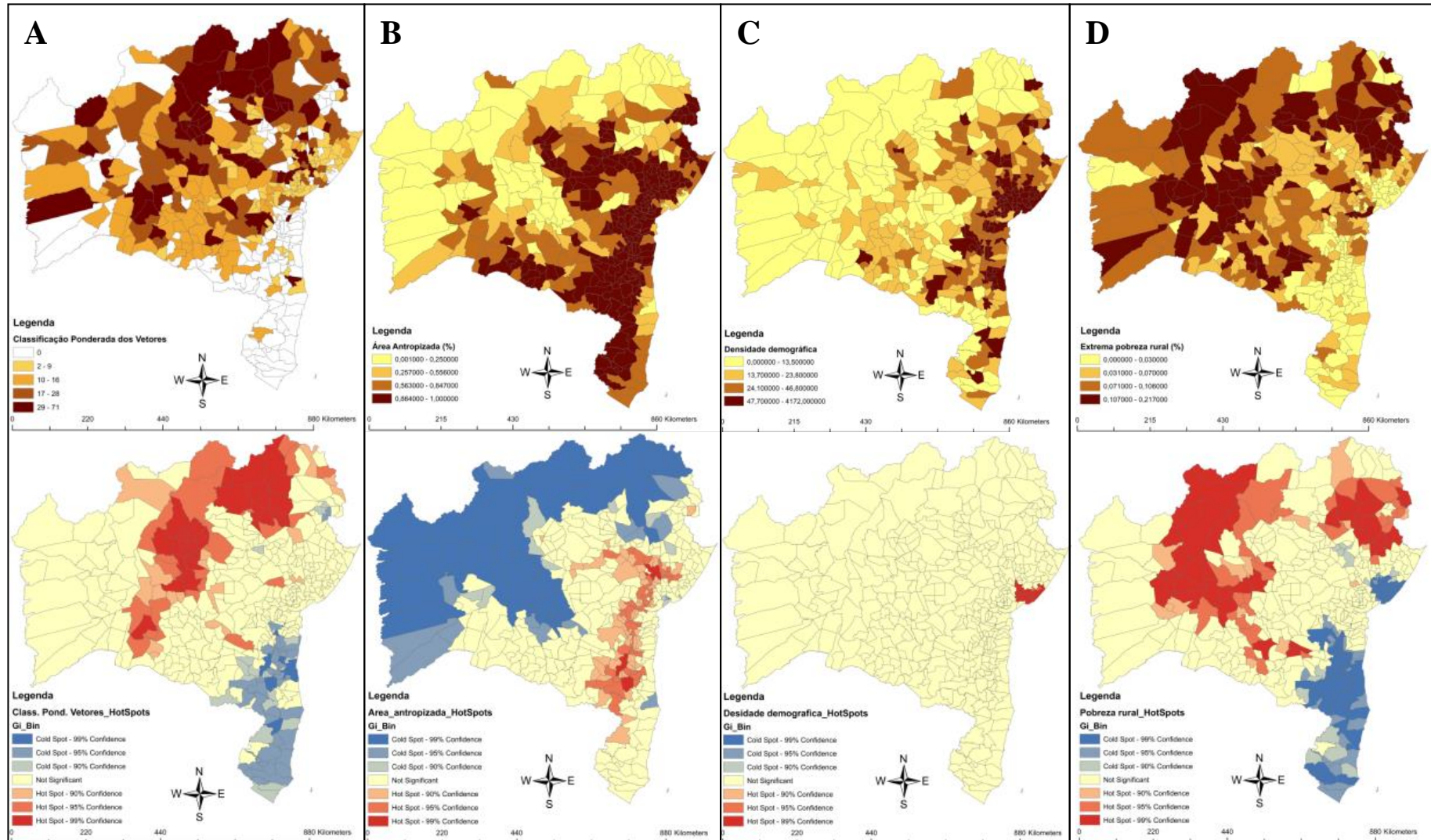


Fig. 2. Distribuição espacial dos valores dos indicadores: entomológico, ambiental, demográfico e econômico analisados: A. TriatoScore; B. Área municipal antropizada; C. Densidade demográfica; D. Pobreza na Zona Rural; Legenda: Na parte superior os dados estão exibidos por quantil; Hot Spot Analysis (Getis-Ord Gi*) dos dados descritos acima;

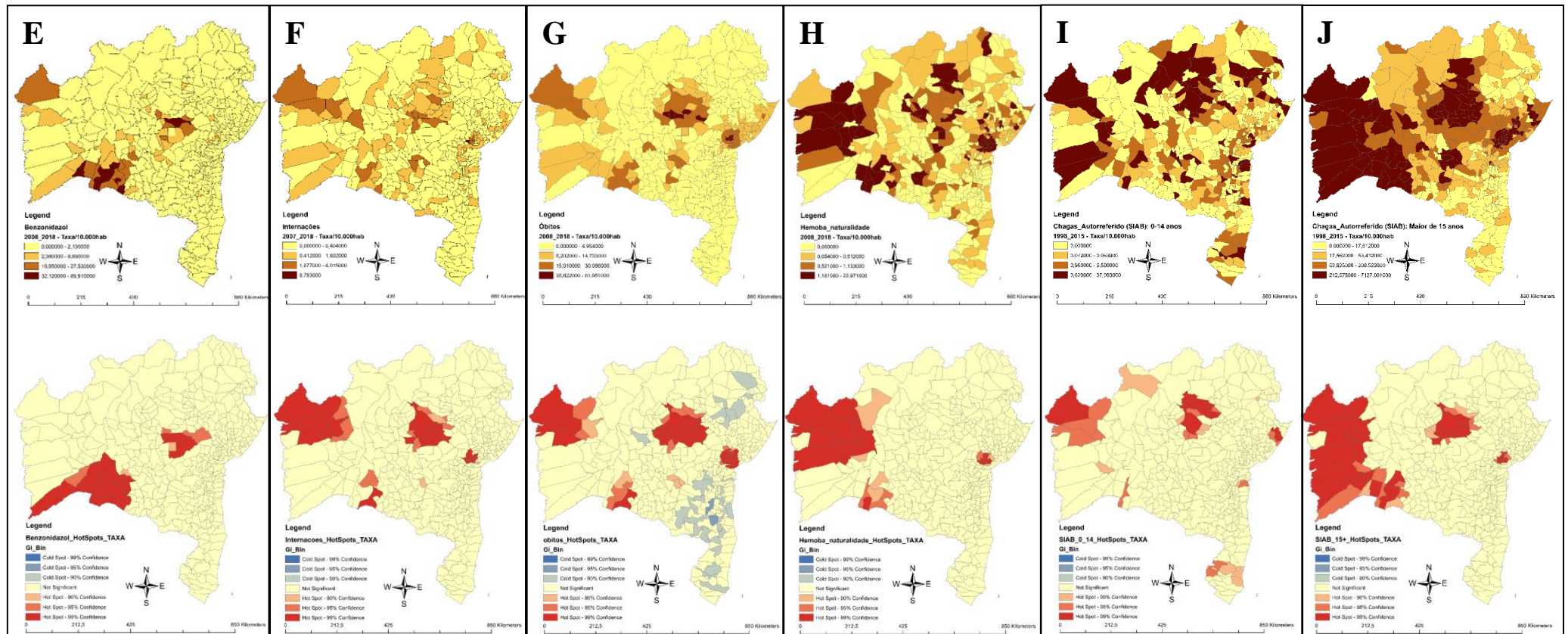


Fig. 3. Distribuição espacial dos valores dos indicadores epidemiológicos analisados: E. Dispensação de Benzimidazol. F. Interações por doença de Chagas; G. Óbitos por doença de Chagas; H. Prevalência de detecção na triagem sorológica da Hemoba (Naturalidade); I. Chagas autorreferido 0-14 anos; J. Chagas autorreferido >15 anos. Legenda: Na parte superior os dados estão exibidos por quantil; *Hot Spot Analysis* (Getis-Ord G_i^*) dos dados descritos acima;

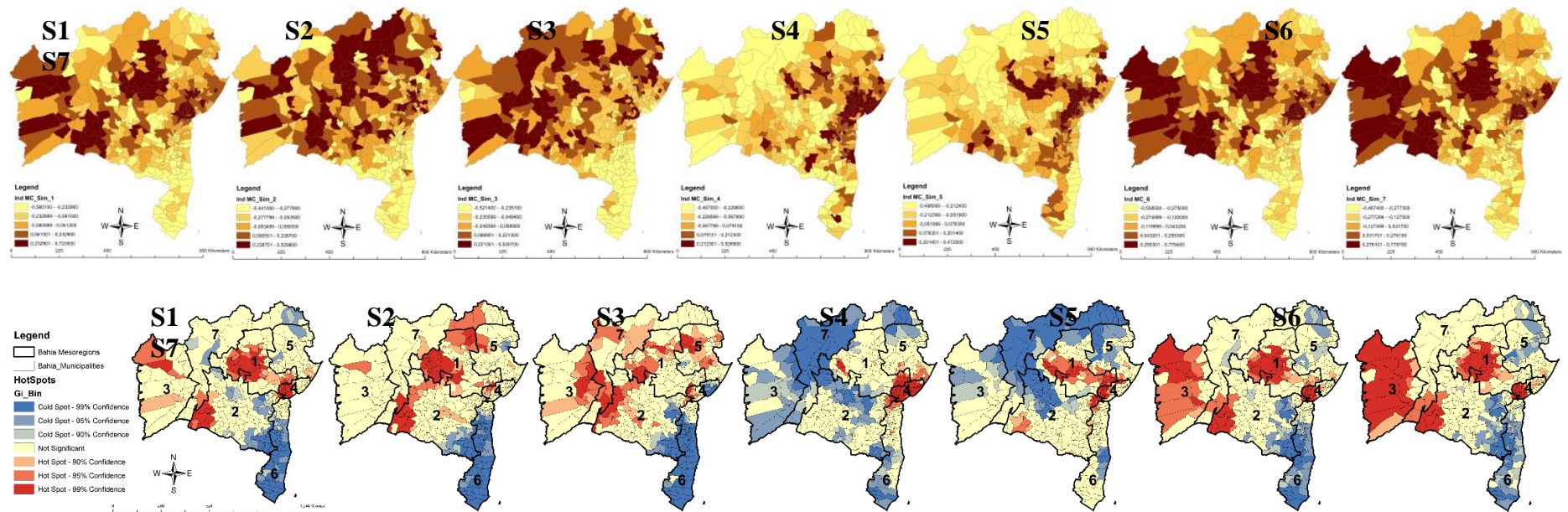


Fig. 4. Simulações da Análise Multicritério de Decisão: Simulações: S1, S2, S3, S4, S5, S6 e S7; Legenda: Na parte superior os dados estão exibidos por quantil; na parte inferior são apresentadas as *Hot Spot Analysis* (Getis-Ord G_i^*), agrupados por mesorregião (1-7), dos dados descritos logo acima; os pesos dos indicadores usados nas simulações S1-S7 estão descritos na Tabela 2.

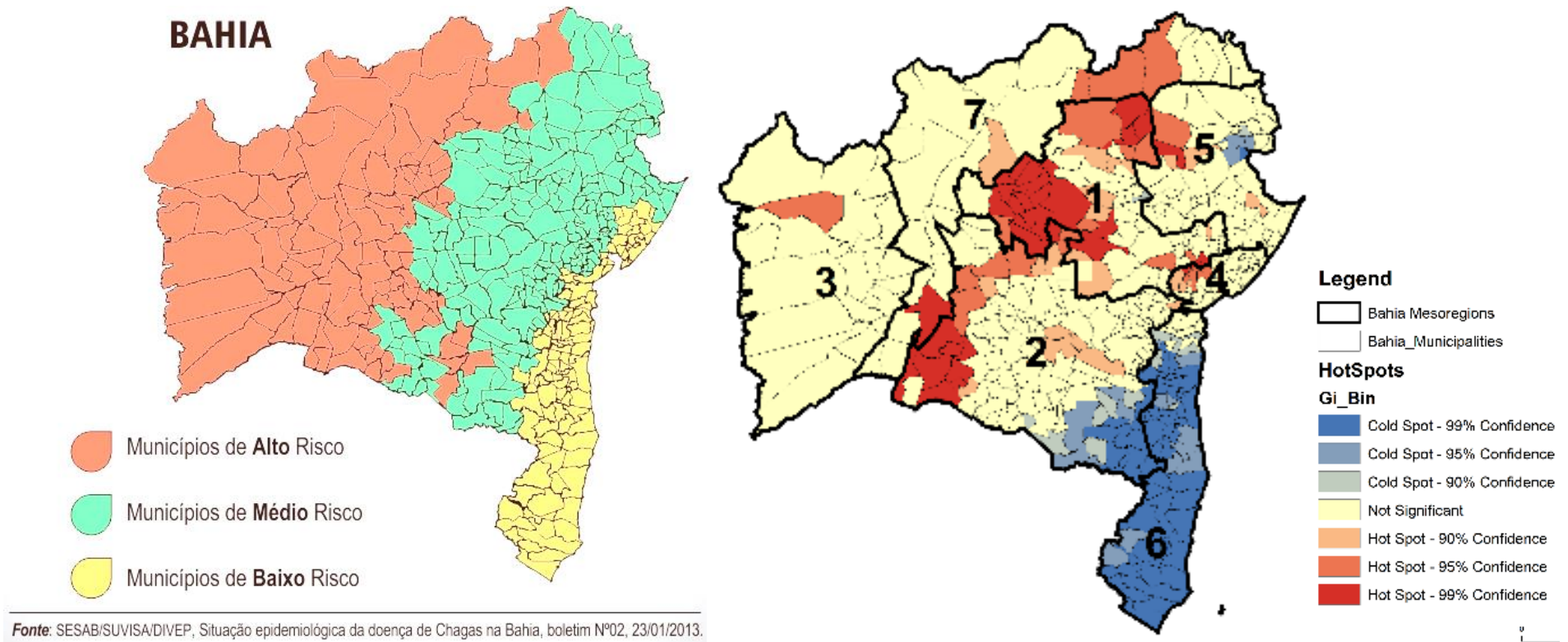


Fig. 5. Comparação entre a classificação atual de risco de transmissão da doença de Chagas e modelo de vulnerabilidade de transmissão vetorial do *T. cruzi* na Bahia. A. Distribuição dos municípios segundo grau de risco. B. Modelo de vulnerabilidade proposto (Simulação S2).

Appendice 1

Lista dos municípios de alta vulnerabilidade na Bahia, organizado por ordem alfabética.

América Dourada; Andorinha; Angical; Aracatu; Barra da Estiva; Barreiras; Barro Alto; Bom Jesus da Lapa; Boquira; Cabaceiras do Paraguaçu; Cafarnaum; Campo Formoso; Canarana; Candiba; Cansanção; Castro Alves; Caturama; Central; Conceição da Feira; Correntina; Cotegipe; Curaçá; Feira de Santana; Guanambi; Ibipeba; Ibititá; Iramaia; Iraquara; Irará; Irecê; Itaberaba; Itaguaçu da Bahia; Itiúba; Ituaçu; Jacobina; Jaguarari; Jequié; João Dourado; Juazeiro; Jussara; Lamarão; Lapão; Macaúbas; Maragogipe; Matina; Miguel Calmon; Mirangaba; Mulungu do Morro; Mutuípe; Olindina; Oliveira dos Brejinhos; Palmas de Monte Alto; Paramirim; Paripiranga; Pindaí; Pindobaçu; Piraí do Norte; Piritiba; Presidente Dutra; Riacho de Santana; Ruy Barbosa; Salvador; Santa Brígida; Santa Rita de Cássia; Santa Teresinha; Santana; Santo Amaro; São Félix; São Gabriel; São Gonçalo dos Campos; São Sebastião do Passé; Seabra; Senhor do Bonfim; Serrinha; Souto Soares; Tabocas do Brejo Velho; Tucano; Uibaí; Utinga; Wagner;

6. DISCUSSÃO GERAL

Nesta pesquisa realizamos: (a) um inquérito entomológico para determinação das frequências de infecção e fontes alimentares dos vetores; (b) uma proposta para classificação para triatomíneos e territórios geográficos, de forma lógica e reproduzível; (c) um estudo comparativo de dados históricos das populações de vetores; e (d) uma avaliação para identificar áreas vulneráveis à transmissão vetorial de *T. cruzi*, contemplando indicadores entomológicos, ambientais, socioeconômicos, demográficos e epidemiológicos.

No primeiro manuscrito desta pesquisa realizamos a coleta de vetores de *T. cruzi* em diversas áreas do estado da Bahia; assim como, a avaliação de indicadores entomológicos, tais como, o índice de infecção natural por *T. cruzi* e, as fontes alimentares dos triatomíneos selecionados. Identificamos 15 diferentes espécies durante o período do estudo. As espécies que mais encontramos no ambiente doméstico (intra e peridomiciliar) dos municípios amostrados foram: *Triatoma sordida*, *Triatoma pseudomaculata* e *Triatoma brasiliensis*. O percentual de municípios com ocorrência dessas três espécies foi de 90,24%. As espécies identificadas colonizando o ambiente intradomiciliar foram *T. sordida*, *T. pseudomaculata* e *T. juazeirensis*, em municípios de diferentes regiões do estado. A espécie *P. megistus* foi observada apenas de forma intrusiva nas residências e o *T. infestans*, em apenas um município. Estas observações demonstram o sucesso relativo no controle das espécies domésticas e domiciliadas, com a quase eliminação de *T. infestans* dos municípios da Bahia. O estudo revela também, a capacidade vetorial de outras espécies em colonizar o ambiente domiciliar, a exemplo de *T. juazeirensis*, *T. sordida* e *T. pseudomaculata*.

Espécies domiciliadas como o *T. infestans* e *P. megistus* foram amplamente combatidas no Brasil, o que causou redução na infestação e na ocorrência dos triatomíneos nos municípios em diversas regiões do Brasil por estas espécies (Silva *et al.*, 2012; Mendonça *et al.*, 2015). Apesar disso, ainda são registrados focos residuais de *T. infestans* em algumas regiões da Bahia

(Jannuzzi e Miranda, 2009; Hongoh *et al.*, 2011) e do Brasil (Perez *et al.*, 2014), e com esses focos, serão diversos os desafios enfrentados pelos programas de controle vetorial (Committee, 2001).

As espécies *T. sordida*, *T. pseudomaculata* e algumas do complexo *T. brasiliensis* (lato senso), entretanto, têm demonstrado a capacidade de ocupar o ambiente domiciliar, seja o peridomicílio e seus anexos (Pires *et al.*, 1999; Maeda *et al.*, 2012) ou mesmo, o intradomicílio (Mendonça *et al.*, 2015), sendo encontrados sob camas e entre fendas das casas de taipa, e algumas vezes associados a surtos de transmissão oral da doença de Chagas (Dias, J. *et al.*, 2008).

A infecção natural por *T. cruzi* foi de aproximadamente 10% considerando todos os triatomíneos analisados; e variou de acordo com a espécie avaliada e com o ambiente de coleta do triatomíneo. *T. cruzi* foi observado em vetores infectados por todas as regiões do estado da Bahia, inclusive, no ambiente intradomiciliar. Pesquisadores demonstraram que a avaliação da infecção dos triatomíneos por *T. cruzi*, pelo exame de compressão abdominal, pode apresentar resultado subestimado (Haidamak *et al.*; Minuzzi-Souza *et al.*, 2018) e que a alta taxa de infecção dos vetores sinantrópicos pode ser um sinal de alarme para a transmissão domiciliar de *T. cruzi* (Silveira, Ribeiro e Amorim, 2016).

Os resultados de infecção natural observados foram similares aos obtidos em estudos recentes realizados em Pernambuco (Silva *et al.*, 2012), São Paulo (Cominetti *et al.*, 2014), Mato Grosso do Sul (Cominetti *et al.*, 2014), Ceará (Gonçalves e Freitas, 2009) e mesmo na Bahia (Mendonça *et al.*, 2015). A espécie *T. sordida* já foi identificada infectada e também, colonizando o ambiente intradomiciliar; corroborando com pesquisas recentes na Bahia (Mendonça *et al.*, 2015), entretanto, essa ocorrência ainda não foi descrita nos estados citados acima. Tal fato demonstra a necessidade da autonomia regional dos programas de controle

vetorial e combate à transmissão de *T. cruzi* durante a implementação das políticas do PNCDC (Jannuzzi *et al.*, 2009; Salvatella *et al.*, 2014).

A espécie *T. infestans*, encontrada em galinheiro, não foi observada infectada pelo *T. cruzi* (Brandão, H. *et al.*, 2015), uma vez que todos os exemplares analisados foram coletados em uma única colônia, em galinheiro próximo a unidades domiciliares (Guarneri *et al.*, 2000; Pereira *et al.*, 2006).

As fontes alimentares mais frequentes nos triatomíneos foram aves, que além da alta prevalência (>75%) demonstraram também ampla distribuição espacial na Bahia, fato observado em outras pesquisas (Sarquis *et al.*, 2010; Ribeiro *et al.*, 2015). As aves constituem um importante elo no processo de domiciliação dos triatomíneos, pois é um recurso alimentar encontrado amplamente no ambiente silvestre (Almeida *et al.*, 2002) e comumente criadas em galinheiros ou soltas no peri e intradomicílio das residências (Rego *et al.*, 2006; Hernandez *et al.*, 2013), favorecendo a ocorrência de colônias nestes ambientes (Da Silva *et al.*, 2011; Ricardo-Silva, A. *et al.*, 2016).

Observamos triatomíneos alimentados de “Cão”, “Homem” e “Gato” respectivamente com 6%, 1% e 1% de frequência. Estudos demonstram o importante papel dos animais domésticos na manutenção da circulação de *T. cruzi* dentro das UDs, aumento no número de picadas aos humanos, e aumento do risco de transmissão vetorial de *T. cruzi* ao homem (Pizarro e Stevens, 2008; Gottdenker *et al.*, 2012; Curtis-Robles *et al.*, 2015), e a observação de DNA humano no intestino dos triatomíneos infectados demonstra áreas consideradas sob risco para transmissão de *T. cruzi*.

As espécies encontradas com DNA humano em seu conteúdo abdominal foram *T. brasiliensis*, *T. juazeirensis* e *T. sordida*. Diversas espécies de triatomíneos do complexo *Triatoma brasiliensis* demonstraram competência em transmitir *T. cruzi* ao homem no seu ambiente domiciliar (Guarneri *et al.*, 2000; Fuente *et al.*, 2007; Sarquis *et al.*, 2010; Batista *et*

al., 2013; Costa *et al.*, 2014; Correia, 2015; De Souza, 2015). Com relação à distribuição espacial das observações de fontes alimentares e de *T. cruzi*, foi observada a formação de um agrupamento de observações de triatomíneos infectados e alimentados com DNA humano ou de animais domésticos na região Nordeste da Bahia, nas regiões de Juazeiro, Curaçá e Irecê.

No segundo manuscrito desta tese desenvolvemos um novo índice entomológico. O TriatoScore é uma medida de um único dígito do risco entomológico da doença de Chagas que (i) abrange espécies nativas e não nativas de triatomíneos, (ii) aborda a questão de registros de ocorrência de vetores localmente incompletos e (iii) é projetado para apoiar tomada de decisão na escala espacial mais relevante para sistemas descentralizados de controle-vigilância. Nossa abordagem aproveita o melhor conhecimento disponível sobre ecologia-comportamento e distribuição-biogeografia de espécies individuais de triatomíneos para calcular TriatoScores locais (por exemplo, municipais), que por sua vez podem ser usados para estratificar e mapear o risco entomológico em unidades espaciais maiores. Quando baseada em TriatoScores padronizados, a estratificação de risco torna-se um exercício fundamentalmente dinâmico – as mudanças nas faunas vetoriais locais (ou em nosso conhecimento sobre elas) são automaticamente contabilizadas à medida que as unidades espaciais locais são reatribuídas aos estratos de risco em relação ao risco médio recalculado em todas as unidades. Embora a versão mais básica do TriatoScore use apenas dados vetoriais, a integração de informações epidemiológicas, demográficas, ambientais ou operacionais é simples. O TriatoScore pode, portanto, tornar-se uma adição útil a caixa de ferramentas de vigilância e controle de vetores da doença de Chagas.

Os valores do TriatoScore foram, em geral, mais altos em municípios dominados por ecorregiões secas a semiáridas, mais baixos em municípios onde predominam florestas úmidas e intermediários em municípios dominados por savana-campos sazonalmente secos. O maior risco entomológico na Caatinga e Mata Atlântica reflete tanto uma riqueza particularmente alta

de espécies de triatomíneos quanto o fato de que muitas dessas espécies são frequentemente encontradas infestando ou invadindo casas (Tabela 1).

Enquanto a porção leste do Cerrado que cobre o oeste da Bahia também é bastante rica em espécies, pelo menos três das 11 espécies de triatomíneos que ocorrem lá (*Panstrongylus diasi*, *Cavernicola pilosa* e *Psammolestes tertius*) raramente são encontradas dentro ou ao redor das casas. Na Bahia, a maior diversidade de triatomíneos se encontra ao longo do vale do Rio São Francisco, e o mapeamento do TriatoScore revelou um padrão de maior risco entomológico naquela região. Em contraste, as savanas montanhosas Campos Rupestres da Serra da Mangabeira abrigam apenas três espécies de triatomíneos, e os valores do TriatoScore foram baixos em dez municípios (com ~10% a ~50% do território correspondente aos Campos Rupestres) localizado ao longo de uma estreita faixa diagonal sudeste-noroeste no centro do estado. Finalmente, 6 das 10 espécies de triatomíneos que ocorrem nas ecorregiões costeiras mais úmidas não parecem capazes de infestar as casas de forma estável. Portanto, os valores do TriatoScore são particularmente baixos ao longo da costa central e sul da Bahia. As análises geoespaciais confirmaram esses padrões ao mostrar (i) um hotspot leve de maior risco entomológico em municípios ao longo do vale do São Francisco e nas serras que o delimitam, (ii) um hotspot raso de menor risco entomológico em municípios ao longo do vale do São Francisco costa centro-sul e (iii) duas áreas separadas de agrupamento não significativo de valores do TriatoScore: (a) a região oeste dominada pelo Cerrado e (b) a região dominada pela Caatinga a leste do São Francisco mais a costa norte mais seca.

Ressaltamos ainda a importância da correta identificação taxonômica dos exemplares de triatomíneos coletados para o sucesso desta abordagem, dessa forma, deverão ser reforçados os treinamentos das equipes municipais e estaduais para determinação taxonômica dos triatomíneos, bem como o uso de materiais didáticos dedicados a este fim (SOUSA *et al.*, 2020).

Nossa perspectiva é que os serviços de vigilância utilizem o TriatoScore na priorização das ações de controle de triatomíneos no Brasil. Atualmente, a indicação do controle químico é baseada somente na ocorrência de triatomíneos em ambiente domiciliar, não levando em consideração outros critérios incluídos no TriatoScore. Adicionalmente, a utilização do TriatoScore permite gerar mapas para indicar as áreas que merecem mais atenção da vigilância entomológica. A validação do TriatoScore poderá ser realizada ao comparar indicadores epidemiológicos da doença de Chagas nos municípios com alto e baixo valor do TriatoScore. Por exemplo, espera-se encontrar maior número de casos agudos, internações por doença de Chagas em municípios com maiores valores do TriatoScore. O serviço de vigilância entomológica também pode usar o mapeamento dos *hotspots* indicados pelo TriatoScore para guiar a distribuição de inseticidas ou cobertura das áreas de melhoria habitacional.

A utilização de indicadores entomológicos para classificar os triatomíneos utilizando variáveis incorporadas a um índice (TriatoScore) para a vigilância e controle de triatomíneos é uma iniciativa importante para monitorar o potencial de transmissão vetorial nos municípios. A abordagem apresentada pode se tornar uma forma alternativa para classificação de triatomíneos a partir das informações disponíveis no serviço de vigilância entomológica, num sistema de valores, de forma a direcionar ações de controle vetorial. A utilização do TriatoScore é promissora, pois leva em consideração diversas informações importantes para a transmissão de *T. cruzi* ao homem, além de ser reprodutível, permitindo a associação das informações de diversas regiões. Esperamos que a utilização do TriatoScore seja uma ferramenta relevante para a tomada de decisões políticas relacionadas ao controle dos vetores da doença de Chagas.

No terceiro manuscrito demonstramos que a ocorrência das espécies de triatomíneos sinantrópicos na Bahia mudou após 44 anos de ações sistematizadas de controle iniciadas em 1975. Entre 1957 e 1971, *P. megistus* era a espécie predominante. Após a certificação

internacional de eliminação da transmissão da doença de Chagas por *T. infestans* em 2006, *T. sordida* e *T. pseudomaculata* foram as espécies mais frequentes na Bahia.

De forma geral, observamos clara redução da ocorrência das espécies com maior importância epidemiológica na transmissão de *T. cruzi* nas décadas de 1960 e 1970. No período A, *T. infestans* ocorria em 9% dos municípios e representava 3,7% dos triatomíneos coletados enquanto que *P. megistus* ocorria em 42% dos municípios com abundância relativa de 62% dos triatomíneos coletados (SHETLOCK; SERAFIM, 1972). No período B, *T. infestans* foi registrado em apenas sete municípios em colônias residuais (ARAÚJO *et al.*, 2014; BRANDÃO *et al.*, 2015), representando 0,4% dos triatomíneos coletados. Além disso, a ocorrência de *P. megistus* reduziu muito na Bahia, principalmente nos municípios do recôncavo baiano e região metropolitana de Salvador. A redução da ocorrência de *P. megistus* também foi observada em outros estados brasileiros (PASSOS; SILVEIRA, 2011; SILVA *et al.*, 2012; DIAS, J. V. *et al.*, 2016). Três hipóteses poderiam explicar a redução da ocorrência de *P. megistus* na região metropolitana de Salvador. Primeiro, o controle químico foi realizado com sucesso ao longo de quatro décadas com sucesso resultando na eliminação das populações domésticas de *P. megistus*. Segundo, os processos de urbanização intensa nesses municípios resultaram em desmatamento e fragmentação de áreas de mata atlântica, habitat natural da espécie (GURGEL-GONCALVES *et al.*, 2012). Terceiro, houve melhoria habitacional, com redução progressiva da ocorrência de casas de adobe/pau a pique (RODRIGUES; SALVADOR, 2011; PEREIRA, 2013), ambiente favorável para colonização de *P. megistus*. A eliminação de *T. infestans* em vários municípios do oeste e região central na Bahia é explicada pelo Plano de Eliminação do *Triatoma infestans*, que foi intensificado em 2004 com ações de aplicação de inseticidas no ambiente domiciliar, seguidas de pesquisa e captura de triatomíneos, que foram concluídas em ~500 mil casas (DIAS, 2007). Entretanto, observa-se que colônias residuais da espécie foram detectadas em outros municípios (ARAÚJO *et al.*, 2014;

BRANDÃO *et al.*, 2015) o que exige monitoramento constante para eliminação definitiva de *T. infestans* da Bahia. Dessa forma, acreditamos que as atividades de vigilância e controle de triatomíneos devem ser priorizadas nesses municípios. Apesar das limitações do uso de base de dados referenciais, o uso de informações dos sistemas de vigilância é uma abordagem comum nas diferentes regiões do Brasil (SILVEIRA *et al.*, 2001; CARVALHO *et al.*, 2011; SILVEIRA, 2011; VILLALOBOS *et al.*, 2011).

Na Bahia, além do controle químico realizado a partir de 1975, e intensificado a partir de 1991, com objetivo de eliminar *T. infestans*, ações sociais implementadas pelo Governo Federal, como o Programa de Aceleração de Crescimento (PAC), que visam a melhoria da qualidade habitacional, com substituição das casas de taipa por residências de alvenaria e reboco nas paredes, também podem ter influenciado na redução colonização domiciliar por *T. infestans* e outras espécies domiciliares (PEREIRA, 2013).

Apesar do sucesso das ações de combate aos triatomíneos, diversas espécies nativas, consideradas secundárias na transmissão da doença de Chagas, foram registradas em um número maior de municípios, com ocorrência no interior das casas e frequentemente colonizando o peridomicílio, alguns dos quais estando infectados e muitos deles estando alimentado de animais domésticos e mesmo sangue humano (RIBEIRO *et al.*, 2019). No período A, *T. sordida* ocorria em 26% dos municípios e representava 23% dos triatomíneos coletados enquanto *T. pseudomaculata* ocorria em 14% dos municípios com abundância relativa de 2,5% dos triatomíneos coletados. No período B, *T. sordida* foi registrado em 38% municípios e representou ~82% dos triatomíneos. *T. sordida* tem sido a espécie mais abundante, com maior frequência no intra e peridomicílio de casas em diferentes regiões da Bahia. Em situação semelhante, *T. pseudomaculata* foi a espécie que ocorreu em maior número de municípios do estado no período B (42%). Aparentemente, as espécies *T. sordida* e *T. pseudomaculata* tiveram a sua distribuição espacial no estado da Bahia pouco impactada pelas

sistemáticas ações de combate vetorial. Observou-se maior ocorrência de *T. sordida* no oeste baiano e região centro-sul da Bahia onde há áreas de cerrado, bioma de origem de populações naturais dessa espécie (MAEDA *et al.*, 2012; GALVÃO, 2014; GALVAO; JUSTI, 2015). A maior ocorrência de *T. pseudomaculata* nos municípios do oeste (áreas de cerrado) e da região central (caatinga) da Bahia está de acordo com predições de ocorrência da espécie baseada em variáveis ambientais (GURGEL-GONCALVES *et al.*, 2012). O número de exemplares das espécies *T. sordida* e *T. pseudomaculata* somados superaram 90% de todos os triatomíneos coletados no estado entre 2006 e 2019.

Na última etapa desta tese, manuscrito quatro, realizamos uma análise de vulnerabilidade a nível municipal para o Estado da Bahia, através da análise multicritério de decisão (AMD) e geoestatística, utilizando indicadores entomológicos, ambientais, socioeconômicos (PIB e percentual de domicílios na zona rural em situação de extrema pobreza - 2010), ambientais e epidemiológicos do PNCDCh: (a) dispensação de benzonidazol; (b) autorização de internação hospitalar; (c) Óbitos por doença de Chagas; (d - e) doença de Chagas auto referido; (f - g) residência e naturalidade de pacientes detectados na triagem sorológica do Hemoba; de forma a desenvolver a análise multicritério de decisão (AMD) para avaliação da vulnerabilidade de transmissão vetorial de *T. cruzi* por vetores sinantrópicos no Estado da Bahia. A abordagem multicritério de decisão tem sido amplamente utilizada em pesquisa, para entender os determinantes da doença e especialmente para ajudar gestores em saúde na tomada de decisão (Haidamak *et al.*; Silveira, Ribeiro e Amorim, 2016).

Os mapas resultantes da AMD indicaram a existência de municípios com alta vulnerabilidade para transmissão domiciliar de *Trypanosoma cruzi* em praticamente todo o território do Bahia, com exceção da Região Sul, onde a grande maioria dos municípios foi classificada como de baixa vulnerabilidade em praticamente todas as simulações realizadas. O

indicador pobreza na zona rural demonstra que a Mesorregião Sul da Bahia se destaca por apresentar menor pobreza, em relação às outras mesorregiões avaliadas.

A existência de áreas vulneráveis à transmissão vetorial de *T. cruzi* por triatomíneos sinantrópicos do Brasil, através da AMD, já havia sido descrito (VINHAES *et al.*, 2014), chamando atenção para o grande número de municípios vulneráveis em contraste com o baixo número de casos agudos e crônicos notificados (VINHAES *et al.*, 2014). De forma a aprimorar o proposto por VINHAES *et al.* (2014), detalhamos nesta pesquisa as classes de indicadores, aprimoramos a classificação ponderada dos vetores (TriatoScore), levando em considerações importantes aspectos regionais da fauna dos vetores de *T. cruzi*. Ademais, consideramos os indicadores epidemiológicos disponíveis no sistema de vigilância estadual (SESAB-DIVEP) para a avaliação de áreas vulneráveis à transmissão de *T. cruzi* através da AMD, bem como utilizamos ferramentas de geoprocessamento específicas para delimitação de áreas de *hotspots* e *coldspots* (*Getis-Ord Gi Statistic*), facilitando o processo de localização de municípios vizinhos e vulneráveis.

A classificação municipal de risco de transmissão de *T. cruzi* oficial na Bahia classifica 97 municípios (23,3%) como de alto risco, 219 (52,5%) como de médio risco, e 101 (24,2%) como de baixo risco. Essa classificação foi desenvolvida a nível central, pelo ministério da saúde, entretanto, não considera características regionais importantes, como a diversidade vetorial e os indicadores epidemiológicos regionais, ou mesmos os indicadores sociodemográficos apresentados. Na simulação 02, por exemplo, que atribui maior peso ao indicador vetorial (Figura 3B, Tabela 2), a AMD classificou 151 (36%) municípios com algum nível de vulnerabilidade pela análise dos scores obtidos na AMD, e com apoio da análise de *hotspots* e *coldspots* identificamos os agrupamentos de municípios altamente vulneráveis e vizinhos, através da análise do GiZScore e número de vizinhos das feições analisadas, sendo 63 (15%) considerados municípios de alta vulnerabilidade, e agrupados em quatro diferentes

hotspots, localizados nas regiões centro sul, vale do São Francisco, metropolitana de Salvador e em espacial a região centro-norte, que apresentou maior número de municípios considerados como de alta vulnerabilidade, além de recorrência em todas as simulações geradas.

Por fim, a estratégia apresentada de analisar diferentes indicadores através da AMD e geoestatística foi exitosa em estabelecer regiões específicas mais vulneráveis no Estado da Bahia, tangíveis à ação dos sistemas de vigilância e controle do agravo, além de ser uma metodologia reprodutível e escalonável. Entretanto, buscando estratégias para lidar com limitações comuns no uso de dados secundários, tais como a não notificação e ou subnotificação dos indicadores avaliados, e limitações da técnica de AMD, sugerimos que outras estratégias, não cartesianas, como a lógica fuzzy possa ser utilizada para obtenção de projeções mais precisas e imparciais.

7. CONCLUSÕES

7.1 Infecção por *T. cruzi* e padrões alimentares de triatomíneos capturados no Estado da Bahia entre 2013 e 2014

Triatomíneos infectados por *T. cruzi* permanecem amplamente distribuídos na Bahia; a maioria dos triatomíneos se alimentou de aves, mas humanos, cães e gatos também foram fontes alimentares desses insetos principalmente na região Nordeste da Bahia, mantendo assim o risco potencial de transmissão de *T. cruzi*. Apesar da maioria dos triatomíneos ter sido capturada no peridomicílio, foram detectadas colônias de *Triatoma sordida*, *T. pseudomaculata* e espécies do complexo *T. brasiliensis* dentro das casas. Os inquéritos entomológicos, associados à vigilância molecular, e geoestatística são úteis para revelar a infecção de *T. cruzi* e fontes alimentares de triatomíneos sinantrópicos, identificando áreas com risco para a transmissão de *T. cruzi* ao homem e animais domésticos.

7.2 TriatoScore: um indicador composto para vigilância de triatomíneos.

A eliminação histórica de *Triatoma infestans* de 125 municípios reduziu os TriatoScores em ~27% (intervalo, 20–44%); oito municípios relataram *T. infestans* desde que a Bahia foi certificada livre da transmissão de *Trypanosoma cruzi* por esta espécie não nativa. Os estratos de risco entomológico baseados nos TriatoScore concordaram bem com os estratos oficiais de risco para doenças da Bahia, mas os TriatoScores sugerem que a classificação oficial provavelmente subestima o risco em 42 municípios. Dos 152 municípios que não notificaram triatomíneos em 2006-2019, dois e 71 tiveram TriatoScores correspondentes, respectivamente, a risco entomológico alto e moderado. O TriatoScore pode ajudar os gerentes de controle e vigilância a avaliar e estratificar com flexibilidade o risco entomológico da doença de Chagas em escalas operacionalmente relevantes. Integração de dados eco epidemiológicos, demográficos, socioeconômicos ou operacionais (sobre, por exemplo, frequências de infestação de residências ou infecções por

vetores em escala local, mudança no uso da terra e urbanização, condições de moradia, pobreza ou funcionamento de sistemas de controle e vigilância) também é direto. O TriatoScore pode, assim, se tornar uma adição útil à caixa de ferramentas de controle e vigilância de bugs triatomíneos.

7.3 Ocorrência de triatomíneos sinantrópicos na Bahia após 40 anos do programa de controle de vetores

Houve clara redução da ocorrência de *P. megistus* e *T. infestans* e aumento da abundância relativa e distribuição geográfica de *T. sordida* e *T. pseudomaculata* após 40 anos de programa de controle vetorial na Bahia. A alta frequência de outras espécies de triatomíneos nos municípios da Bahia e a grande abundância de *T. sordida* nos últimos anos evidencia a necessidade de reforçar as ações permanentes de vigilância entomológica da doença de Chagas.

7.4 Identificação de municípios mais vulneráveis para transmissão vetorial de *T. cruzi* no Estado da Bahia.

Municípios com alta vulnerabilidade para transmissão vetorial de *T. cruzi* foram identificados em praticamente todo o território da Bahia, com exceção da região Sul, onde a grande maioria dos municípios foi classificada como de baixa vulnerabilidade. Os municípios mais vulneráveis foram localizados nas Regiões Centro-Sul, Vale do São Francisco, Metropolitana de Salvador e principalmente na Região Centro-Norte, que apresentou maior número de municípios considerados como de alta vulnerabilidade, além de ser uma área recorrente, independente das simulações realizadas. A combinação da AMD com a geoestatística facilitou no processo de localização de agrupamento de municípios vizinhos e altamente vulneráveis, delimitando áreas tangíveis para as ações de vigilância e controle da transmissão da doença de Chagas.

Outras estratégias, não cartesianas, como a lógica fuzzy podem ser utilizadas para obtenção de projeções mais precisas e imparciais, e lidar com limitações comuns no uso de

dados secundários, tais como a não notificação e ou subnotificação dos indicadores avaliados por parte dos municípios, e limitações da técnica de AMD.

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FORMULÁRIO DE CAMPO – ENTOMOLOGIA

Página 2 de 2

4 DADOS ECOLÓGICOS		(Digitar no Banco de Dados 1 – Formulário de Campo)																																																																																																																																																															
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Local

Data

Pesquisador / Técnico de Campo

9.2. Apêndice 2 – Formulário digital do Google docs utilizado para dupla digitação e informatização dos dados coletados em campo – Digitação um e dois.

FORM. DE CAMPO - ENTOMOLOGIA

DIGITAÇÃO 1

*Obrigatório

1. INFORMAÇÕES GERAIS

Digite abaixo as informações gerais de seu formulário.
Item 1 do seu formulário de campo.

Nº do formulário de campo *

Digite o Número do Formulário de Campo

Sua resposta

Nome do Digitador *

Selecione seu nome

Escolher

1.1. Nome do Coletor *

Digite o nome do coletor (técnico de campo)

Sua resposta

1.2. Nº SIAPE *

Nº SIAPE do técnico de campo

Sua resposta

1.3. DIRES *

Digite a DIRES onde foi realizada a coleta

Escolher

1.4. Data da Coleta

Digite o dia da coleta com números e dois dígitos. Ex: 23
Data

dd/mm/aaaa

PRÓXIMA

FORM. DE CAMPO - ENTOMOLOGIA

DIGITAÇÃO 2

*Obrigatório

1. INFORMAÇÕES GERAIS

Digite abaixo as informações gerais de seu formulário.
Item 1 do seu formulário de campo.

Nº do formulário de campo *

Digite o Número do Formulário de Campo

Sua resposta

Nome do Digitador *

Selecione seu nome

Escolher

1.1. Nome do Coletor *

Digite o nome do coletor (técnico de campo)

Sua resposta

1.2. Nº SIAPE *

Nº SIAPE do técnico de campo

Sua resposta

1.3. DIRES *

Digite a DIRES onde foi realizada a coleta

Sua resposta

1.4. Data da Coleta

Digite o dia da coleta com números e dois dígitos. Ex: 23
Data

dd/mm/aaaa

PRÓXIMA

9.4. Apêndice 4 – Coautoria de artigo publicado: *Trypanosoma cruzi* and *Leishmania* spp. infection in Wildlife from Urban Rainforest Fragments in Northeast Brazil.

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TRYPANOSOMA CRUZI AND LEISHMANIA SP. INFECTION IN WILDLIFE FROM URBAN RAINFOREST FRAGMENTS IN NORTHEAST BRAZIL

Inidra Trüeb,¹ Ricardo D. Portela,² Carlos R. Franke,¹ Ianei O. Carneiro,¹ Gilmar J. Ribeiro Jr.,³ Rodrigo P. Soares,⁴ and Stella Maria Barrouin-Melo^{1,5,6}

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ABSTRACT: *Trypanosoma cruzi* and *Leishmania* sp. are important protozoan parasites for humans and animals in the Americas, causing Chagas disease and cutaneous or visceral leishmaniasis, respectively. These vector-borne diseases affect permanent and transient populations in developing tropical countries that exhibit favorable conditions for the perpetuation of the parasite cycle. Our objective was to investigate the occurrence of infection with these parasites in wild animals from urban rainforest fragments in the city of Salvador, the largest city in the northeast region of Brazil. Sixty-five wild animals were captured, clinically examined, and sampled for parasite detection by PCR and culture. Ten different mammalian genera were identified, being 58% (38/65) marsupials. The prevalence of *T. cruzi* and *Leishmania* sp. infections was 13% and 43%, respectively. Both parasites were detected by PCR in 11% (7/65), three of which were also double infected as determined by culture. Among the 28 animals found infected with at least one parasite (43%, 28/65), 68% (19/28) were marsupials, two specimens were *Callithrix* sp. (7%), and one was *Trinomys* sp. (3%). Most infected animals (89%) had no clinical signs of disease. We found that healthy free-living animals from urban rainforest fragments harbored pathogenic trypanosomatids and should be included in epidemiology studies of diseases in big cities in tropical countries, as these cities grow and engulf rainforest remnants.

Key words: Callithrix, Chagas disease, leishmaniasis, *Trinomys*, urban forests, wildlife infection.

INTRODUCTION

With the expansion of urban centers and the use of natural areas for agriculture or livestock breeding, native forest areas have been gradually fragmented, affecting ecosystems (Grisotti 2010). Ecological disturbances create an imbalance in parasite-wildlife cycles, reaching humans in areas next to forest fragments (Epstein and Price 2009). In this context, wild animals play an important role in the urban transmission of vector-borne zoonoses, maintaining parasite transmission cycles (de Almeida Curi et al. 2014). Life cycles of Trypanosomatidae protozoa are examples of this process, because they share epidemiological characteristics related to vector and

reservoir adaptations to urban environments (Souza et al. 2009). Enzootic infections in wildlife are a risk for public health, because after feeding in wild animals, vectors can easily reach urban humans (Dias-Lima and Sherlock 2000). Therefore, the monitoring of infectious diseases in these animals, some of them endangered species, is important for surveillance and control of emerging and reemerging diseases (McFarlane et al. 2012).

Trypanosoma cruzi and *Leishmania* sp. are parasites commonly found in developing tropical countries, such as Brazil, in areas that nowadays include large cities as well as in areas favored by tourists. Invertebrate Triatominae and Phlebotominae vectors transmit *T. cruzi* and *Leishmania* sp., respectively, to

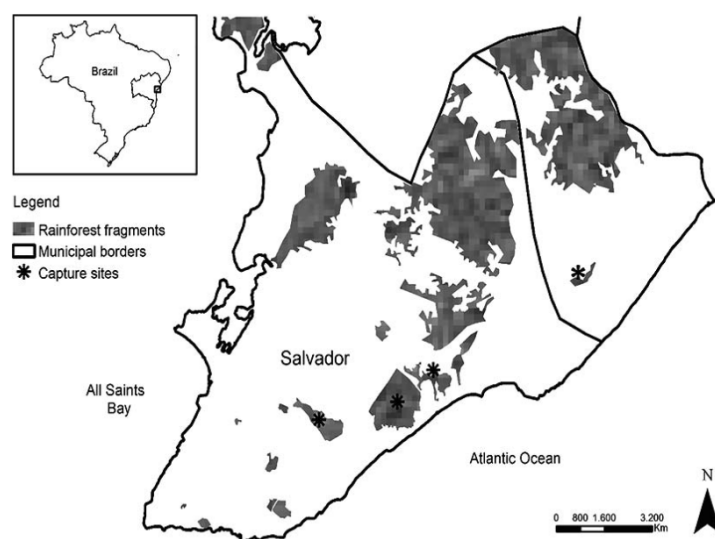


FIGURE 1. Map of Salvador metropolitan region, Bahia State, Brazil ($12^{\circ}58'13''S$, $38^{\circ}30'45''W$). Urban rainforest fragments are shown in dark gray. Asterisks mark the four sites where wild animals were captured, examined, and sampled for parasitological and molecular diagnosis of infection by *Trypanosoma cruzi* and *Leishmania* sp. Clear areas represent the urbanized surface of the city, which has almost 3 million inhabitants.

humans and animals, causing Chagas disease and cutaneous or visceral leishmaniasis, which are among the greatest public health problems in the Americas (Thompson et al. 2010). Because of the increasing anthropic action on the environment, such vectors and wild reservoirs are in close contact with peridomestic and urban regions, following the occupation of recently deforested areas by humans (de la Fuente et al. 2008).

There are no human vaccines for Chagas disease or cutaneous or visceral leishmaniasis, diseases that have spread as fast as accelerated urbanization and occupation of rain forest areas have taken place in Brazil and other tropical countries. We describe the detection and prevalence of *T. cruzi* and *Leishmania* sp. in wild mammals captured from urban rainforest fragments located in Salvador, Brazil.

MATERIALS AND METHODS

Animal capture and identification

We worked in different rainforest fragments in the metropolitan region of Salvador, Bahia State, Brazil ($12^{\circ}58'13''S$, $38^{\circ}30'45''W$). Salvador has a

population of 2,921,087 inhabitants, making it the third-largest Brazilian city (Instituto Brasileiro de Geografia e Estatística 2015). The studied areas included Atlantic rainforest remnants that have undergone important anthropic influences, being progressively reduced and isolated by the growth of the city.

From January 2013 to December 2013, 20 capture efforts were made in four urban rainforest fragments, with five efforts in each of the studied areas (in January, April, July, October, and December), covering the rainy and the dry seasons. These collection sites in forest fragments inside the city of Salvador were chosen considering their neighboring areas, which are densely populated and had poor sanitary infrastructure, with no delimited borders between the rainforest area and the neighboring houses (Fig. 1). For free-living animal capture, 20 Sherman traps and 20 Tomahawk traps (Rosaminas, Piraúba, Brazil) were strategically placed in the rainforest, with banana, pineapple, and smoked pig fat used as bait. Traps were placed 20 m from each other in a central area of each rainforest fragment; the objective was to be as far as possible from the borders to avoid human destruction of the traps and because the wild animals might be frightened by human presence. The taxonomic classification of animals captured was made as previously described (Reis et al. 2011), and when it was not

TABLE 1. Number, by genus and species, of free-living wild mammals captured and examined for trypanosomatid (*Trypanosoma cruzi* and *Leishmania* sp.) infection, in rainforest urban fragments from Salvador City, Brazil, during 2012 and 2013.

Mammal	n (% total animals)
<i>Didelphis</i> sp. (opossum)	22 (33.8)
<i>Micoureus</i> sp. (opossum)	16 (24.6)
<i>Cerdocyon thous</i> (crab-eating fox)	5 (7.7)
<i>Nasua nasua</i> (coati)	1 (1.5)
<i>Dasyppus</i> sp. (armadillo)	5 (7.7)
<i>Callithrix</i> sp. (marmoset)	5 (7.7)
<i>Mus musculus</i> (mouse)	2 (3.1)
<i>Rattus rattus</i> (rat)	4 (6.2)
<i>Trinomys</i> sp. (rodent)	3 (4.6)
<i>Oecomys</i> sp. (rat)	2 (3.1)
Total	65 (100)

possible to perform complete species identification, genus classification was assumed.

Samples collection and clinical evaluation

Captured animals were anesthetized (Santiago et al. 2007) to undergo clinical examination by veterinarians with the objective to search for signs of disease such as mucosal paleness, congestion or jaundice, emaciation, lymph node enlargement, skin lesions, discharges from natural orifices, presence of ectoparasites, and sampling. Blood was collected from the tail vein, jugular vein, or femoral artery using vacuum tubes containing EDTA as an anticoagulant. Fine needle aspirations of spleen and liver were performed using sterile materials, adapting the method described for canids (Barrouin-Melo et al. 2006). All animals had their left ear marked through the excision of a 1–2 mm “V”-shaped fragment of the ear tip with the objective of avoiding data duplication and were returned to their habitat. Samples were refrigerated until laboratory processing.

All procedures were performed under the license of the Brazilian Institute of Environment and Renewable Resources–Sistema de Autorização e Informação em Biodiversidade (IBAMA–SISBIO no. 32058) and were approved by the Ethics Committee on Animal Use from the Federal University of Bahia (license no. 19/2011).

Parasite isolation and culture

Culture for parasite isolation was performed using 18 samples taken from the captured animals. Briefly, samples of whole blood, spleen, or hepatic puncture were cultivated in a biphasic

medium containing 1.5 mL of blood-agar solid medium and 2 mL of Schneider’s medium (Sigma-Aldrich, St. Louis, Missouri, USA) supplemented with 20% of fetal bovine serum (Life Technologies, Grand Island, New York, USA) and antibiotics. The cultures were incubated for 6 wk at 25 C and observed by optical microscopy once per week. Positive cultures were subjected to DNA extraction for PCR evaluation and parasite identification. Thirteen animal samples—five splenic samples, five liver samples, and eight blood samples—were analyzed by culture. These samples were taken when it was possible to 1) collect a sufficient amount of blood for the procedures and/or 2) perform liver or spleen puncture.

PCR for *T. cruzi* and *Leishmania* sp. detection

A PCR for *Leishmania* sp. detection was performed on genomic DNA extracted from blood samples from all 65 animals, as previously described (Rocha et al. 2010), using primers D1 (5′-GGGGAGGGCCGTTCTGCGAA-3′), D2 (5′-CCGCCCTA TTTACACCAACCCC-3′), and D3 (5′-GGCCCACTATATTA CACCAACCCC-3′). This PCR reaction amplifies the *Leishmania braziliensis*, *Leishmania infantum*, and *Leishmania amazonensis* kDNA minicircle (120 bp) genomic region, with high specificity and sensitivity.

A PCR for *T. cruzi* molecular detection was carried out as described (Leça-Junior et al. 2013) in blood samples from all 65 studied animals using the primers S35 (5′-AAATAATGTACGGGGGATGTCATGA-3′) and S36 (5′-GGTTCCGATTGGGTTGGTGT-3′). These primers are specific to *T. cruzi* kDNA conserved regions and were previously described as sensitive and specific tools for the parasite detection and classification (Avila et al. 1990).

RESULTS

A total of 65 free living mammals were captured (Table 1). The specimens belonged to 10 different genera, being mostly marsupials (58%, 38/65), which were 34% (22/65) from *Didelphis* and 25% (16/65) from *Micoureus*. There was a more intense trapping of wild animals during the rainy season (from May to August), and a similar number of animals were trapped in each of the studied areas.

Among the 65 animals tested by PCR, 28 were positive for at least one of the parasites: 41% (27/65) were positive for *Leishmania* sp.

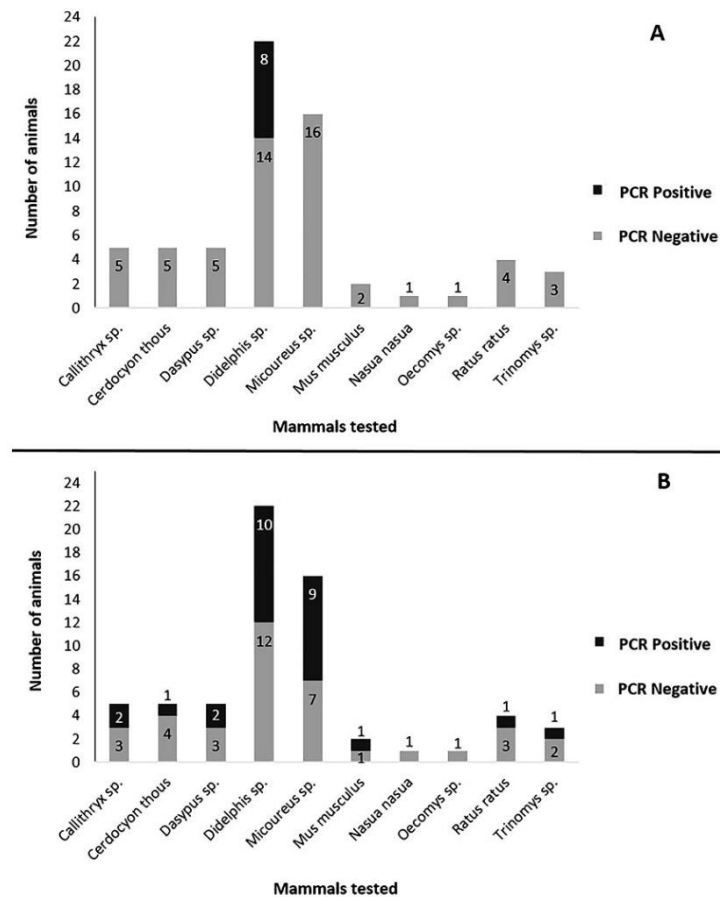


FIGURE 2. Molecular detection of *Trypanosoma cruzi* (A) and *Leishmania* sp. (B) in blood cells from wildlife mammals captured in Salvador metropolitan region during 2013. Numbers inside/above the columns represent the number of positive or negative animals for each assay.

and 12% (8/65) for *T. cruzi*. Seven animals (11%, 7/65) were positive for both parasites. All animals whose samples were positive for *T. cruzi* by PCR belonged to the genus *Didelphis* sp. (Fig. 2A). Marsupials also showed positive results by PCR for *Leishmania* sp.: 36% (10/28) were *Didelphis* and 32% (9/28) were *Micoureus* (Fig. 2B). Infections by both parasites could be detected in trapped animals throughout the whole experiment period.

Among the 13 animals whose samples were tested by culture for parasite isolation, three (all *Didelphis* sp. marsupials) had positive flagellated forms as observed by microscopy, including one from spleen, one from liver, and

one positive isolation was obtained from a blood sample. All samples that were positive by culture were positive for both *Leishmania* sp. and *T. cruzi*-specific DNA amplification, demonstrating concomitant infection of the two parasites in the same host.

Clinical evaluation showed that 88% (57/65) of the animals were clinically healthy, having no apparent signs of disease. Skin alterations found in 18% (12/65) of the animals were characterized as small fresh abrasions in the snout or tail, apparently caused by attempts to escape from the trap. Other skin changes suggested clashes caused by territorialism fights or reactions to ectoparasite bites.

TABLE 2. Clinical signs and parasitological diagnosis of *Trypanosoma cruzi* and/or *Leishmania* sp. in wild mammals from rainforest urban fragments in Salvador City, Brazil, during 2013.

Animal	Genus/species	PCR		Isolation		Clinical signs
		<i>T. cruzi</i>	<i>Leishmania</i> sp.	<i>T. cruzi</i> and <i>Leishmania</i> sp.		
AV001	<i>Didelphis</i> sp.	Positive	Positive	Positive (HP) ^a	None	
AV002	<i>Didelphis</i> sp.	Positive	Positive	NC ^b	None	
AV003	<i>Didelphis</i> sp.	Positive	Positive	Positive (SP) ^c	None	
AV004	<i>Didelphis</i> sp.	Positive	Negative	Negative (HP)	Exfoliative dermatitis	
AV005	<i>Didelphis</i> sp.	Positive	Positive	Negative (SP)	Exfoliative dermatitis	
AV006	<i>Didelphis</i> sp.	Positive	Positive	Negative (HP)	None	
AV007	<i>Didelphis</i> sp.	Positive	Positive	Negative (SP)	None	
AV008	<i>Didelphis</i> sp.	Negative	Positive	Negative (HP)	None	
AV009	<i>Didelphis</i> sp.	Positive	Positive	NC	None	
AV013	<i>Dasyppus</i> sp.	Negative	Positive	NC	None	
AV020	<i>Micoureus</i> sp.	Negative	Positive	NC	None	
AV021	<i>Micoureus</i> sp.	Negative	Positive	NC	None	
AV022	<i>Mus musculus</i>	Negative	Positive	NC	None	
AV023	<i>Micoureus</i> sp.	Negative	Positive	NC	Cyanosis, diarrhea, hypothermia	
AV026	<i>Didelphis</i> sp.	Positive	Positive	Positive (BC) ^d	None	
AV033	<i>Callithrix</i> sp.	Negative	Positive	NC	None	
AV035	<i>Micoureus</i> sp.	Negative	Positive	NC	Focal alopecia	
AV039	<i>Rattus rattus</i>	Negative	Positive	NC	None	
AV045	<i>Micoureus</i> sp.	Negative	Positive	NC	Focal alopecia	
AV050	<i>Micoureus</i> sp.	Negative	Positive	NC	None	
AV051	<i>Didelphis</i> sp.	Negative	Positive	NC	None	
AV057	<i>Trynomis</i> sp.	Negative	Positive	NC	None	
AV059	<i>Callithrix</i> sp.	Negative	Positive	NC	None	
AV063	<i>Dasyppus</i> sp.	Negative	Positive	NC	None	
AV067	<i>Cerdocyon thous</i>	Negative	Positive	NC	None	
AV067	<i>Micoureus</i> sp.	Negative	Positive	NC	None	
AV069	<i>Micoureus</i> sp.	Negative	Positive	NC	None	
AV072	<i>Micoureus</i> sp.	Negative	Positive	NC	None	

^a HP = hepatic puncture.

^b NC = not collected.

^c SP = splenic puncture.

^d BC = blood culture.

Nineteen animals (29%/19/65) had ectoparasites, such as fleas, ticks, or insect larvae and eggs, and most of these animals (13/19) were *Didelphis*. An opossum (*Micoureus* sp.) had severe diarrhea at the time of capture, as well as bradycardia and pale mucous tissues, the last being an indicator of anemia.

None of the animals with negative PCR results showed clinical abnormalities. Table 2 shows PCR-positive animals and their respec-

tive clinical signs. The *Micoureus* sp. that presented diarrhea, hypothermia, and cyanosis also tested positive for *Leishmania* sp. infection by PCR. Two other *Micoureus* that presented multifocal alopecia, a common clinical sign of leishmaniasis as described in dogs and humans, were also positive for *Leishmania* sp. by PCR. Two of the positive *Didelphis* for *Leishmania* sp. infection by PCR also exhibited exfoliative dermatitis.

Most PCR-positive animals (82%, 23/28) showed no clinical signs that could be related to trypanosomatid infections.

DISCUSSION

Among the wild animals identified as *Leishmania* sp. reservoirs in Brazil, the most thoroughly studied have been wild canids and marsupials of the genus *Didelphis*. However, only one out of the five canids analyzed in the present study was positive for *Leishmania* sp. Other authors also observed low frequencies of positivity in wild canids from endemic areas for *Leishmania* infection (Courtenay et al. 2002; Ferreira et al. 2013). These findings endorse the idea that the role of canids in the epidemiological cycle of this parasite may not be more important than that of other wild mammals, even though they indicate that canids are at least potential reservoirs. In fact, the concept of potential reservoirs has been redescribed as those that are able to maintain and act as a source of the parasite in nature (Roque et al. 2014). Nevertheless, this aspect was beyond the scope of this study and would require some studies with larger numbers of canids to answer the question. The characterization of the *Leishmania* spp. encountered in wild mammals would be relevant, but the assay to distinguish *Leishmania* species requires a large sample (de Andrade et al. 2006), and for ethical and methodological reasons it was impossible to retrieve such samples from the wild animals we tested. Since the PCR used in our study is specific for *L. braziliensis*, *L. infantum*, and *L. amazonensis* kDNA minicircle (120 bp) genomic region (Rocha et al. 2010), it is worth saying that the *Leishmania* isolates from these animals are pathogenic and involved in public health problems.

Didelphis sp. marsupials presented the highest percentage of positivity for *Leishmania* sp. (37%) in the present study. Our data may corroborate the results of previous studies that demonstrate evidences that these marsupials could be important wild reservoirs of the parasite in urban fragments of forests

(Santiago et al. 2007). Other species of marsupials, such as *Micoureus*, have not been well described as parasite reservoirs. Nevertheless, other authors have reported a *Leishmania* sp. infection prevalence of 7.5% of in *Micoureus paraguayanus* from an isolated forest reserve in São Paulo, Brazil (Quintal et al. 2011). In our study, 56% of captured *Micoureus* were positive for *Leishmania* sp. The methodological approach may represent a reason for such different frequencies, since we performed diagnostic tests on blood, spleen, and liver, while the former authors used only skin samples. Perhaps even more critically, the environmental characteristics of the studied areas differed between the studies, suggesting that wild animals living in rainforest fragments engulfed by the city could be more susceptible to, or at least more exposed to, infections than animals from a forest reserve. In fact, according to previous studies (Santiago et al. 2007), animals inhabiting periurban areas may present a higher prevalence when compared to those captured in conserved forest areas. Other authors have described findings of infection under similar conditions in some countries from Africa (Bousslimi et al. 2012) and in other areas within Brazil (Leça-Junior et al. 2013). These reports indicate the need for better planning of public health and environmental policies.

A *Rattus rattus*, a *Mus musculus*, and a *Trinomys* sp. were found positive for *Leishmania* sp. and represented 11% of *Leishmania* sp.-positive animals in the present study. There are few studies describing rodents as natural hosts of *Leishmania*, but recently a report described natural infection of wild *M. musculus* and *R. rattus* in an endemic area for *L. infantum* in Europe (Navea-Pérez et al. 2015). Some other species have been cited as reservoirs of *Leishmania* and *T. cruzi* (Marcelino et al. 2011; Botto-Mahan et al. 2012). *Rattus norvegicus* have been found positive for *Leishmania* sp. at the frequency of 36.3% in Minas Gerais, another leishmaniasis-endemic state in Brazil (Marcelino et al. 2011). However, no *R. norvegicus* was captured in our study sites. There are no reports of infection by trypanosomatids in the rodent

Trinomys sp.; ours may be the first report of natural infection with *Leishmania* sp. in this species.

We observed concomitant infections with *Leishmania* sp. and *T. cruzi* in 17% 6/35(6/35) positive samples from 28 animals. Coinfection with trypanosomatids has been reported previously in wild animals (Ramirez et al. 2002). De Araújo and collaborators (2013) found mixed infections with *T. cruzi*, *Trypanosoma rangeli*, and *Leishmania* sp. in whole blood and bone marrow of an anteater (*Tamandua tetradactyla*). *Didelphis* was the only genus diagnosed positive for both parasites, *Leishmania* sp. and *T. cruzi*, in our study, indicating the possible importance of this marsupial in the transmission of both parasites. Yet two out of the five armadillos (*Dasyppus* sp., family Xenarthra) we captured and examined were positive only for *Leishmania* sp.

Among the wild animals described in our study, two out of five (40%) free-living *Callithrix* sp. were positive for *Leishmania* sp. There have been no previous reports of free-living *Callithrix* infected with *Leishmania* sp. in Brazil. The susceptibility of *Callithrix* sp. to infection by *Leishmania* sp. has been investigated by experimental infection (Carneiro et al. 2012). However, in contrast to the results of those authors, the naturally infected primates we examined showed no clinical signs of leishmaniasis. Together with the finding of *Leishmania* infection in *Trinomys* sp., our finding of *Leishmania* in *Callithrix* sp. may indicate the possibility that wild animals may become hosts for such an important parasite, as environmental changes affect their habitats. The subject of newly infected species in areas that have been modified by human action should be investigated more thoroughly. We hypothesize that rainforest remnants located inside urban regions with poor sanitary infrastructure may become focuses of infection and represent risks for the human population as well as for endangered animal species.

Bahia State is endemic for canine and human leishmaniasis and Chagas disease. A previous study found a 30% seroprevalence of

humans infected by *Leishmania* in Camaçari, a town very close to Salvador (Cunha et al. 1995). Recently the same area was again studied 20 years later, and a 46% prevalence was found. In addition, individuals residing in houses with a sand backyard had significantly lower rates of *Leishmania* infection, when compared to those with a different type or no backyard (Maia et al. 2016). Despite being surrounded by areas with a high incidence of the disease, Salvador is not considered an endemic area, because no proved autochthonous cases have been described so far. For Chagas disease, some cities close to Salvador are considered endemic areas (Mota et al. 1990).

A missing step in the epidemiology of the forest remnants in Salvador yet to be determined is to ascertain the vectors species involved. *Lutzomyia longipalpis*, a species complex, is a very urbanized and anthropophilic species rarely found in forest remnants (Soares and Turco 2003; Salomón et al. 2015). This is in accordance with other vector studies in Gafanhoto Park, Minas Gerais, Brazil, which did not find *L. longipalpis* in periurban-forested areas (Margonari et al. 2010). Also, no study has reported the finding of *L. longipalpis* in Salvador (Magalhães-Junior et al. 2012). However, the scenario for the vectors of cutaneous leishmaniasis is completely different, as they are often associated with forests and peridomestic areas. Such vectors may include *Lutzomyia whitmani* and *Lutzomyia intermedia*, very anthropophilic species commonly found in the neighboring region of Corte de Pedra in Bahia State, Brazil (Soares et al. 2010). Vector surveys in our study sites are still needed to determine the species, frequency, fluctuation, and percentage of infected insects. Considering that some of the reservoirs observed in the present study could act as potential reservoirs for sandflies, Salvador must be at risk at least for cutaneous leishmaniasis.

In the present study, *T. cruzi* infection was detected only in *Didelphis* sp. marsupials. A recent study described the infection by *T. cruzi* in marsupials and chiroptera from Maranhão State, Brazil, but these animals

were captured in deep forest areas (da Costa et al. 2015). Also, Rosypal et al. (2014) reported that wild canids from Tennessee, US, present *T. cruzi* specific antibodies, but again these animals did not belong to periurban forest areas. The overlap between wild and domestic environments where *T. cruzi* circulates is increasing and the risk factors for Chagas disease and the boundaries between wild and domestic transmission cycles have been redefined in tropical countries (Herrera 2014). Our study reinforces this situation, because it showed that *Didelphis* sp. marsupials, living in forest fragments inside the third largest Brazilian city, were infected with *T. cruzi*. These marsupials are very common in those wild environments, have a large contact with the population living close to those areas, and are commonly captured in suburban streets and backyards. Not surprisingly, Ribeiro et al. (2015) found *T. cruzi*-infected triatomines in suburban areas of Salvador city. The present study, which reports data on natural infection by *T. cruzi* in wild mammals in related areas, reinforces that the parasite is circulating between the vectors and wild reservoirs in forest fragments inside the city.

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9.5. *Apêndice 5 – Coautoria de artigo publicado: Prevalence of Infection of Biomphalaria glabrata by Schistosoma mansoni and the risk of urban Schistosomiasis mansoni in Salvador, Bahia, Brazil.*

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Major Article

Prevalence of Infection of *Biomphalaria glabrata* by *Schistosoma mansoni* and the risk of urban Schistosomiasis mansoni in Salvador, Bahia, Brazil

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Abstract

Introduction: *Biomphalaria glabrata* is considered to be responsible for the incidence of schistosomiasis in Brazil. Therefore, surveillance of areas where schistosomiasis is prevalent is fundamental for public health planning. This study was aimed to evaluate *B. glabrata* populations in water bodies of the city of Salvador, determine their distribution, estimate the prevalence of *Schistosoma mansoni* infections, characterize shed cercariae, and identify transmission foci. **Methods:** Malacological surveys were carried out in 17 water collections from Salvador. Snail species were identified based on shell and mantle characteristics. Snails were evaluated for *S. mansoni* infection by exposure to light and via real time polymerase chain reaction (qPCR) using *S. mansoni*-18S rRNA subunit specific primers. **Results:** 1,403 *B. glabrata* were collected. Classical cercarial shedding indicated that 5 snails (0.4%) were positive for *S. mansoni*. A higher prevalence of infections was found in Horta de Saramandaia (5.5%) and Lagoa do IAT (1.9%). Non-*Schistosoma* larvae, such as Xiphidiocercaria, Strigeidae, Spirorchiidae and Clinostomidae, were observed in 3.2% of the snails. *S. mansoni* DNA was detected in 6.2% snails via qPCR. **Conclusions:** *B. glabrata* is widely distributed in Salvador, as indicated by 7 water collections associated with a risk of schistosomiasis transmission. To our knowledge, this is the first study to identify *B. glabrata* eliminating cercariae of Clinostomidae, Strigeidae, and Spirorchiidae in Salvador. We propose that qPCR may be employed in combination with classical cercarial shedding. Estimating *S. mansoni* prevalence in snails by only considering the results of light exposure method classical into account may underestimate the problem.

Keywords: Schistosomiasis. *Biomphalaria glabrata*. Prevalence. cercarial types.

INTRODUCTION

Schistosomiasis, a water-transmitted tropical disease (NTDs)—caused by trematode parasites of the genus *Schistosoma*—that remains largely neglected. Several parasites of this genus, such as *S. haematobium*, *S. japonicum*, *S. intercalatum*, *S. mekongi*, and *S. mansoni* are epidemiologically relevant and are capable of parasitizing humans. In Brazil,

human schistosomiasis is caused by *S. mansoni*, which is responsible for the intestinal and hepatic forms of this disease^{1,2}.

Schistosomiasis mansoni, which affects some 240 million individuals worldwide, causes a parasitic disease considered as the third most important socioeconomic and public health issue. In Brazil, schistosomiasis remains an important public health issue due to its prevalence throughout the national territory. According to the "National Survey of Prevalence of Schistosomiasis mansoni and geohelminthosis", conducted among schoolchildren in Brazil, 14 states were found to be endemic for schistosomiasis³. Among the Brazilian states endemic for schistosomiasis, Bahia has the second highest prevalence with the largest endemic area, which included 251 out of 417 municipalities, including the city of Salvador⁴.

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The life cycle of *S. mansoni* is complex, and involves definitive hosts (vertebrates) and intermediate hosts (snails). Previously, transmission of schistosomiasis was found primarily in rural areas. However, intense migratory flows of people from rural endemic areas to urban areas and rapid urbanization contributes to the spreading of parasitic diseases to urban areas. Furthermore, large-scale distribution of the intermediate host—snails of the genus *Biomphalaria*—favors territorial expansion of this disease via the establishment of schistosomiasis transmission foci. In Brazil, 11 species and 1 subspecies of *Biomphalaria* have been described, of which, the following 3 are considered natural hosts of *S. mansoni*: *B. glabrata*, *B. tenagophila*, and *B. straminea*⁵.

The distribution of the snail vector is directly correlated with the distribution of schistosomiasis cases⁶. Since the snails are necessary for transmission, updated information on the distribution and characterization of the snail population is essential and contributes directly to the orientation, planning and development of surveillance as well as to the adoption of proper control measures for schistosomiasis. This study was aimed at evaluating *B. glabrata* populations in the water collections from the city of Salvador in order to determine their distribution, identify foci of schistosomiasis transmission to determine the prevalence of *S. mansoni* infection and to characterize shed cercariae.

METHODS

Study site and selection of collection points

The study was conducted in Salvador, capital of the State of Bahia, Northeastern region of Brazil (-12.9704; -38.5124); (Figure 1A). Salvador is divided into 12 regions, termed Sanitary Districts, for the purpose of public health administration. Samples were collected from 17 lentic or lotic water collection points distributed in 8 Sanitary Districts of Salvador, namely Boca do Rio, Brotas, Cabula/Beiru, Centro Histórico, Itapuã, Pau da Lima, São Caetano/Valéria and Subúrbio Ferroviário (Table 1; Figure 1B). Five of the sites were in the process of undergoing urban renewal or major construction with little community contact and were therefore eliminated from the assessment. All sites had permanent collections of water throughout the year and were at, or near, points where the human population had significant contact with the water.

Malacological survey and mollusk maintenance

The malacological surveys were conducted between June and December of 2017, in accordance with the technique described by Oliver and Schneiderman⁷. The density of collected snails was made by dividing the number of planorbids collected at each point by the number of collectors that collected in the 10-minute period. The snails were transported to the Gonçalo Moniz Institute (IGM-FIOCRUZ) and kept in glass aquaria with dechlorinated water. The snails were fed on alternate days with thoroughly washed fresh lettuce.

Natural infection survey and snail identification

Snails were placed individually in jars containing 4 mL of filtered, dechlorinated tap water. Screening for *S. mansoni*

cercariae and other larval trematodes was carried out via weekly exposure to light (60W/4 hours) over a period of 4 weeks. Snails remaining negative at the end of this period, were analyzed for another 10 d. Positive snails were examined using a stereoscopic microscope, and live cercariae were stained with 5% lugol. Cercarial types were identified according to the criteria established by Alves Pinto and Lane de Melo⁸. Shell crushing was not performed because the soft body portion of *Biomphalaria glabrata* was required for morphological identification of the species. All snails were morphologically identified according to Paraense⁹.

PCR analysis

The prevalence of *S. mansoni* infection in a randomly selected sub-group of snails was evaluated via real time polymerase chain reaction (qPCR). DNA extraction from snails was conducted using a DNeasy[®] Blood and Tissue Kit (QIAGEN[®], Germany), following the manufacturer's instructions. *S. mansoni*-specific primers were used to amplify the 18S rRNA subunit as follows: Schfo 111 (5' - CGATCAGGACCAGTGTTCAGC - 3') and Schre 111 (5' - GACAGGTCAACAAGACGAACTCG - 3'), as described by Gomes,¹⁰ and qPCR was carried out on an ABI PRISM 7000 system (Applied Biosystem, CA, US). The total qPCR reaction volume of 25 µL consisted of 7.5 µL H₂O, 12.5 µL Syber Green, 2 µL of the two amplification primer, 1 µL ROX and 2 µL of template DNA. PCR was performed under the following cycles: 50°C for 2 min, followed by 40 cycles of amplification (95°C for 2 min, 95°C for 15 s, and 60°C for 30 s). Negative controls were used for each reaction, and a standard curve was constructed using a sample of *S. mansoni* DNA isolated from worms. All reactions were performed in duplicate. ABI PRISM software (version 1.1) was used for the analysis and interpretation of results.

Results

General distribution of *Biomphalaria glabrata*

A total of 1,403 *B. glabrata*, the only vector species found in this study, were collected from 12 water collections (Table 2). Of these, 730 snails survived at the end of 40 d of malacological analyzes, representing a survival rate of 52%. These snails were morphologically identified and submitted for DNA extraction. The highest snail survival rate, that of 69% survivors, was observed in the water collections of Dique do Cabrito and Lagoa do Urubu (Table 2).

B. glabrata was found to be distributed in 8 Sanitary Districts of the city of Salvador. In the Sanitary District of Cabula/Beiru, in particular, where the Horta de Saramandaia is located, 410 snails were collected. In the Boca do Rio Sanitary District, where Parque Pituacu is located, a total of 294 snails were collected, and in the São Caetano/Valéria Sanitary District, 289 snails were collected in Horta de São Bartolomeu.

Larvae of trematodes found in *B. glabrata*

Of the 730 snails that survived parasitological analyses, 5 snails shed *S. mansoni* cercariae (Figure 2A). Notably, 25 snails shed only non-*Schistosoma* larvae as follows: Xiphidiocercaria (Figure 2B1-3); Strigeidae (Figure 2E), Spirorchidae (Figure 2D), and Clinostomatoide (Figure 2C).

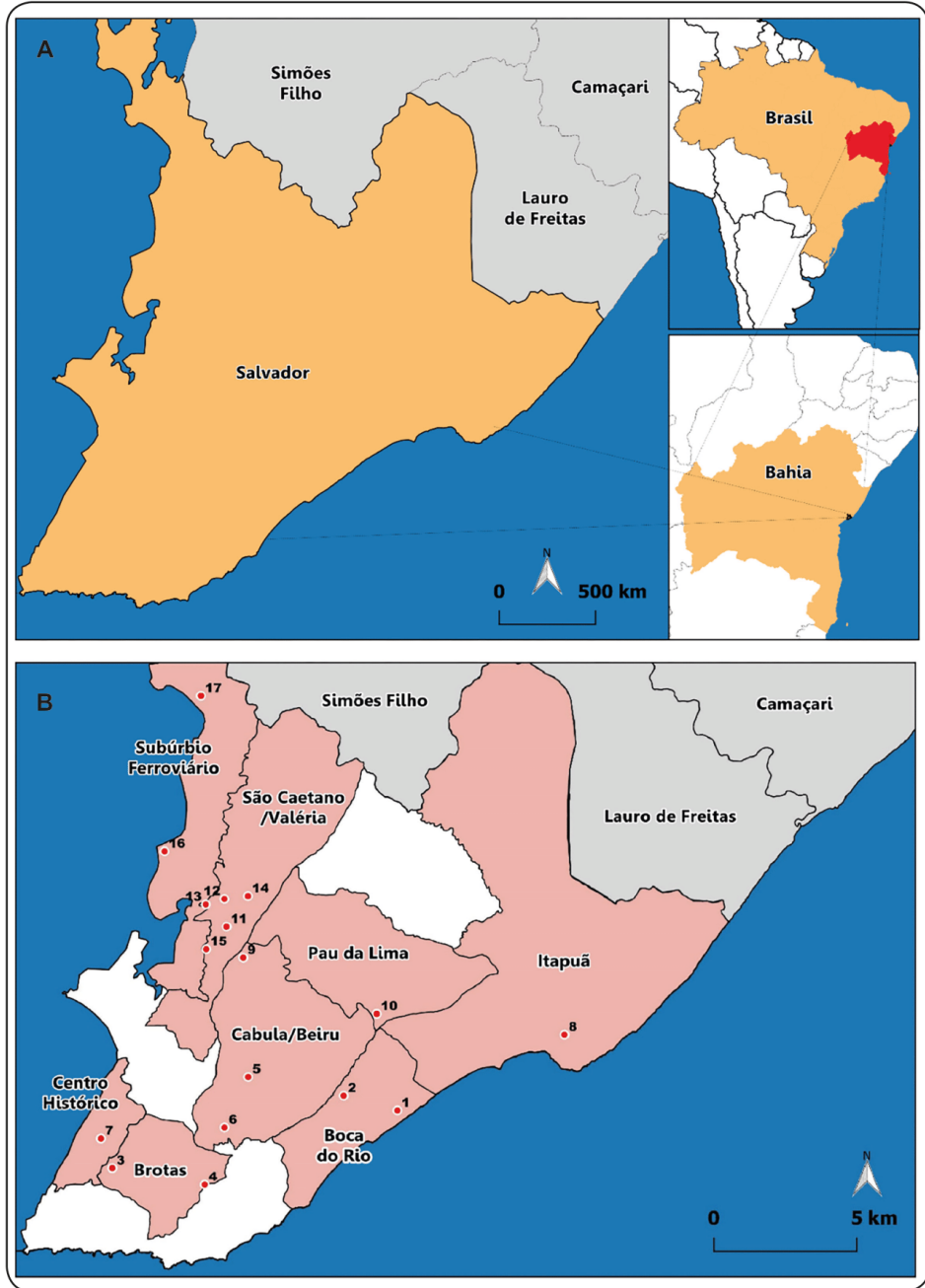


FIGURE 1: (A): study site and (B): distribution of water collection in the Sanitary District of the city of Salvador.

TABLE 1: Characterization of collection points in the Sanitary District study sites, type of water collection and presence of vegetation.

Sanitary District	Point	Study Site	Classification
Boca do Rio	1	Parque Pituáçu	Stream
	2	Bate Facho	Stream
Brotas	3	Dique do Tororó	Dike
	4	Avenida ACM	Ditch
Cabula/Beiru	5	Alameda Flamengo	Lagoon
	6	Horta Saramandaia	Vegetable garden channel
Centro Histórico	7	Rua Nossa Senhora de Lourdes	Stream
Itapuã	8	Lagoa do Abaeté	Lagoon
Pau da Lima	9	Lagoa do Urubu	Lagoon
	10	Lagoa do IAT	Lagoon
São Caetano/Valéria	11	Rua São Rafael	NA
	12	Horta São Bartolomeu	Vegetable garden channel
	13	Rua das Fontes	NA
	14	Rio do Cobre	River
	15	Dique do Cabrito	Dike
Subúrbio Ferroviário	16	Rua Gevârsio Cerqueira	Vegetable garden channel
	17	Rua Ray Charles	NA

NA: not applicable.

S. mansoni cercariae were found only in 2 water collections: the Lagoa do IAT, in the Sanitary District of Pau da Lima; and Horta de Saramandaia, located in the Sanitary District of Cabula/Beiru, which were 5.5% and 1.9% positive, respectively. Additionally, in Horta de Saramandaia, 4.3% snails shed *Xiphidiocercaria*. In Dique do Cabrito, 1 snail shed *Clinostomidae* cercariae and 1 snail shed *Spirorchidae* cercariae. Positivity rate in both cases was 4.3%. The highest positivity was observed in Lagoa do Urubu, with 31% of snails shedding *Strigeidae* cercariae (Table 3).

Molecular detection of *S. mansoni*

Of the 1403 snails collected, 626 were used for molecular detection of *S. mansoni* via qPCR. Only product amplifications with a melting temperature equal to that of the positive control, $C_t < 35$, and a correlation coefficient (r^2) of 0.99 were considered positive. All negative controls were negative in all experiments. The sensitivity of qPCR for detection of *S. mansoni* infections was 100% while specificity was 94.5% compared with the results of the light exposure method.

Of the 626 samples, 39 were considered positive, representing a positivity of 6.2%. Of these, only 5 (0.8%) were positive by the light exposure method. No snails that had

eliminated other cercarial types were found to be positive for *S. mansoni* via qPCR, while none of the snails were found to be infected with 2 species of cercariae.

Among the 12 water collections containing *B. glabrata*, 5 (41.7%) were positive for *S. mansoni* only, via qPCR as follows: Parque Pituáçu, Avenida ACM, Rua Nossa Senhora de Lourdes, Horta de São Bartolomeu and Dique do Cabrito. The highest positivity via qPCR was observed in the water collection of the Dique do Cabrito, followed by Av. ACM, Lagoa do IAT and Rua Nossa Senhora de Lourdes.

Water collections that were previously determined to be positive for *S. mansoni*, via light exposure, were found to be even more positive for *S. mansoni* via qPCR. In Lagoa do IAT, only 5.5% of snails were found to be positive via the light exposure method, whereas 16.6% were found to be positive via qPCR. Similarly, the water collection of Horta de Saramandaia, which indicated a 1.9% positivity via the light exposure method, showed a positivity of 4.8% via qPCR (Table 4).

DISCUSSION

The malacological survey, conducted by the current study, demonstrated that *B. glabrata* was present in 70.6% of the water collections examined. Most snails were present in streams and

TABLE 2: Total *B. glabrata* counts per water collection, amount, and percentage (%) of alive snails after 40 days of laboratory maintenance.

Sanitary District	Collection Site	Collected Snails	Live Snails after 40 days
Boca do Rio	Parque Pituauçu	294	145 (49%)
	Bate Facho	22	13 (59%)
Brotas	Dique do Tororó	3	2 (66%)
	Avenida ACM	84	48 (57%)
Cabula/Beiru	Alameda Flamengo	0	0
	Horta Saramandaia	410	205 (50%)
Centro Histórico	Rua Nossa Senhora de Lourdes	49	27 (55%)
Itapuã	Lagoa do Abaeté	0	0
Pau da Lima	Lagoa do Urubu	42	29 (69%)
	Lagoa do IAT	33	18 (54%)
São Caetano/Valéria	Rua São Rafael	0	0
	Horta São Bartolomeu	289	144 (49%)
	Rua das Fontes	-	-
	Rio do Cobre	58	28 (48%)
	Dique do Cabrito	33	23 (69%)
Subúrbio Ferroviário	Rua Gevârsio Cerqueira	86	48 (56%)
	Rua Ray Charles	0	0
Total		1403	730

TABLE 3: Cercarian types found in specimens of *Biomphalaria glabrata* in the water collections of Salvador.

Sanitary District	Water Collection	Positive Snails	Cercarial Types	Positivity (%)
Cabula/Beiru	Horta de Saramandaia	4/205	<i>S. mansoni</i>	1.9%
		9/205	Xiphidiocercaria	4.3%
Pau da Lima	Lagoa do Urubu	9/29	Strigeidae	31%
	Lagoa do IAT	1/18	<i>S. mansoni</i>	5.5%
São Caetano/Valéria	Dique do Cabrito	1/23	Spirorchidae	4.3%
		1/23	Clinostomidae	4.3%

ditches, which together represented 50% of the water collections sampled.

The highest concentration of *B. glabrata* was observed in the water collections of Horta de Saramandaia and Horta de São Bartolomeu. “Horta is Portuguese for “garden”, which in Salvador often implies a large area under cultivation for local

and commercial production. Although *Biomphalaria* snails are commonly found in natural water collections, highest population densities are usually observed in artificial breeding sites such as drainage and irrigation ditches associated with human activity¹¹. Constant irrigation of vegetable gardens provides ideal breeding grounds for *Biomphalaria* spp¹².

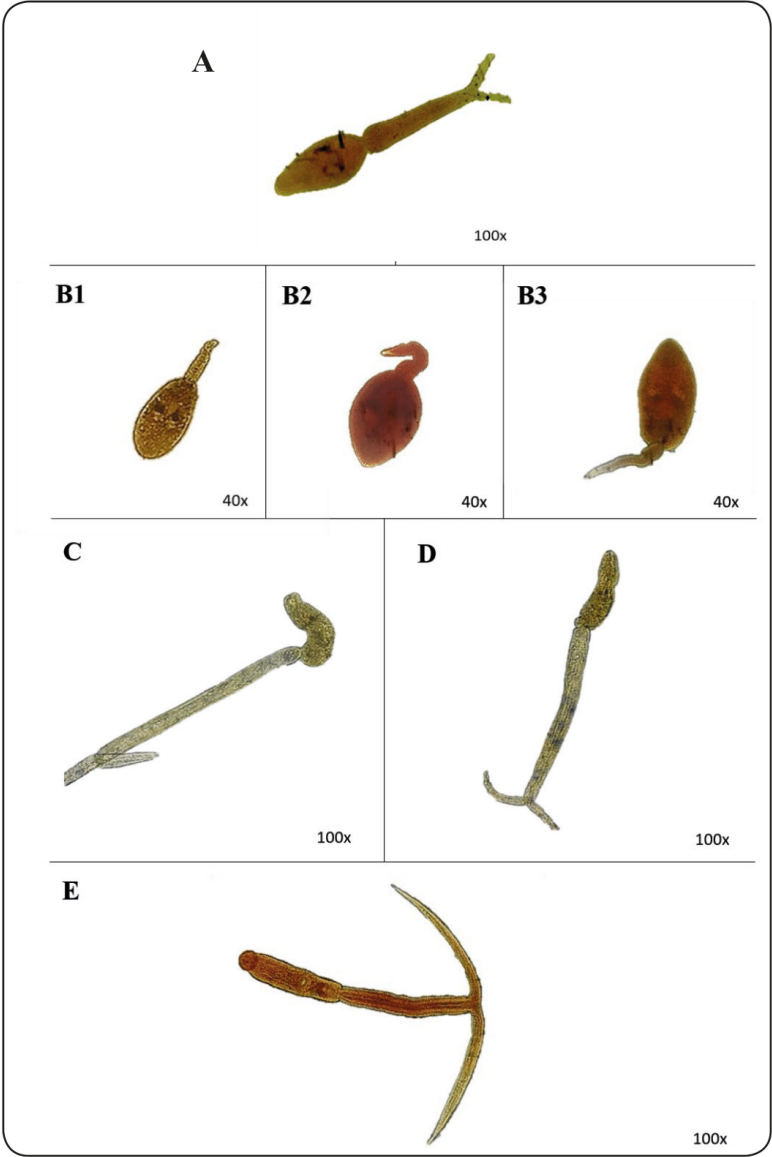


FIGURE 2: Larvae of trematodes found in *B. Glabrata*. (A): Cercaria *S. mansoni*; (B): Xiphidiocercaria: (B1): Cercariae Lutzii; (B2,3): Cercária Santense; (C): Clinostomidae, (D): Spirorchidae (E): Strigeidae.

TABLE 4: Infection Rates obtained via qPCR and light exposure methods in surviving *B. glabrata* samples.

Sanitary District	Collection Site	<i>B. glabrata</i>	Infection Rate		qPCR (+)
			Light Exposure Method		
			<i>S. mansoni</i>	Outros	
Boca do Rio	Parque Pituauçu	100	–	–	5 (5%)
	Bate Facho	13	–	–	–
Brotas	Dique do Tororó	2	–	–	–
	Av. ACM	48	–	–	9 (18,7%)
Cabula/Beiru	Horta Saramandaia	145	4 (1,9%)	9 (4,3%)	7 (4,8%)
Centro Histórico	R. N. Senhora de Lourdes	28	–	–	3 (10,7%)
Pau da Lima	Lagoa do Urubu	29	–	9 (31%)	–
	Lagoa do IAT	18	1 (5,5%)	–	3 (16,6%)
São Caetano/Valéria	Horta São Bartolomeu	100	–	–	2 (2%)
	Rio do Cobre	28	–	–	–
	Dique do Cabrito	23	–	2 (8,6%)	10 (43,4%)
Subúrbio Ferroviário	R. Gevârsio Cerqueira	48	–	–	–
Total		626	5	20	39

Only the water collections from Horta de Saramandaia and Lagoa do IAT were found to be positive for *S. mansoni* via the light exposure method after 30 d, with infection rates of 1.9% and 5.5%, respectively. Given the conditions governing cultivation and irrigation in Horta de Saramandaia, the findings from that location were expected. The presence of channels excavated for irrigation of vegetables, compounded by precarious sanitary conditions of the neighborhood and the high population density of *B. glabrata*, provide the necessary environment for maintaining the life cycle of *S. mansoni* at this site. Furthermore, in 2015, the Zoonoses Control Center (CCZ), identified *B. glabrata* specimens which shed *S. mansoni* cercariae. In the Lagoa do IAT region, similar conditions that were favorable for maintaining the *S. mansoni* life cycle, such as residential sewage flushed directly into the water collection and residents living with schistosomiasis, were observed.

The qPCR confirmed that all water collections found to be positive via the light exposure method, were also positive via *S. mansoni* DNA. Furthermore, classical methods combined with PCR were able to detect higher levels of infection prevalence. These findings were corroborated by the results of previous studies. Jannotti-Passos and Souza¹³ used LS-PCR in association with light exposure to determine the prevalence of *S. mansoni* infection following 7 and 42 d exposure of *B. straminea* and *B. tenagophila* to miracidia. Although other studies evaluated infections in other species of *Biomphalaria*, using different PCR techniques, their results corroborate those found in the current study, since apparent infection prevalence increased from 20% to 55% in *B. straminea*, and from 45% to 67.6% in *B. tenagophila*.

Positivity for *S. mansoni* seen via PCR and the absence of cercarian elimination may be explained away as being due

to snail immune system activity. Non-successful infections, which do not lead to the elimination of cercariae, are detected by PCR, because parasite DNA is not completely degraded¹⁴. This phenomenon may also be explained by the fact that some primary sporocysts either degenerate or are encapsulated by hemocytes, leading to unsuccessful infections. Thus, sporocysts play a fundamental role in disease progression, since the production levels of cercariae are directly associated with the development and concentration of sporocysts in the snail¹⁵.

Moreover, late development of the immune response to *S. mansoni* may lead to a delay in cercarian release. Significant tissue changes which occur in infected *Biomphalaria* prevent the elimination of cercariae. Focal and diffuse proliferation of hemocytes accompanied by an expansion of the extracellular matrix in a manner similar to that seen in granulomas, was observed in *B. glabrata*¹⁶. Lemos and Andrade¹⁷ proposed that these tissue changes may develop gradually in infected snails that had previously eliminated cercariae. However, these tissue changes do not guarantee complete eradication of the infection, since some sporocysts that remain may be able to complete the development cycle of the parasite, whereby cercariae may be released at any time within 9 months following infection¹⁶.

Late release of cercariae may also occur due to reproduction between susceptible and resistant snails, which influences the timing of *S. mansoni* development in the snail. A study of *B. glabrata*, generated by crossing resistant and susceptible species, reported that descending snails exhibited a delayed pre-patent phase, which could last up to 10 months¹⁸. Additionally, such late releases may also be related to sporocytogenesis¹⁹. Jourdan and Théron observed that changes that compromise the production of cercariae, such as secondary sporocyst migration

to ectopic regions (cephalopodal region and kidney), may occur during sporocystogenesis²⁰. This phenomenon has been observed in partially resistant *B. glabrata*, with delays in the release of cercariae up to 7 months²¹.

To our knowledge, this is the first record of other cercarian types, such as Strigeidae, Clinostomidae and Spirorchiidae, in the city of Salvador. Alves Pinto and Lane de Melo reported the presence of Spirorchiidae and Clinostomidae cercariae in the 3 schistosomiasis transmitter species in the state of Minas Gerais⁷. Clinostomidae cercariae are considered to be parasites of the oral cavity of birds, but accidental human infections have been reported²². Strigeidae cercariae have also been identified in the States of Maranhão, Minas Gerais and Rio de Janeiro²³⁻²⁵.

The presence of *B. glabrata* shedding Xifidiocercariae was observed in the water collections of Subúrbio Ferroviário in Salvador by the CCZ in 2017. Previous studies have already evaluated the presence of this cercarian type in *Biomphalaria* spp. from other sites^{26,27}. This cercarian type, which has not been found to be responsible for any clinically important disease, has been considered as a source of biological control for mosquito larvae²⁸.

The absence of coinfection in snails that were observed in this study may be due to cercarian antagonism, which leads to competition between larvae of different trematodes and results in a reduction in the number of parasites able to complete development. However, simultaneous elimination of cercariae during coinfections have been observed in *S. mansoni* and *Cercaria lutzi* coinfections exclusively in *B. tenagophila*²⁴.

B. glabrata was not found in the water collections of Alameda Flamengo, Lagoa do Abaeté, Rua São Rafael, Rua das Fontes and Rua Ray Charles. Three of these locations were undergoing major public construction work, such as sanitary sewer placement or street paving. Considering that parasitic diseases reflect sanitary conditions as well as hygiene habits of a population, these results demonstrated that effective public interventions is fundamental for improving living conditions as well as for preventing and regulating parasitic diseases²⁹.

A limitation of this study was the reduction of snail survival rates during weekly malacological analyses that lasted 30 d. This suggests that the duration of the analysis may have influenced *B. glabrata* survival, as it is possible that snails that did not survive were parasitized by *S. mansoni*, may have had different susceptibility profiles or differences in the amount of miracidia penetrated³⁰.

In the future, we hope to assess more water collections in the city of Salvador, in order to evaluate infections in snails using a combination of conventional and molecular techniques. An additional goal is to evaluate resistance and susceptibility profiles of these snails.

Our results indicate that *B. glabrata* is widely distributed in the city of Salvador, and 7 of its water collections carry a risk of schistosomiasis transmission. In addition, we propose that qPCR may be utilized to evaluate *S. mansoni* infections in *B. glabrata* during the pre-patent phase. It is evident that estimating *S. mansoni* prevalence in snails by taking only the light exposure method classical into account may underestimate the issue. To

the best of our knowledge this is the first study of *B. glabrata* eliminating Clinostomidae, Strigeidae, and Spirorchiidae cercariae in Salvador.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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9.6. Apêndice 6 – Coautoria de artigo publicado: *Seroprevalence of Trypanosoma cruzi infection among blood donors in the state of Bahia, Brazil.*

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Major Article

Seroprevalence of *Trypanosoma cruzi* infection among blood donors in the state of Bahia, Brazil

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Abstract

Introduction: Chagas disease (CD) affects 5.7-7.0 million individuals worldwide, and its prevalence reached 25.1% in the state of Bahia, Brazil. There is an association between the prevalence of CD, the socioeconomic status of the population, and the risk of re-emergence due to non-vectorial transmission, such as blood transfusion. This study determined the seroprevalence of *T. cruzi* infection among blood donors in the state of Bahia, located in northeastern Brazil, and their epidemiological profile during a 10-year period. **Methods:** We performed a descriptive cross-sectional study involving a database review. Data were collected from patients with non-negative results for *T. cruzi* infection during a 10-year period. **Results:** A total of 3,084 (0.62%) samples were non-negative for *T. cruzi* infection in an initial serological screening, and 810 (0.16%) samples were non-negative in the second screening. The correlation between infection and age (30 years or older) and between infection and lower educational level (12 years or less) in the first and second screening was statistically significant. The seroprevalence of *T. cruzi* infection was higher in men in the first screening. In addition, 99.52% of the municipalities of Bahia had at least one case of CD. Livramento de Nossa Senhora and Salvador presented the highest disease prevalence and recurrence, respectively. **Conclusions:** The seroprevalence of *T. cruzi* infection in these populations was lower than that found in other studies in Brazil but was comparatively higher in densely-populated areas. The demographic characteristics of our population agreed with previous studies.

Keywords: Chagas disease. Blood donor. Seroepidemiological studies.

INTRODUCTION

Human Chagas disease (CD), also known as American trypanosomiasis, is a vector-borne tropical disease caused by the protozoan hemoflagellate *Trypanosoma cruzi*. CD was discovered and initially described by Dr. Carlos Chagas in 1909, at Lassance, Minas Gerais, Brazil¹.

In Latin American countries, CD causes 12,000 to 14,000 deaths annually² and affects 5.7 to 7.0 million people³. More affordable traveling conditions from Latin America to non-

endemic areas and increasing international migration flows have increased *T. cruzi* infection beyond the borders of Latin America, and CD reached Europe, North America, and Oceania countries⁴⁻⁶.

The seroprevalence of *T. cruzi* infection decreased in Latin America (from 6.49% to 1.28%) and Brazil (from 6.96% to 0.21%) between 1970 and 2006⁷ as a consequence of control campaigns in endemic countries, which helped reduce vectorial and transfusion transmission. Despite this decreasing trend in endemic areas, neglected regions have a higher number of cases and infected individuals.

In Brazil, the overall prevalence of *T. cruzi* infection reached 4.2% between 2000 and 2002 in the northeast, and the infection rate was higher than 5%^{8,9}. More recently, micro-outbreaks of CD occurred via oral transmission in municipalities in the interior of the states of Santa Catarina and Bahia¹⁰.

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Vectorial transmission is more common in endemic areas, in which the parasite is transmitted by contact with feces or urine of infected blood-sucking triatomine bugs (Hemiptera, Reduviidae). Other transmission routes include the consumption of contaminated foods or beverages, tissue and organ transplantation, mother-to-child transmission during pregnancy, laboratory accidents, induced or criminal infection, and blood transfusion. The transmission of *T. cruzi* via blood transfusion has increased because of urbanization and modernization of medicine¹¹ and is the predominant route of transmission in both endemic and non-endemic areas¹², especially in countries in which screening tests for CD are not mandatory in blood banks. Furthermore, *T. cruzi* tolerates extreme conditions, such as low temperatures (−80 to 4°C), and freezing and thawing¹³, indicating that the parasite can be transmitted in all blood components, including granulocytes, red blood cells, and plasma.

T. cruzi infection occurs predominantly in individuals living in rural areas⁸. However, the epidemiological status of CD has changed as a consequence of migration flows from rural areas to the periphery of large cities, where the presence of forest remnants increases the risk of disease transmission¹⁴⁻¹⁷.

Human CD is classified as a neglected transmitted disease, primarily affecting communities with higher social vulnerability, characterized by low income, poor housing and hygiene conditions, and low levels of schooling. For this reason, among parasitic diseases, CD is the most strongly associated with population vulnerability, in which the majority of seropositive individuals have these risk factors¹⁸.

Studies on CD should assess ecological, biological, social, and economic parameters to identify risk factors associated with disease development and progression. Furthermore, these studies reduce the risk of infection by producing knowledge for scientific and technological innovations.

Technologies capable of reducing the risk of transmitting infectious diseases through transfusion are critical to prevent the spread of contagious agents and ensure the quality of blood for transfusion¹⁹. Studies of discarded serological tests are capable of creating relevant technologies to improve the quality of blood transfusion. Serological screening has been used in several countries to improve public health programs. However, this type of testing is facing ethical, operational, and financial difficulties²⁰.

Training the teams responsible for collecting pre-donation information is critical, given that adequately collected epidemiological data may help avoid transmission and decrease costs in the public health system.

In addition to avoiding transmission, identifying individuals with suspected disease allows researchers to identify regions with a higher risk of transmission and the most relevant epidemiological characteristics associated with seropositivity. Moreover, identifying places of birth and residence with higher prevalence may help characterize areas with a higher risk of transmission in the state of Bahia.

Previous studies with blood donors reported that the percentage of non-negative results was higher among married

individuals, people in stable relationships⁷, and subjects with lower education levels²¹. The percentage of infection was higher in men²²⁻²⁵, although one study found that the rate was higher in women²¹. The number of cases was higher in older age groups, especially in individuals older than 30 years^{7,22,23,25,26}. Moreover, the percentage of infection between men and women differed according to age group²².

The objective of this study was to determine the seroprevalence of *T. cruzi* infection and describe the epidemiological profile of blood donors from the Hematology and Hemotherapy Foundation of Bahia (HEMOBA) with non-negative results for *T. cruzi* infection detected by screening tests during a 10-year period (February 2008 to February 2018) in the state of Bahia. The specific objectives were to determine the most relevant epidemiological characteristics of blood donors with inconclusive results for CD and identify the municipalities of origin of this population.

METHODS

Study design: Descriptive cross-sectional study involving a database review.

Target population: All blood donors from HEMOBA in the state of Bahia, Brazil, examined during the study period. HEMOBA is the official blood service in Bahia, and its units cover all health macro-regions in the state;

Diagnostic criteria: Non-negative results for *T. cruzi* infection;

Selection criteria:

- **Inclusion:** Donors with non-negative results for *T. cruzi* infection detected in serological screening tests performed at HEMOBA;
- **Exclusion:** Donors with negative serology in the second screening;

Variables: Gender, age, place of residence, place of birth, education level, racial group, marital status, number of annual blood donations, and test results in the first and second screening;

Clinical selection of candidates and release of blood bags:

To minimize the risk of *T. cruzi* infection via blood transmission, the Brazilian Ministry of Health has issued several ordinances, the most recent being Ordinance 5, which was published in the Official Gazette of the Union on September 28, 2017²⁷ and lists the criteria for the clinical selection of candidates for blood donation. HEMOBA created a standard computerized questionnaire (Hemovida System) following these criteria and disclosed the data for the study. The methodology used for the release of blood bags followed the recommendation of the Ministry of Health according to Ordinance 5.

Serological screening: High sensitivity tests are mandatory. Between January 1991 and January 2015, screening tests for CD among blood donors at HEMOBA used anti-*T. cruzi* antibodies and ELISA from MUREX (FRA) and BIOMÉRIEUX (FRA). Since February 2015, HEMOBA adopted serological screening by chemiluminescence using IgG and IgM class antibodies. These antibodies were purchased from ABBOTT (IL, USA)

until February 25, 2016, and from DIASORIN (ITA) since then to date. HEMOBA uses the same serological test in donors in the second screening and sends them to a referral unit in case of positivity.

Statistical analysis: Descriptive analyses were performed using the statistical package Epi Info 2000 (CDC, Atlanta, GE, USA). The χ^2 test was used to compare proportions, and a level of significance of 5% ($p < 0.05$) and a confidence interval of 95% were adopted in all tests. Qualitative variables were expressed as absolute numbers and relative frequency.

Georeferencing: The information was georeferenced using the software Qgis version 20.0 to identify groups with high rates of non-negative results for *T. cruzi* infection according to the place of residence. The natural breaks method with five class intervals was used because the variables were discrete.

Ethics

The adopted procedures were in accordance with the ethical standards of the Research Ethics Committee of the Gonçalo Moniz Institute (FIOCRUZ, Bahia, Brazil) and with the Helsinki Declaration of 1964, revised in 1975, 1983, 1989, 1996, and 2000. The consent form was waived because the analysis was based on a database. However, no personal identification data were used to ensure the complete anonymity of blood donors. The research did not cause any physical, psychic, moral, intellectual, social, cultural, or religious risk to blood donors.

RESULTS

During the 10-year study period (February 22, 2008 to February 21, 2018), 494,010 donors and 816,346 blood samples were examined at HEMOBA. In a preliminary analysis, 3,084 (0.62%) inconclusive samples were identified. All samples reactive for *T. cruzi* are considered non-negative by HEMOBA. Patients whose test results were non-negative or inconclusive were advised to return to HEMOBA to undergo a second screening. This orientation follows a protocol in which the blood center sends up to three letters to the donor's registered address, with an interval of 30 days between each of them. A total of 1,486 patients returned to the blood center for a second screening, of whom 810 (0.16%) were considered non-negative for *T. cruzi* infection.

Among the non-negative samples, 1,334 (43.26%) were analyzed by chemiluminescence using IgG and IgM antibodies and 1,750 (56.74%) by ELISA using anti-*T. cruzi* antibodies in the first screening, whereas 671 (45.15%) samples were analyzed by chemiluminescence using IgG and IgM antibodies and 815 (54.85%) by ELISA using anti-*T. cruzi* antibodies in the second screening.

The group of individuals positive for *T. cruzi* infection in the first and second screening (810) was analyzed by gender, age, racial group, marital status, educational level, place of residence, and place of birth.

In the first screening, the prevalence of non-negative results for *T. cruzi* infection was 0.62% (3048 samples) and was higher in men (PR, 1.0919; 95% CI, 1.02–1.17) and in blood donors aged 30 years or older (PR, 1.5863; 95% CI, 1.4676–1.7147).

The prevalence of non-negative results in donors with less than 12 years of schooling was 32% (PR, 1.3236; 95% CI, 1.2040–1.4550) when compared with those with at least 12 years of education. There was no statistically significant association between the place of birth and residence and the prevalence of non-negative serology in this population (Table 1).

The analysis of data from the second screening indicated that the prevalence of non-negative results for *T. cruzi* infection was 0.15% (810 samples). The prevalence was higher in blood donors aged 30 years or older (PR, 3.4497; 95% CI, 2.8547–4.1689) and donors with 12 years of schooling or less (PR, 2.6943; 95% CI, 2.1154–3.4317). There was no statistically significant association between non-negative serology, gender, and the place of birth and residence in Bahia (Table 1).

The data on the prevalence of non-negative results according to the marital status and racial group are shown in Table 2. Given the high miscegenation of the population in Bahia, it is difficult to classify the study sample into racial groups. Notwithstanding, there was no significant association between prevalence rates and racial groups.

The data on the place of residence of blood donors with non-negative results for *T. cruzi* infection were georeferenced and summarized in cartographic representations. The prevalence rates during the 10-year period in the first and second screening performed at HEMOBA is shown in Figure 1.

During the study period, 415 municipalities of Bahia were identified as places of residence of blood donors who presented at HEMOBA. Only the municipalities of Almadina and Jiquiriçá were not represented in the samples in this period. In addition, 608 cities outside of Bahia were identified as places of residence, with 11,656 donors (1.43%); however, these donors were not included in the geographical representations because these cities were outside the cartographic limit of the state.

According to the data from the first screening, the municipalities with the highest prevalence were Livramento de Nossa Senhora, located in the center-south of the state ($n=2$, 100%), Cotegipe ($n=14$, 6.31%), Wanderley ($n=17$, 6.69%), Sebastião Laranjeiras ($n=5$, 7.25%), Mulungu do Morro ($n=8$, 7.92%), Jaborandi ($n=4$, 8.33%), and Lajedinho ($n=1$, 10%). In the second screening, the municipalities with the highest prevalence were Livramento de Nossa Senhora ($n=2$, 100%), Boa Vista do Tupim ($n=1$, 3.85%), Wanderley ($n=11$, 4.33%), Botuporã ($n=1$, 4.7%), and Lajedinho ($n=1$, 10%).

The places of residence with at least one non-negative result per year between February 2008 and February 2018 in the second screening are shown in Figure 2.

The municipalities with the highest number of recurrences was Salvador (state capital) (11 out of 11 years), Feira de Santana (located in the north-central region of the state) (9 out of 11 years), Barreiras (8 out of 11 years), and Santo Antônio de Jesus (8 out of 11 years).

DISCUSSION

Human CD is endemic in Brazil, despite the success of government initiatives adopted in the past few decades.

TABLE 1: Factors associated with *T. Cruzi* infection in the first and second screening.

Characteristics	Seroprevalence of <i>T. cruzi</i> infection (2008-2018)									
	First screening					Second screening				
	Total	%*	PR	(95% CI)	p	Total	%*	PR	(95% CI)	p
Total	3084	0.62				810	0.16			
Sex										
Male	1908	0.65	1.0919	(1.0150-1.1733)	p < 0.05	470	0.16	0.9298	(0.8088-1.0689)	ns
Female	1176	0.59	1			340	0.17	1		
Age, years										
≥ 30	2196	0.44	1.5863	(1.4676-1.7147)	p < 0.001†	683	0.14	3.4497	(2.8547-4.1689)	p < 0.001†
< 30	888	0.28	1			127	0.40	1		
Place of residence is located in Bahia										
Yes	3049	0.38	1.2657	(0.9075-1.7653)	ns	803	0.10	1.6667	(0.7922-3.5066)	ns
No	35	0.30	1			7	0.06	1		
Place of birth is located in Bahia										
Yes	2832	0.38	1.0743	(0.9447-1.2218)	ns	753	0.10	1.2629	(0.9649-1.6529)	ns
No	252	0.35	1			57	0.08	1		
Years of education										
≤ 12	2573	0.66	1.3236	(1.2040-1.4550)	p < 0.001†	738	0.19	2.6943	(2.1154-3.4317)	p < 0.001†
> 12	511	0.50	1			72	0.07	1		

*% of total blood donors that belong to this category between 2008 and 2018. CI, confidence interval; ns, not significant. † Significant in a multivariate logistic regression analysis.

TABLE 2: Seroprevalence distribution according to marital status and racial group.

Characteristics	Seroprevalence of <i>T. cruzi</i> infection (2008-2018)			
	Sample 1		Sample 2	
	Total	%*	Total	%*
Total	3084	0.62	810	0.16
Marital status				
Married	1299	0.75	373	0.22
Separated	10	0.89	4	0.36
Divorced	57	0.62	20	0.22
Judicially separated	10	0.71	2	0.14
Single	1634	0.55	386	0.13
Widow	41	1.29	17	0.01
Others	33	0.50	8	0.12
Racial group				
Caucasian	172	0.59	45	0.15
Brazilian Caucasian	1069	0.60	277	0.16
Indian	4	0.27	1	0.07
Mixed	1422	0.66	400	0.18
Black	417	0.60	87	0.13
Asian	0	0	0	0

*% of total blood donors that belong to this category between 2008 and 2018.

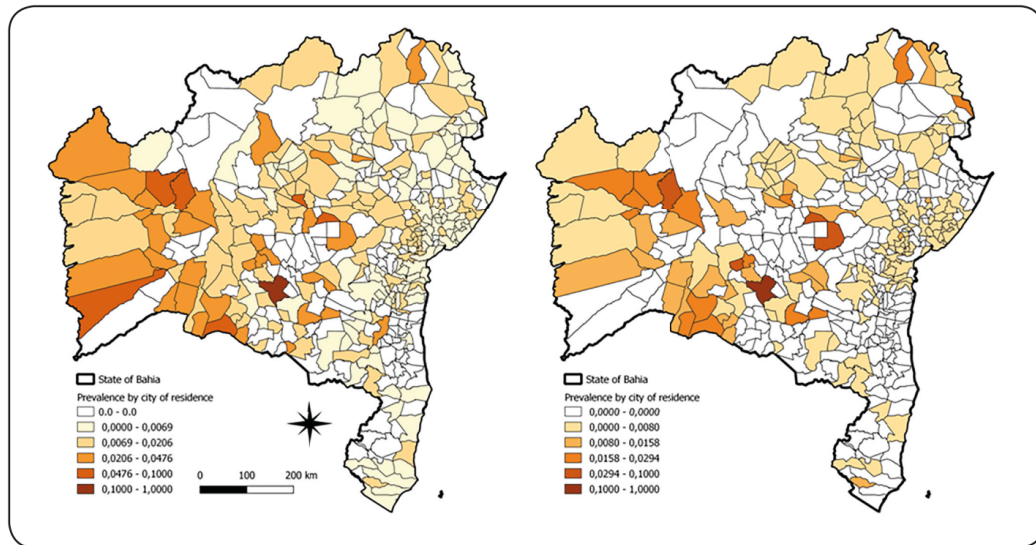


FIGURE 1: Seroprevalence of *Trypanosoma cruzi* infection in the state of Bahia in the first screening (left) and second screening (right) between 2008 and 2018.

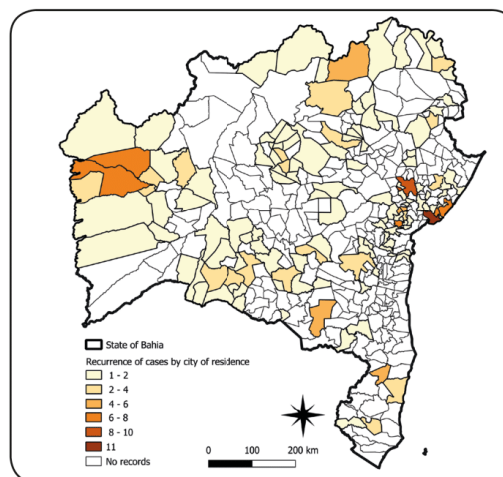


FIGURE 2: Recurrence of *Trypanosoma cruzi* infection (second sample) per year by municipality in the state of Bahia, Brazil, between 2008 and 2018.

Measures for eradicating vectorial transmission are constantly debated. However, much remains to be done because the disease is transmitted by other routes, including blood transfusion, which has gained importance in the epidemiological literature. Studies investigating the prevalence of CD in blood donors can provide quantitative epidemiological data and guide interventions in high-risk areas and population groups.

Among a population of 494,010 donors and 816,346 blood donations during the 10-year period, 3,084 (0.62%) samples were non-negative in the first screening. Among 1,486 donors, 810 (0.16%) samples were non-negative in the second screening. These rates may be underestimated because, despite strong guidance on the importance of second screening, many donors did not return to the blood units and therefore were not included in the analysis.

The prevalence rates were lower than those found in other studies conducted in Brazil but were relatively higher in densely-populated regions. Dias et al. (2000) found that the seropositivity for *T. cruzi* infection between 1987 and 1994 ranged from 0.77% to 2.22% in Bahia and from 0.69% to 0.88% in the northeast region of Brazil. Dias (1998) reported that the seropositivity between 1988 and 1990 in Brazil was 0.97% and decreased in the following years: 0.7–1.1% in 1991, 0.7% in 1993, and 0.75% in 1994; the seropositivity in the northeast of Brazil was 0.36% in 1994. In contrast, Monich (2016) found that the discard rate of donated blood in Curitiba between 2003 and 2012 varied from 0.6% to 0.9%, whereas Slavov et al. (2016) indicated that the discard rate in the state of São Paulo between 2012 and 2014 was 0.1%. It is worth mentioning that the methodology used in some studies did not involve second screening, and prevalence was calculated using donors with positive serology in the first screening. The prevalence in the present study using this approach was 0.62% (3,084 individuals).

The seroprevalence of *T. cruzi* infection was higher in individuals aged 30 years or older and individuals with 12 years or less of education in the first and second screening, and seroprevalence was significantly higher in men in the first screening. These results agree with previous studies²¹⁻²⁵. Data on seroprevalence rates according to marital status and racial group are summarized in **Table 1**.

With respect to the geographical distribution of the places of residence, 99.52% (415) of the municipalities of the state of Bahia and 608 municipalities outside the state borders had at least one diagnosed case of CD during the 10-years study period. Georeferencing was performed according to the place of residence rather than the place of birth because maternities are unavailable in many cities in the interior of the state. Moreover, the place of residence is a more reliable parameter because it represents, in most cases, the place where individuals are most exposed to infection throughout life.

Georeferencing enabled the identification of municipalities with higher seroprevalence (**Figure 1**). Increased access to blood transfusion services is a contributing factor for higher recurrence. Salvador, the state capital, is home to the HEMOBA headquarter and has the highest number of blood donors and donations, which may explain the higher rate of recurrence in this city. In addition, HEMOBA's blood collection stations were being implemented in municipalities of the state throughout the study period, which might have increased the number of blood donations.

Seroprevalence analysis of CD can accurately assess the risk of transfusion-related CD and the rate of disease transmission²⁸. This study identified high-prevalence regions in Bahia, allowing performing serological and epidemiological surveys in these areas and collecting epidemiological data to formulate health policies.

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Conflicts of Interests

The authors declare that there are no conflicts of interest associated with this study.

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9.7. *Apêndice 7 – Coautoria em capítulo de livro publicado: Diagnose e distribuição das espécies dos triatomíneos da Bahia.*



■ Diagnose das espécies de triatomíneos ocorrentes na Bahia

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Nesse capítulo é apresentado um breve resumo, na forma de uma ficha, para cada uma das espécies de triatomíneos que foram assinaladas na Bahia, destacando dentre os diversos aspectos intrínsecos para cada espécie, suas principais características morfológicas e métricas, seu nível de importância como vetor transmissor de *Trypanosoma cruzi*, frequência de encontro, distribuição, desenvolvimento e habitats. Para cada espécie é fornecido para consulta uma fotografia geral da espécie com escala em tamanho real e um mapa de distribuição geográfica.

Para a maioria das espécies de triatomíneos é apresentado um mapa da distribuição geográfica onde os pontos de coordenada da espécie são plotados sobre uma camada de bioma do Estado da Bahia. Para a confecção dos mapas foram utilizadas informações do banco de dados entre os anos 1957-1971⁽⁴⁾, dados do programa de vigilância Estadual dos vetores da doença de Chagas entre os anos, 2007- 2015, e os registros de ocorrência das espécies em ambiente domiciliar entre os anos 2007 e 2014, obtidos no banco de dados de triatomíneos do laboratório de entomologia do LACEN/BA.

Em todos os períodos analisados, os triatomíneos e suas informações foram coletados de forma geral, por três vias:

- Notificação de triatomíneos: realizada pela própria população, que leva o inseto suspeito até um Posto de Informação de Triatomíneo (PIT), instalado em locais estratégicos dos municípios, como escolas e postos de saúde;
- Atendimento à notificação: procedida pelos agentes que trabalham no Programa de Controle da doença de Chagas de cada

município e são dependentes da etapa anterior;

- Pesquisa ativa: neste caso, é realizada a procura de vetores em todas as unidades domiciliares da localidade, ou parte dela, independente da população ter realizado ou não alguma notificação.




Para as informações, utilizados nesse capítulo que relaciona os aspectos da biologia, importância vetorial e distribuição geográfica das espécies, foram consideradas as observações dos autores dessa obra e dos servidores que trabalham nas capturas em campo. Além disso, os diversos trabalhos publicados na literatura ratificaram tais observações^(4, 11, 73-102). Informações adicionais também foram obtidas em guias, chaves e Atlas de identificação de triatomíneos publicados para outros Estados do Brasil^(2, 5, 7, 8, 46, 103-105).

Parabelminus yurupucu Lent & Wygodzinsky, 1979

Características diagnósticas: Coloração geral negra, antena marrom, hemiélitro com manchas amareladas na base da membrana e adjacente ao cório; cabeça duas vezes mais longa que larga, distintamente mais longa que o pronoto; pronoto em forma de sino; escutelo sem processo apical; fêmures medianos e posteriores com um par de processos espinhosos cada.

Biologia: espécie endêmica da Bahia. Ocorre em bromélias e troncos de árvores com ninhos de roedores, onde provavelmente alimenta-se de répteis e anfíbios. Encontrada em associação com *Triatoma tibiamaculata* e *Rhodnius domesticus*.

Importância vetorial (I.V): Espécie rara e estritamente silvestre.

Escala Tamanho Real	 9,5 a 9,7 (fêmeas) 8 a 9 mm (machos) 8,85mm Média de Tamanho	I.V. 
Progressão		Sem informações



Galvão, C., 2014 (modificado)

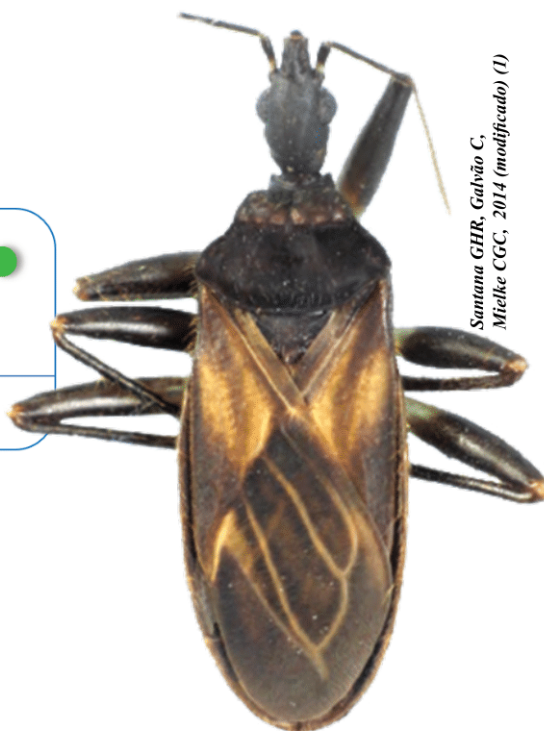
Cavernicola pilosa Barber, 1937

Características diagnósticas: Coloração geral negra com manchas amareladas no hemiélitro; corpo piloso; cabeça fusiforme em vista dorsal, em vista lateral muito convexa dorsalmente; processo apical do escutelo muito pequeno, dirigido para cima; conexivo com todos os segmentos da mesma cor; ocelos muito pequenos; conexivo escuro.

Biologia: encontrada em cavernas e ocas de árvores habitados principalmente por morcegos. A infecção natural desse triatomíneo por *Trypanosoma cruzi* tem sido observada.

Importância vetorial (I.V): baixa. Há registros raros em domicílios. Foi registrada apenas uma vez na Bahia.

Escala Tamanho Real		I.V. ●
	12 a 13,8 mm (fêmeas) 11 a 11,5 mm (machos) 12,4mm Média de Tamanho	
Progressão		92 dias



Santana GHR, Galvão C,
Mielke CGC, 2014 (modificado) (1)

P *sammolestes tertius* Lent & Jurberg, 1965

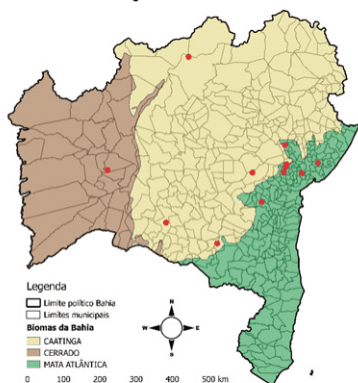
Características diagnósticas: Coloração geral marrom-amarelada com manchas irregulares marrom-escuras ou negras; cabeça ligeiramente mais longa que larga; região antecular com 2,5 vezes o comprimento da pós-ocular; ângulos ântero-laterais do pronoto muito curtos, não proeminentes.




Biologia: frequentemente encontrada em ninhos de aves dos gêneros *Phacellodomus*, *Pseudoseisura* e *Anumbius*, conhecidas regionalmente como graveteiro, João-graveto, João-tenenem, cochicho, *titiri* e casaca-de-couro. Essa espécie põe seus ovos aderidos aos gravetos usados pelas aves para construir seus ninhos. Comportamento que pode influenciar na sua dispersão passiva, pelos pássaros.

Importância vetorial (I.V): Baixa



Santos & Sousa, 2016



Escala Tamanho Real		I.V. 
	12 a 13,5 mm (fêmeas) 11,5 a 12,5 mm (machos) 12,5mm Media de Tamanho	
Progressão		165 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016

BANCO DE DADOS:

1957-1972: (4)

2007- 2015: Programa de Vigilância Estadual

2007 – 2014: Entomologia do Lacen- BA

Rhodnius nasutus Stål, 1859

Características diagnósticas:

Coloração geral marrom-amarelada, ligeiramente alaranjada; cabeça mais longa que o pronoto; antenas de coloração marrom-avermelhada, com ápice do segundo e metade apical do terceiro artícuo mais escuros; ângulos ântero-laterais proeminentes. Escutelo escuro com 1 + 1 carenas claras; pernas uniformemente marrom-avermelhadas; conexivo com manchas não muito nítidas na região ântero-lateral de cada segmento.




Biologia: essa espécie é característica da caatinga, ocorrendo em palmeiras principalmente da espécie *Copernicia prunifera* (carnaúba). Além de outras espécies de palmeiras e espécies arbóreas da Caatinga. Na Bahia, há registros dessa espécie dividindo habitat com *Rhodnius neglectus* e *Triatoma sordida*.

Importância vetorial (I.V):

A ocorrência de espécimes adultos e infectados por *Trypanosoma cruzi* em outros Estados brasileiros, principalmente no Ceará



Santos & Sousa, 2016

Escala Tamanho Real		I.V. 
	14 a 18 mm (fêmeas) 12,5 a 16,5 mm (machos) 15,25mm Média de Tamanho	
Progressão		209 dias

Rhodnius neglectus Lent, 1954

Características diagnósticas:

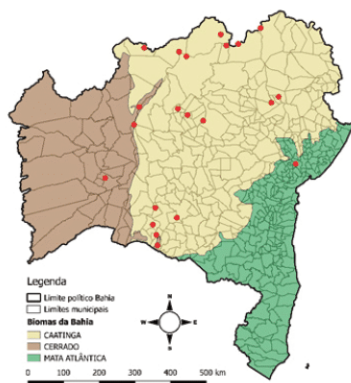
Coloração geral marrom-clara com marcas marrom-escuras na cabeça, pronoto, escutelo, cório e conexivo e áreas amareladas ventralmente no abdome, no conexivo, nas coxas e trocânteres; cabeça distintamente mais longa que o pronoto; ângulos ântero-laterais proeminentes; pernas marrom-escuras, coxas, trocânteres e anel basal da tíbia amarelados; conexivo dorsal com metade ou 2/3 anteriores marrom, o resto amarelado.

Biologia: frequentemente encontrada em palmeiras dos gêneros *Attalea* (babaçus), *Acrocomia* (macaúbas), *Mauritia* (buritis) e *Copernicia* (carnaúbas), além de ninhos de pássaros graveteiros (*Phacellodomus*).

Importância vetorial (IV): Baixa. Nesse Estado a invasão e colonização peridomiciliar, principalmente em galinheiros foi assinalada e espécimes infectados foram detectados em São Desidério e Ibotirama



Santos & Sousa, 2016



Escala Tamanho Real		I.V.
	18,5 a 20 mm (fêmeas)	
	17,5 a 19 mm (machos)	
	18,75mm Média de Tamanho	
Progressão		340 dias



Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Rhodnius domesticus Neiva & Pinto, 1923

Características diagnósticas: Coloração geral marrom-alaranjada com manchas mais escuras em várias áreas do corpo e pernas; cabeça escura, tão longa ou ligeiramente mais longa que o pronoto, o qual possui carenas submedianas de coloração amarelo-luminosa; ângulos ântero-laterais não proeminentes; hemiélitro cor de palha e membrana fracamente pigmentada; pernas estreitas com coxas, trocânteres e fêmures uniformemente castanho-escuros, tíbias mais claras com anelação apical escura.

Biologia: essa espécie é característica da Mata Atlântica, ocorrendo em bromélias, ninhos de roedores, ocos de árvores e palmeiras.

Importância vetorial (I.V): baixa. Espécie rara em ambiente domiciliar. Na Bahia, espécimes adultos foram detectados recentemente em casas do município de São Félix, no Recôncavo Baiano.

<p>Escala Tamanho Real</p>	 <p>I.V. ●</p> <p>16,5 a 18 mm (fêmeas) 15 a 17 mm (machos) 16,5 mm Média de Tamanho</p>
<p>Progressão</p>	 <p>95 dias</p>



Santos & Sousa, 2016

Panstrongylus megistus (Burmeister, 1835)

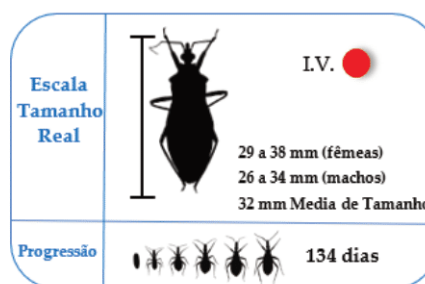
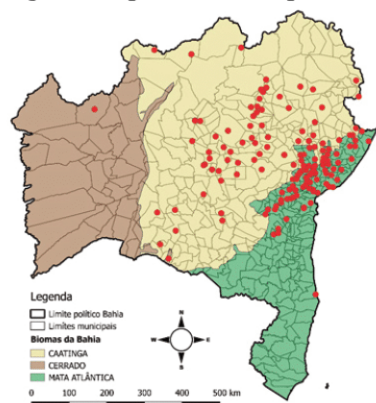
Características diagnósticas: Coloração geral negra com manchas vermelhas no pescoço, pronoto, escutelo, cório e conexivo; pronoto com lobo anterior negro e posterior negro com 2 + 2 grandes manchas vermelhas; processo do escutelo curto, arredondado, cônico; pernas negras; segmentos do conexivo com mancha escura na parte anterior.

Biologia: vivem em ocos de árvores e palmeiras, onde geralmente se alimentam de mamíferos, principalmente marsupiais (*Didelphis* spp.). No entanto, apresenta hábito alimentar variado, alimentando-se também em aves, cães, gatos, roedores, bovinos, caprinos, suínos e humanos.

Importância vetorial (I.V): Após o controle de *Triatoma infestans*, tem sido considerada a espécie com maior importância epidemiológica no Brasil. É a quarta espécie mais capturada na Bahia.



Santos & Sousa, 2016



Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do LACEN- BA

Panstrongylus lenti Galvão & Palma, 1968

Características diagnósticas: Menor espécie do gênero *Panstrongylus*. Apresenta coloração geral marrom-clara levemente avermelhada. Pronoto com tubérculos disciais pequenos, mas distintos; tubérculos laterais obsoletos; escutelo com processo apical tão longo quanto o corpo do escutelo; ângulos humerais arredondados; ângulos ântero-laterais bem desenvolvidos, subcônicos; cabeça 1,5 vezes mais curta que larga e mais curta que o pronoto; pernas uniformemente marrom amareladas claras; placas conexivais III e IV com pequenas manchas escuras próximas ao bordo anterior.

Biologia: habitats silvestres; biologia desconhecida.

Importância vetorial (I.V): baixa devido ao encontro raro da espécie. Foram assinalados registros em municípios da região centro-sul na Bahia e um encontro na região do litoral norte.



Galvão, C., 2014 (modificado)



Escala Tamanho Real		I.V. ●
	19 mm (fêmeas) 18 mm (machos) 16,5 mm Media de Tamanho	
Progressão		688 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Panstrongylus geniculatus (Latreille, 1811)

Características diagnósticas:

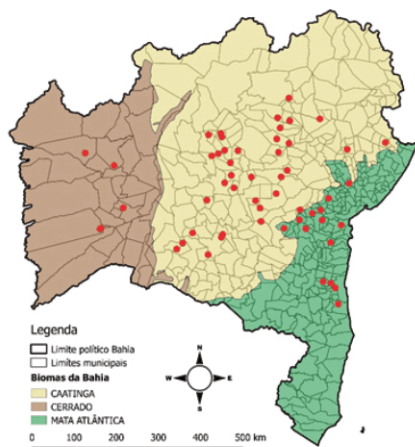
Coloração geral marrom-clara ou marrom-alaranjada, com áreas escuras em várias partes do corpo; pronoto marrom-alaranjado, com lobo anterior com marca central negra em forma de trevo de quatro folhas; processo do escutelo alongado, cilíndrico e afilado na ponta; lobo posterior com faixa preta ao longo da margem posterior, excluindo a região humeral; coxas e trocânteres amarelados ou alaranjados; fêmures escuros, avermelhados no ápice; ventre do abdome claro com série longitudinal de manchas pretas.




Biologia: encontrado em tocas de tatus e pacas, em cavernas, sob cascas de árvores, próximo a ninhos de aves e em várias espécies de palmeiras.

Importância vetorial (I.V): Média. Já foram assinaladas colonizando casas e adultos foram encontrados infectados por *Trypanosoma cruzi* na Bahia.



Santos & Sousa, 2016



Escala Tamanho Real		IV. 
	22,5 a 29,5 mm (fêmeas) 22 a 28 mm (machos) 16,5 mm Média de Tamanho	
Progressão		387 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Panstrongylus lutzi (Neiva & Pinto, 1923)

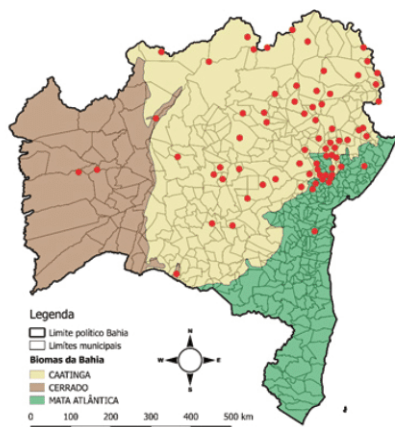
Características diagnósticas: Coloração geral marrom-amarelada, com marcas marrom-escuras na cabeça, pronoto, pleura, escutelo, cório e conexivo; rostro com segundo segmento mais longo que o primeiro; lobo anterior do pronoto com tubérculos disciais bem desenvolvidos e marcas escuras; processo do escutelo alongado, cilíndrico e afilado na ponta; asa anterior com cório tão escuro quanto a membrana, com a base e o ápice mais claros; pernas uniformemente marrom-avermelhadas, um pouco mais escuras no ápice; fêmures anteriores e medianos com espinhos.




Biologia: Característica da caatinga: na área silvestre essa espécie é encontrada em tocas de tatus, rochas habitadas por mocós (*Kerodon rupestris*). No peridomicílio alimenta-se de forma muito variada e apresenta altos índices de infecção por *Trypanosoma cruzi*.

Importância vetorial (I.V): Média. A ocorrência de espécimes adultos e infectados por *Trypanosoma cruzi* já foi assinalada na Bahia e em outros Estados.



Santos & Sousa, 2016



Escala Tamanho Real		I.V. 
		28 a 29 mm (fêmeas) 24 a 28,5 mm (machos) 26,5 mm Média de Tamanho
Progressão		688 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Panstrongylus diasi Pinto & Lent, 1946

Características diagnósticas:

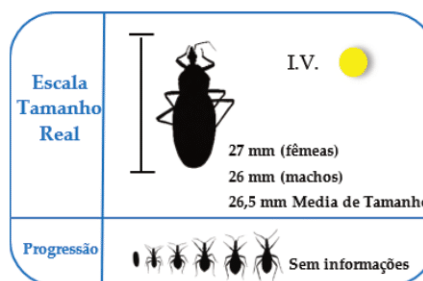
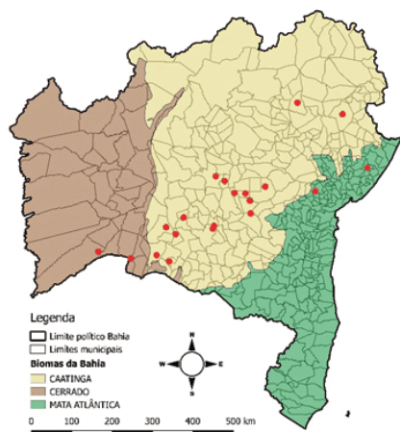
Coloração geral marrom-amarelada com áreas marrom-escuras no pronoto, escutelo, cório e conexivo; rostró com segundo segmento mais longo que o primeiro; cabeça muito mais curta que o pronoto; lobo anterior do pronoto com tubérculos disciais obsoletos ou ausente; processo do escutelo alongado, cilíndrico e afilado na ponta; asa anterior com cório tão escuro quanto a membrana, com a base e o ápice mais claros; fêmures anteriores e medianos com dois ou três espinhos cada

Biologia: habitats silvestres; biologia desconhecida.

Importância vetorial (I.V): Média. Tem sido encontrada em ambiente domiciliar na Bahia (região centro-sul) e outros Estados brasileiros, porém, dificilmente estabelece colônias. A infecção natural desse triatomíneo por *Trypanosoma cruzi* tem sido observada em ambiente domiciliar na Bahia.



Santos & Sousa, 2016



Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Triatoma rubrofasciata (De Geer, 1773)

Características diagnósticas:

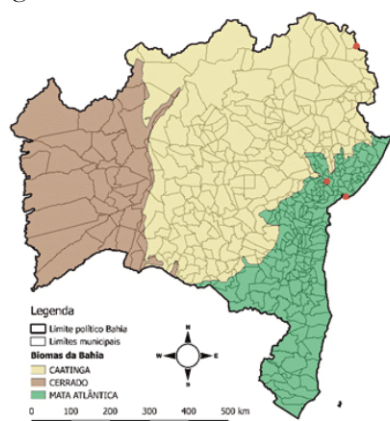
Coloração geral castanho-escuro ou negra com manchas amarelas, alaranjadas ou avermelhadas. Cabeça fortemente granulosa dorsalmente, uniformemente negra, ligeira mente mais curta que o pronoto; primeiro segmento da antena ultrapassando consideravelmente o ápice do clipeo; pronoto preto com margens laterais inteiramente vermelhas; conexivo escuro com manchas claras estreitas envolvendo as suturas intersegmentares; abdome com achatamento mediano longitudinal no ventre. Pernas uniformemente negras.




Biologia: Habitats silvestres desconhecidos. Geralmente espécie intimamente associada a ratos (*Rattus rattus*) e distribuição geográfica associada a zonas portuárias dos trópicos. Única espécie de barbeiro considerada cosmopolita.

Importância vetorial (I.V): Baixa. Embora tenha sido encontrada infectada por *Trypanosoma cruzi*. Na Bahia, sua ocorrência foi registrada em Salvador.



Santos & Sousa, 2016



Escala Tamanho Real		I.V. 
	20 a 25 mm (fêmeas) 19,5 a 24 mm (machos) 22,25 mm Média de Tamanho	
Progressão		228 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA




Triatoma tibiamaculata (Pinto, 1926)

Características diagnósticas:

Coloração geral negra, com manchas alaranjadas ou avermelhadas na cabeça, pescoço, pronoto, escutelo, cório, pernas e conexivo; cabeça negra, tão longa ou ligeiramente menor que o pronoto; primeiro segmento da antena quase atingindo o ápice do clipeo; pronoto escuro com áreas vermelho alaranjadas nas margens laterais e posterior; escutelo negro com ápice avermelhado; ângulos ântero-laterais e um par de faixas sobre as carenas e tubérculos disciais; pernas negras com exceção das tíbias, que são nitidamente amareladas ou alaranjadas, com anelacção escura no ápice; conexivo com manchas transversais negras e alaranjadas ou amareladas, a parte negra ocupa os 2/3 anteriores de cada segmento.

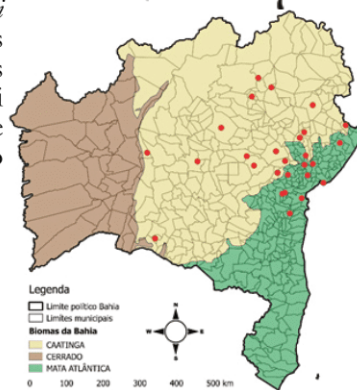
Biologia: espécie silvestre da Mata Atlântica, encontrada em refúgios de marsupiais e roedores, bromélias e palmeiras. Na Bahia ocorre na faixa litorânea, principalmente no Recôncavo Baiano e em Salvador e região metropolitana.

Importância vetorial (I.V): Média. Espécimes infectados por *Trypanosoma cruzi* têm sido frequentemente encontrados em áreas urbanas de Salvador, próximo a remanescentes florestais, onde há presença de palmeiras. Foi indicada como responsável pelo surto de infecção oral de doença de Chagas no Estado de Santa Catarina em 2005⁽¹⁰⁶⁾.

Escala Tamanho Real		I.V. 
	32 a 33,5 mm (fêmeas) 29 a 30 mm (machos) 27,8 mm Média de Tamanho	
Progressão		204 dias



Santos & Sousa, 2016



Fonte: (Ribeiro) – CPOGM; Santos & Sousa– LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do LACEN- BA

Triatoma sherlocki Papa, Jurberg, Carcavallo, Cerqueira & Barata, 2002

Características diagnósticas:

Coloração geral castanho-escuro a negra, com manchas alaranjadas ou avermelhadas semicirculares no conexivo; cabeça negra, mais longa que o pronoto, o qual possui lobo anterior castanho-escuro a negro e bordo do lobo posterior levemente mais claro; pronoto e cório inteiramente pretos; primeiro segmento da antena curto, não atingindo o ápice do clipeo; asas anteriores chegando até a base do sexto tergito abdominal; trocânteres escuros; fêmures escuros com anel laranja avermelhado no centro; tíbias inteiramente escuras.

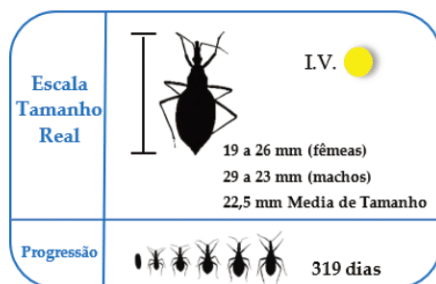
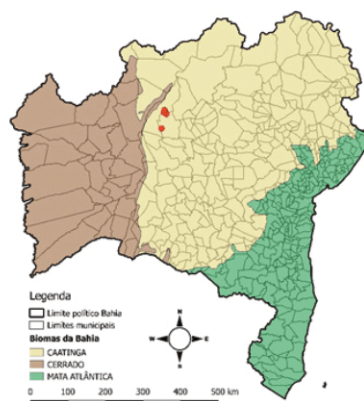
Biologia: espécie associada a rochas.

Importância vetorial (I.V):

Média. Adultos e ninfas infectados por *Trypanosoma cruzi* têm sido encontrados em ambiente doméstico em comunidades de mineradores no município de Gentio do Ouro, indicando que a espécie está em processo de domiciliação nessas áreas.



Santos & Sousa, 2016



Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Triatoma sordida (Stål, 1859)

Características diagnósticas:

Coloração geral de castanho-clara a castanho escura, com manchas amareladas claras na cabeça, pescoço, pronoto, escutelo, hemiélitro, pernas e conexivo; cabeça mais longa que o pronoto; primeiro segmento da antena não atingindo o ápice do clipeo; pronoto marrom com um par de manchas amareladas nos úmeros e com distintos tubérculos laterais e discais; coxas, trocânteres, e maior parte dos fêmures claros; fêmures com manchas claras irregulares no dorso e anel claro próximo ao ápice; tíbias inteiramente escuras; conexivo com manchas negras transversais incluindo a sutura intersegmental e desenhos similares a notas musicais.

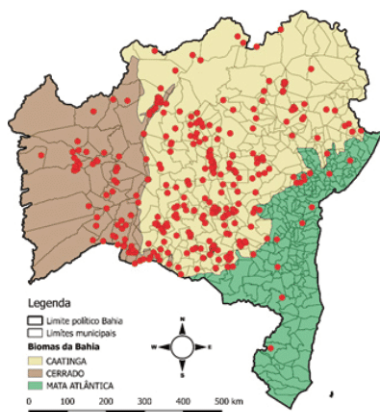
Biologia: no ambiente silvestre, é encontrada sob cascas de árvores e em ninhos de pássaros, principalmente em áreas de cerrado. Em ambiente domiciliar ocorre frequentemente associados a galinheiros, pombais, chiqueiros, currais e pilhas de lenha.

Importância vetorial (I.V):

Média. Devido a seu comportamento predominantemente peridomiciliar, quanto por sua marcada ornitofilia e baixos índices de infecção natural por *Trypanosoma cruzi*.



Santos & Sousa, 2016



Escala Tamanho Real		I.V.
		15 a 20 mm (fêmeas) 14 a 19 mm (machos) 17 mm Média de Tamanho
Progressão		392 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LA-CEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

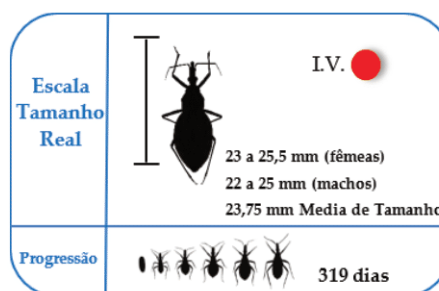
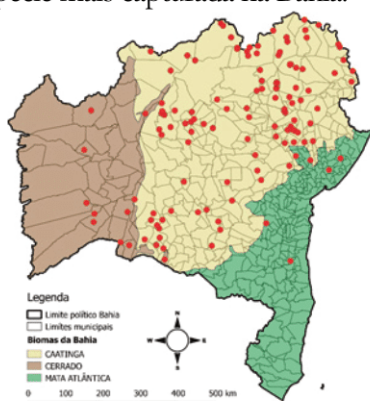
Triatoma brasiliensis (Neiva, 1911)

Características diagnósticas:

Coloração geral castanho-escuro a negra, com manchas amareladas no pescoço, pronoto, pernas, hemiélitro e conexivo; primeiro segmento da antena atingindo o ápice do clipeo; cabeça castanho-escuro e distintamente mais longa que o pronoto, o qual possui faixas longitudinais amarelas, alargando-se para fora das carenas medianas, desde a margem posterior do lobo posterior até o lobo anterior, onde se estreitam; trocânteres predominantemente amarelos; fêmures com anel mediano largo.

Biologia: Espécie encontrada sob rochas, em ambiente silvestre, associa a roedores. Encontrada também colonizando no ambiente domiciliar, além de colônias em galinheiros, currais, muros de pedras, amontoados de tijolos, telhas e madeira.

Importância vetorial (I.V): Alta. Frequentemente encontrada em ambiente domiciliar, sendo considerado o vetor mais importante no nordeste do Brasil, após o controle de *Triatoma infestans*. É a terceira espécie mais capturada na Bahia.



Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Triatoma melanica Costa, Argolo & Felix, 2006

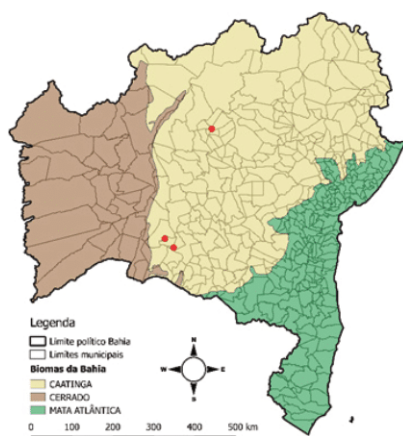
Características diagnósticas: Cor geral negra com áreas amareladas; primeiro segmento da antena atingindo o ápice do clipeo; pronoto com um par de faixas trapezoidais partindo da margem posterior do lobo posterior, mas não atingindo o lobo anterior; membrana do hemiélitro com células internas totalmente negras; trocânteres escuros; fêmures com base escura, claro próximo do centro, usualmente anelados; tíbias com anelação clara perto do ápice.

Biologia: encontrada principalmente no ambiente silvestre (pedregais), mas pode invadir os domicílios, principalmente durante os períodos de seca.

Importância vetorial (I.V): Ainda não existe dados suficientes para atribuir uma importância vetorial no Estado da Bahia.



Santos & Sousa, 2016



Escala Tamanho Real	<p>21 a 24 mm (fêmeas) 20,3 a 24 mm (machos) 22,15 mm Média de Tamanho</p>
Progressão	<p>Sem informação</p>

Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016

BANCO DE DADOS:

1957-1972: (4)

2007- 2015: Programa de Vigilância Estadual

2007 – 2014: Entomologia do LACEN- BA

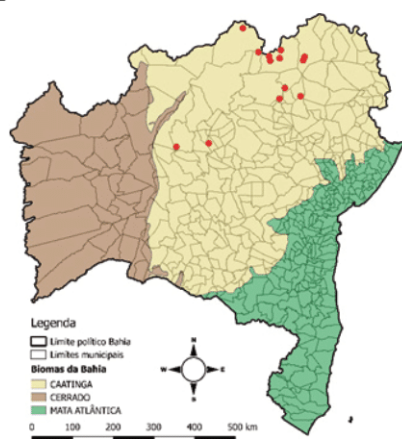
Triatoma juazeirensis Costa & Felix, 2007

Características diagnósticas:

Cor geral negra, com partes amareladas a acastanhadas; primeiro segmento da antena atingindo o ápice do clipeo; pronoto, em geral, inteiramente negro, podendo apresentar um par de pequenos pontos castanhos na parte anterior da carena submediana; membrana do hemiélitro com células internas parcialmente enegrecidas; trocânteres escuros com marcas claras; coxas e fêmures escuros; tíbias com porção levemente amarelada próxima ao ápice.



Biologia: encontrada em rochas no ambiente silvestre, assim como *Triatoma brasiliensis*. Espécie aparentemente endêmica da Bahia, ocorrendo em áreas ao norte do vale do São Francisco e centro-norte baiano.

Importância vetorial (I.V): Ainda não existe dados suficientes para atribuir uma importância vetorial no Estado da Bahia.



Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA



Escala Tamanho Real	 20 a 24 mm (fêmeas) 23 a 25,5 mm (machos) 23,5 mm Média de Tamanho
Progressão	 Sem informação

Triatoma infestans (Klug, 1834)

Características diagnósticas:

Coloração geral negra ou castanho-escuro, com manchas amarela das nas pernas, cório e conexivo; primeiro segmento da antena não atingindo o ápice do clipeo; cabeça tão longa quanto o pronoto o qual também é negro; escutelo negro, fortemente rugoso; pernas totalmente negras, ou com os trocânteres e base dos fêmures amarelados; conexivo preto, cada segmento com grande mancha amarela afastada da sutura anterior, mas estreitamente próxima da fronteira do segmento posterior.

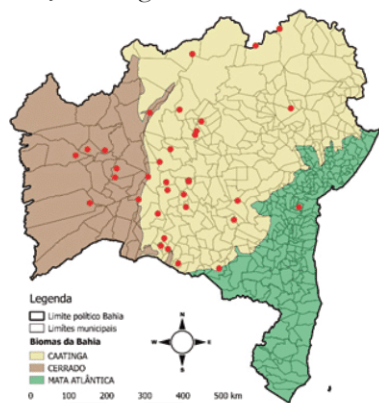
Biologia: espécie quase exclusivamente domiciliar na Bahia, até os programas de controle.

Importância vetorial (I.V): Alta.

Foi o principal alvo nas campanhas de controle vetorial até 2006 no Brasil, quando foi certificado pela Comissão Intergovernamental da Iniciativa do Cone Sul a erradicação dessa espécie. No entanto, focos residuais ainda persistem na Bahia, como no município de Novo Horizonte, até o ano de 2015. Sua eliminação é um dos principais desafios dos serviços de vigilância e controle no Estado.



Santos & Sousa, 2016



Escala Tamanho Real		I.V. ●
		26 a 29 mm (fêmeas) 21 a 26 mm (machos) 23,5 mm Media de Tamanho
Progressão		303 dias

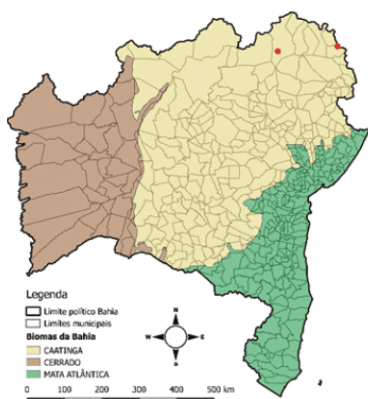
Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Triatoma petrocchiae Pinto & Barreto, 1925

Características diagnósticas: Coloração geral castanho-escura com manchas amareladas; cabeça negra e muito mais longa que o pronoto; primeiro segmento antenal muito curto, não atingindo o ápice do clipeo; terceiro segmento do rostro mais curto que o segundo; pronoto marrom escuro com tubérculos disciais reduzidos e um par de marcas amarelas nos úmeros; cório marrom com manchas amarelas na base e ápice; fêmures e tíbias inteiramente escuros; conexivo marrom escuro nas porções anterior e posterior, amarelo centralmente.

Biologia: encontrada no ambiente silvestre preferencialmente em refúgio de marsupiais conhecidos vulgarmente na Bahia como mocós (*Kerodon rupestris*).

Importância vetorial (I.V): Baixa. A invasão de espécimes adultos nas casas, é rara na Bahia.



Escala Tamanho Real		I.V.
		18 a 23 mm (fêmeas) 17 a 21,3 mm (machos) 20 mm Media de Tamanho
Progressão		Sem informações

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015; Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Triatoma pseudomaculata Corrêa & Espínola, 1964

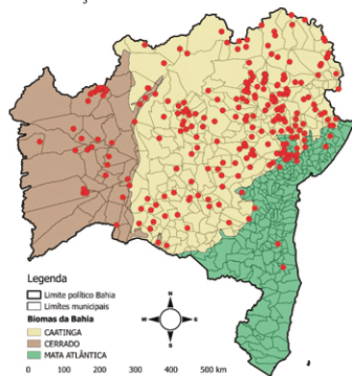
Características diagnósticas: Coloração geral castanho-escuro ou negra, com manchas amareladas, alaranjadas ou avermelhadas; cabeça uniformemente negra, tão longa quanto o pronoto, o qual é castanho-escuro ou negro, com lobo posterior apresentando três pares de manchas amareladas ou alaranjadas; genas ultrapassando o ápice do clipeo; ângulos ântero-laterais amarelo claros, dirigidos lateralmente; lobo anterior do pronoto com tubérculos disciais e laterais; pleura negra, na maioria dos espécimes com manchas alaranjadas na região posterior (ausente em alguns espécimes); pernas uniformemente negras; conxivo amarelado, alaranjado ou avermelhado com faixa escura larga situada sobre a sutura interconexival.




Biologia: comumente encontrada associada a galinheiros em cercas de madeira feitos com galhos secos, em muitos casos estabelecendo grandes colônias. Em ambiente silvestre vive em ocos e sob cascas de árvores secas, onde se alimenta em répteis, aves e pequenos mamíferos.

Importância vetorial (I.V): Média. A invasão de espécimes adultos e ninfas colonizando o intradomicílio tem sido observada na Bahia. Um dos fatores de risco de domiciliação pode ser o transporte passivo de lenha para uso diário, e madeira para construção de cercas.



Santos & Sousa, 2016



Escala Tamanho Real		I.V. 
		19 a 20 mm (fêmeas) 17 a 19 mm (machos) 20 mm Média de Tamanho
Progressão		487 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Triatoma costalimai Verano & Galvão, 1959

Características diagnósticas:

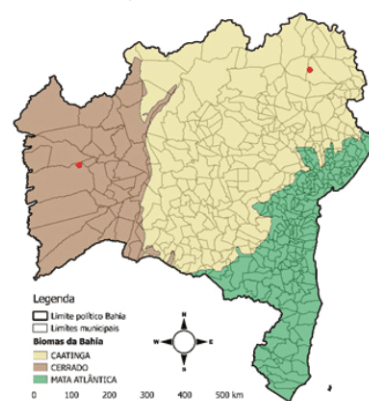
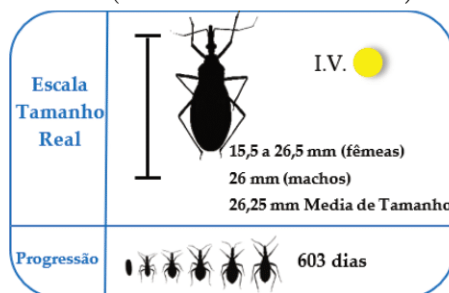
Coloração geral castanho-escuro ou negra, com manchas avermelhadas no pescoço, cório e conexivo; cabeça negra e distintamente mais longa que o pronoto; primeiro segmento da antena curto, não atingindo o ápice do clipeo; pronoto totalmente castanho-escuro ou preto; escutelo com processo apical quase tão longo quanto o corpo principal; pernas uniformemente negras; conexivo castanho-escuro, cada segmento com uma mancha vermelho-alaranjada de tamanho variável situada ao longo do bordo externo; fêmeas e tibiás inteiramente escuros

Biologia: encontrada comumente entre rochas calcárias associada a lagartos, roedores e marsupiais.

Importância vetorial (I.V): Média. Espécie predominantemente silvestre. Porém, ninfas e adultos já foram encontrados no peridomicílio, principalmente em casas próximas a afloramentos rochosos. Na Bahia, existem registros de invasão de espécimes infectados em ambiente intradomiciliar, no oeste baiano (São Desidério e Catolândia).



Santos & Sousa, 2016



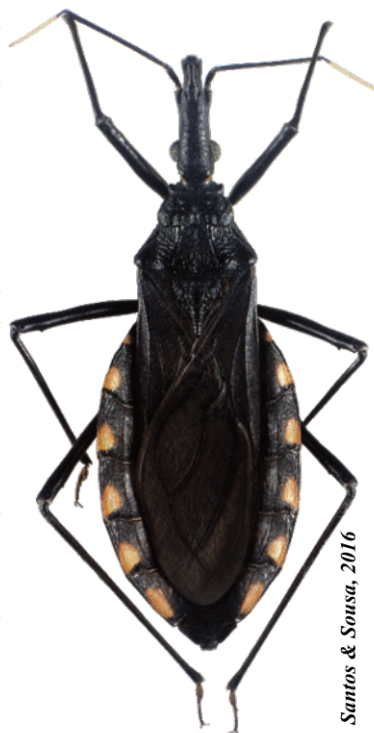
Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA

Triatoma lenti Sherlock & Serafim, 1967

Coloração geral castanho-escura ou negra, com manchas avermelhadas no pescoço, cório e conexivo; cabeça negra e distintamente mais longa que o pronoto; primeiro segmento da antena atingindo o ápice do clipeo; pronoto totalmente castanho-escuro ou preto; escutelo com processo apical quase tão longo quanto o corpo principal; conexivo castanho-escuro, cada segmento com uma mancha vermelho-alaranjada de tamanho variável situada ao longo do bordo externo; fêmures e tíbias inteiramente escuros; manchas do conexivo amarelo alaranjadas.



Biologia: encontrada em domicílios, currais de cabras e entre rochas, em municípios da região centro-sul da Bahia.

Importância vetorial (I.V): Baixa. A colonização em domicílios na Bahia é rara.



Santos & Sousa, 2016



Escala Tamanho Real		I.V. ●
	26,5 a 27,5 mm (fêmeas) 25 a 26 mm (machos) 26,25 mm Média de Tamanho	
Progressão		636 dias

Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007-2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Triatoma lenti Sherlock & Serafim, 1967

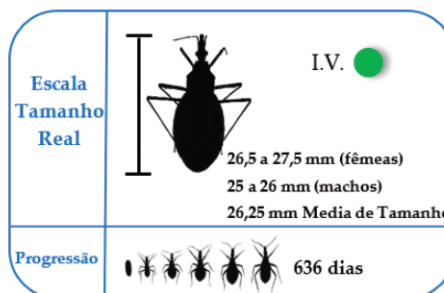
Colorido geral negro, com manchas alaranjadas; cabeça maior que o pronoto sendo este, de cor negra e/ou tonalidade de castanho escuro; escutelo negro com ápice relativamente longo, reto e rugoso; hemiélitros não atingindo o ápice do abdômen; cório com duas manchas alaranjadas disfórmicas em cada lado, uma menor no ápice e uma maior na base; abdômen de cor castanho escura e espiráculos claros; conexivo dorsal discretamente mais largo que o ventral com manchas bem delimitadas alaranjadas em cada segmento.

Biologia: encontrada em domicílios, currais e cabras e entre rochas em municípios da região centro-sul da Bahia.

Importância vetorial (I.V): Baixa.
Colonização rara em domicílios na Bahia.



Santos, Sousa & Ribeiro, 2016
Coleção: Sherlock, CPQGM (modificado)



Fonte: (Ribeiro) – CPQGM; Santos & Sousa – LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Triatoma vitticeps (Stål, 1859)

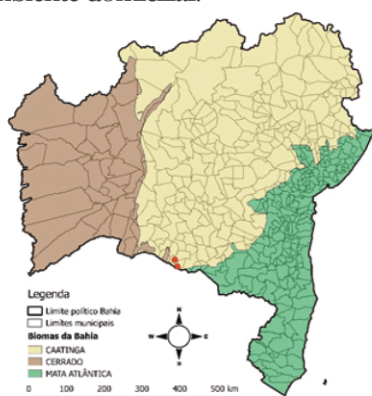
Coloração geral de castanho-escuro a negra, com manchas alaranjadas ou avermelhadas na cabeça, pescoço, pronoto, pleura, escutelo, hemiélitro e conexivo; cabeça castanho-escuro com uma faixa dorsal castanho-avermelhada indo dos ocelos até o clipeo, distintamente mais curta que o pronoto, o qual é castanho-escuro, com manchas avermelhadas; escutelo castanho-escuro, com depressão central avermelhada; pernas negras; conexivo castanho-escuro com manchas transversais alaranjadas ou amareladas menores que as áreas escuras.

Biologia: encontrada em refúgios de marsupiais, roedores, galinheiros e currais. Ocorre em regiões mais úmidas, em áreas de Mata Atlântica na Bahia.

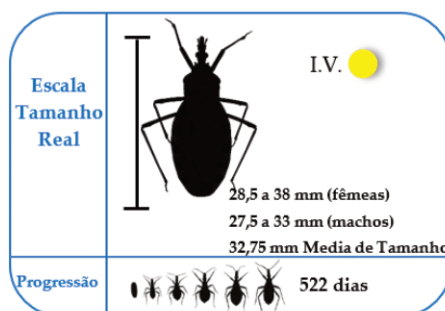
Importância vetorial (I.V): Média. Adultos frequentemente invadem casas, atraídos pela luz; pequenas colônias já foram encontradas em ambiente domiciliar.



Santos & Sousa, 2016



Fonte: (Ribeiro) – CPQGM; Santos & Sousa– LACEN, 2016
 BANCO DE DADOS:
 1957-1972: (4)
 2007- 2015: Programa de Vigilância Estadual
 2007 – 2014: Entomologia do Lacen- BA



T

riatoma melanocephala Neiva & Pinto, 1923

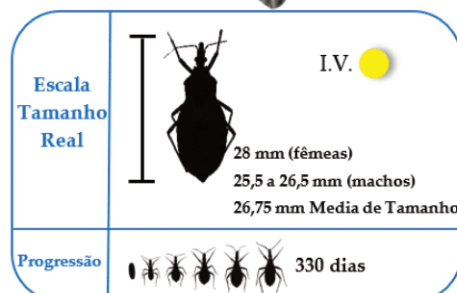
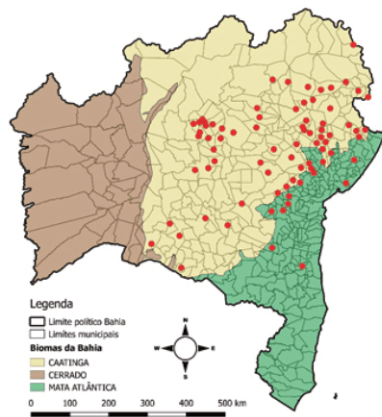
Coloração geral castanho-escuro a negra, com manchas amareladas no pescoço, pronoto, hemiélitros e conexivo; primeiro segmento da antena atingindo o ápice do clipeo; genas no máximo atingindo o ápice do clipeo; cabeça negra, aproximadamente tão longa quanto o pronoto; pronoto marrom a preto com um par de manchas amarelas na metade anterior do lobo posterior; segmentos conexivais com as manchas negras de formato irregular localizadas sobre as suturas intersegmentares; fêmures e tíbias inteiramente escuros; pleuras e ventre do tórax e abdome polidos.

Biologia: Silvestre, associado a bromélias, refúgios de marsupiais, e ocasionalmente no peridomicílio e domicílio. Espécimes silvestres foram coletados em bromélias terrestres, em áreas semiáridas no Nordeste. Estavam associados à *Didelphis albiventris* e infectados por *Trypanosoma cruzi*.

Importância vetorial (I.V): Média. A invasão de espécimes adultos nas casas tem sido muito registrada na Bahia, porém, dificilmente estabelecendo colônias.

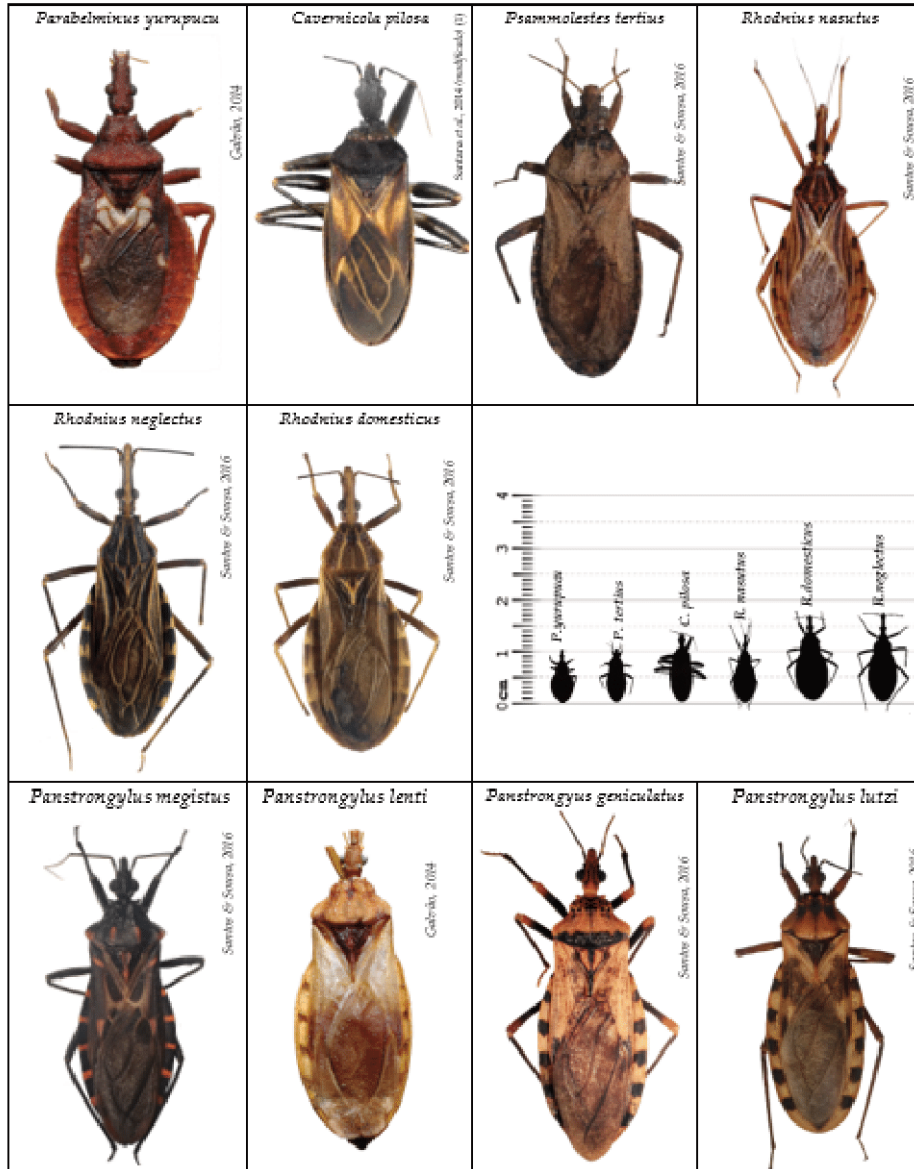


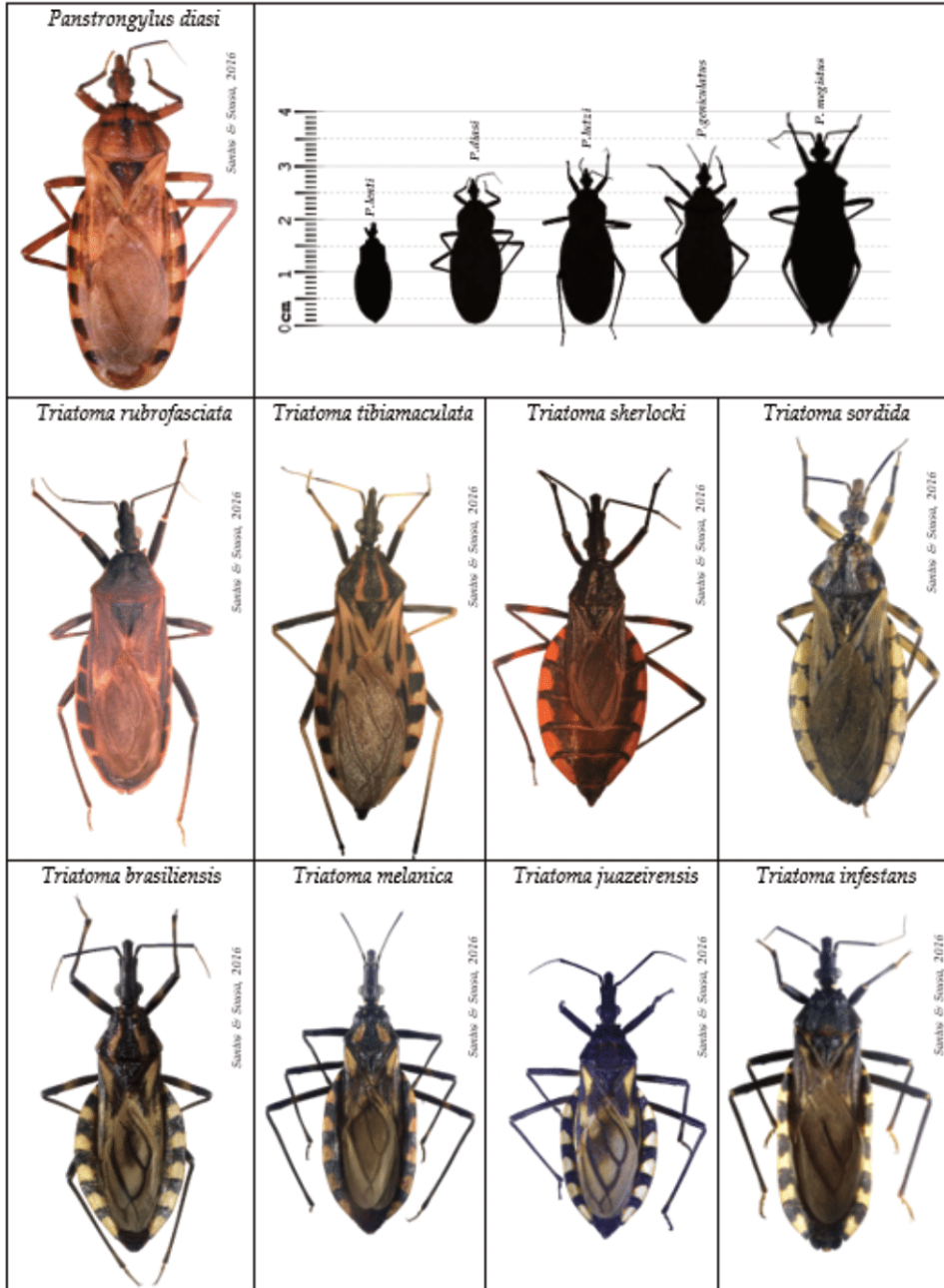
Santos & Sousa, 2016

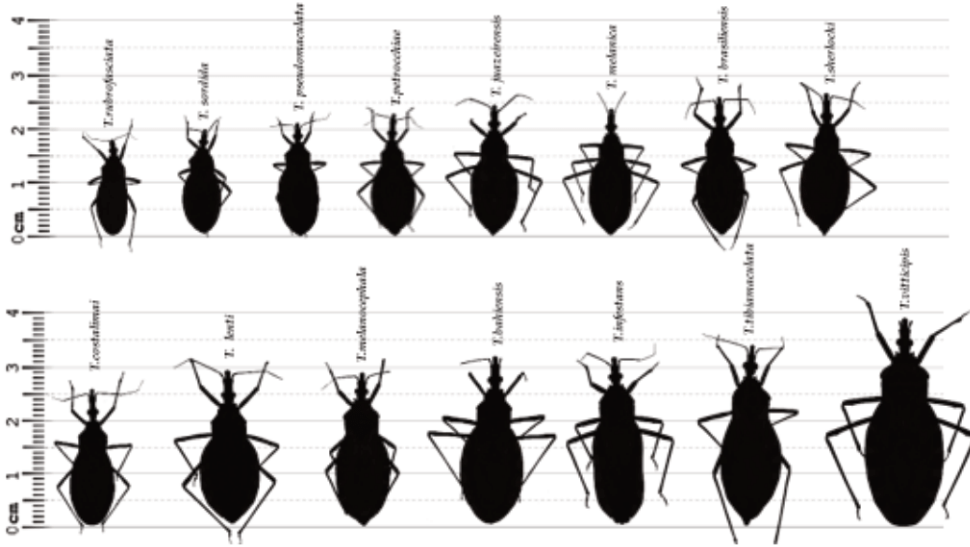
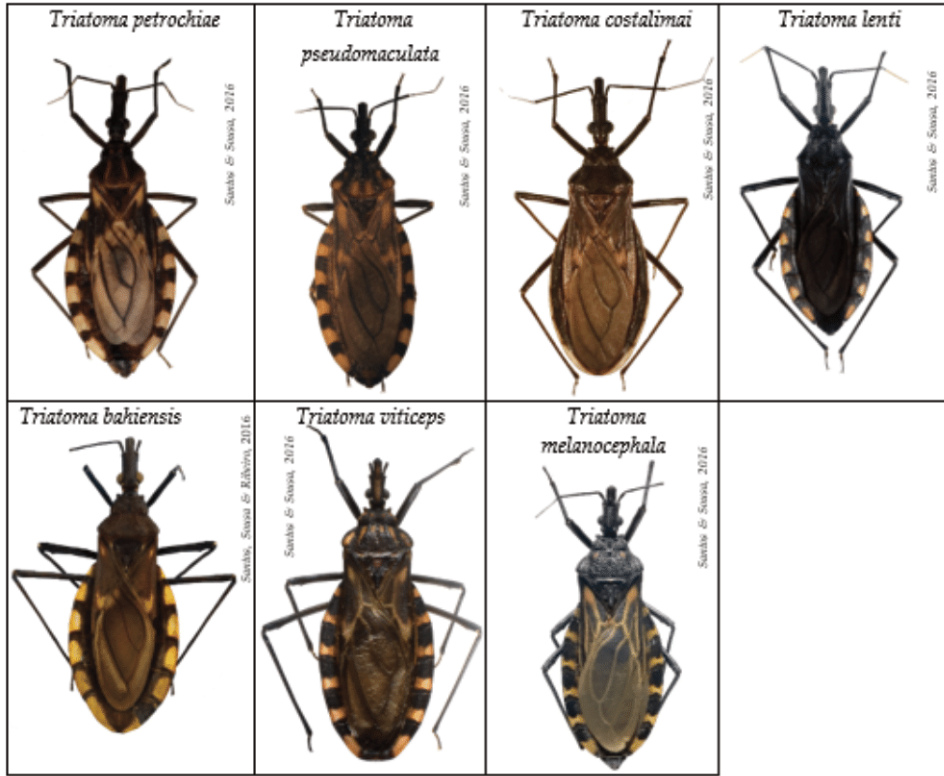


Fonte: (Ribeiro) – CPQGM; Santos & Sousa–LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

Prancha das espécies de triatomíneos da Bahia







Distribuição regional das espécies de triatomíneos da Bahia

BANCO DE DADOS DISTINTOS:
1957-1972: (4) SHERLOCK
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

A atualização e o refinamento dos dados de distribuição regional dos vetores conhecidos na Bahia, assim como no Brasil, é de suma importância, uma vez que, o padrão de transmissão da doença de Chagas vem sofrendo mudanças, sobretudo, após o controle do *Triatoma infestans*. Além disso, mudanças do padrão comportamental de outras espécies de triatomíneos considerados silvestres, vem contribuindo significativamente para composição de incrementos na dinâmica de transmissão da doença de Chagas. Como exemplos, podemos citar a transmissão por via oral e extra-domiciliar. Todas essas informações em conjunto, são fundamentais para a compreensão dos aspectos epidemiológicos relacionados à transmissão do *Trypanosoma cruzi* e devem ser consideradas para orientar as ações de vigilância e controle da doença de Chagas. Nesse sentido se justifica a necessidade do conhecimento mais preciso da distribuição geográfica dessas espécies de triatomíneos.



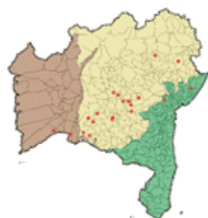
Fonte: (Ribeiro, no prelo) – CPQGM;
Santos & Sosa– LACEN, 2016
BANCO DE DADOS:
1957-1972: (4)
2007- 2015: Programa de Vigilância Estadual
2007 – 2014: Entomologia do Lacen- BA

ESPÉCIE	Nº de MUN
<i>Parabelminius yurupucu</i>	Espécie rara
<i>Cavernicola pilosa</i>	Espécie rara
<i>Psammolestes tertius</i>	10
<i>Rhodnius nasutus</i>	-
<i>Rhodnius neglectus</i>	21
<i>Rhodnius domesticus</i>	-
<i>Panstrongylus megistus</i>	141
<i>Panstrongylus lenti</i>	6
<i>Panstrongylus geniculatus</i>	54
<i>Panstrongylus lutzii</i>	69
<i>Panstrongylus diasi</i>	20
<i>Triatoma rubrofasciata</i>	3
<i>Triatoma tibiamaculata</i>	27
<i>Triatoma sberlocki</i>	1
<i>Triatoma sordida</i>	169
<i>Triatoma brasiliensis</i>	97
<i>Triatoma melanica</i>	2
<i>Triatoma juazeirensis</i>	8
<i>Triatoma infestans</i>	33
<i>Triatoma petrochiae</i>	2
<i>Triatoma pseudomaculata</i>	177
<i>Triatoma costalimai</i>	2
<i>Triatoma lenti</i>	4
<i>Triatoma babiensis</i>	4
<i>Triatoma melanocephala</i>	76
<i>Triatoma vitticeps</i>	2

**Espécies
(n) de municípios**

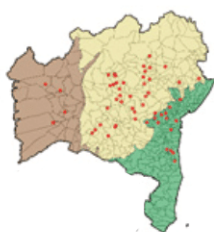
Municípios da Bahia

Panstrongylus diasi



Andaraí; Araças; Boa Vista do Tupim; Botuporã; Candiba; Castro Alves; Cocos; Igaporã; Iramaia; Itaeté; Itiúba; Livramento de Nossa Senhora; Malhada; Nova Redenção; Palmas de Monte Alto; Palmeiras; Riacho de Santana; Rio de Contas; Seabra; Tucano.

Panstrongylus geniculatus



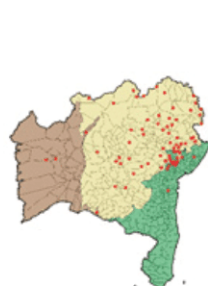
Amargosa; Arataca; Barra do Mendes; Barro Alto; Boa Vista do Tupim; Brejões; Cafarnaum; Caldeirão Grande; Campo Formoso; Canarana; Castro Alves; Coaraci; Correntina; Cotegipe; Crisópolis; Igaporã; Irajuba; Iramaia; Iraquara; Irecê; Itabuna; Itaeté; Itajuípe; Itaquara; Jacobina; Jiquiriçá; Lagoa Real; Lapão; Livramento de Nossa Senhora; Macajuba; Maracás; Matina; Miguel Calmon; Mirangaba; Mulungu do Morro; Nova Itarana; Nova Redenção; Novo Horizonte; Palmeiras; Piraí do Norte; Piritiba; Queimadas; Riachão das Neves; Rio de Contas; Ruy Barbosa; Santana; São Gonçalo dos Campos; Seabra; Serrinha; Tanque Novo; Taperoá; Uibaí; Várzea do Poço; Wagner.

Panstrongylus lenti



Araças; Barra do Mendes; Caturama; Ibipitanga; Ituaçu; Macaúbas.

Panstrongylus lutzj



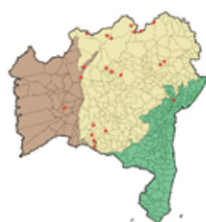
Antônio Cardoso; Araças; Araci; Baianópolis; Barra; Barra da Estiva; Biritinga; Boa Vista do Tupim; Brotas de Macaúbas; Cabaceiras do Paraguaçu; Campo Alegre de Lourdes; Campo Formoso; Candeal; Canudos; Casa Nova; Castro Alves; Conceição da Feira; Conceição do Coité; Coração de Maria; Coronel João Sá; Crisópolis; Curaçá; Euclides da Cunha; Feira de Santana; Governador Mangabeira; Ipecaetá; Ipirá; Iraquara; Itaberaba; Itaeté; Itapicuru; Itatim; Itiúba; Jacobina; Jeremoabo; Juazeiro; Lamarão; Mirangaba; Monte Santo; Morro do Chapéu; Nordestina; Olindina; Ourolândia; Palmeiras; Paripiranga; Paulo Afonso; Pedro Alexandre; Piraí do Norte; Queimadas; Quijingue; Rafael Jambeiro; Rio de Contas; Santa Bárbara; Santa Brígida; Santaluz; Santanópolis; Santo Estêvão; São Desidério; São Gonçalo dos Campos; Sátiro Dias; Seabra; Sento Sé; Serra Preta; Serrinha; Sobradinho; Tanquinho; Urandi; Wagner

Panstrongylus megistus

Acajutiba; Água Fria; Aiquara; Iagoinhas; Amargosa; Amélia; Rodrigues; Andaraí; Anguera; Antônio Cardoso; Antônio Gonçalves; Aporá; Araci; Aratuípe; Baixa Grande; Barra da Estiva; Barra do Mendes; Bonito; Brejões; Cabaceiras do Paraguaçu; Cachoeira; Caém; Caetitê; Cafarnaum; Caldeirão Grande; Campo Alegre de Lourdes; Campo Formoso; Canavieiras; Candeal; Candeias; Candiba; Cardeal da Silva; Castro Alves; Catu; Conceição da Feira; Conceição do Almeida; Conceição do Coité; Conceição do Jacuípe; Conde; Coração de Maria; Coronel João Sá; Cravolândia; Cruz das Almas; Dom Macedo Costa; Elísio Medrado; Entre Rios; Esplanada; Euclides da Cunha; Feira de Santana; Governador Mangabeira; Ibiquera; Inhambupe; Ipecaetá; Ipirá; Iraquara; Irará; Irecê; Itaberaba; Itagi; Itamari; Itanagra; Itaparica; Itaquara; Itiruçu; Itiúba; Ituaçu; Jacobina; Jaguaquara; Jaguarari; Jaguaripe; Jandaíra; Jiquiriçá; Jitaúna; Lafaiete Coutinho; Laje; Lajedinho; Lençóis; Macajuba; Mairi; Maragogipe; Mata de São João; Miguel Calmon; Mirangaba; Monte Santo; Morro do Chapéu; Mundo Novo; Muniz Ferreira; Muritiba; Mutuípe; Nazaré; Ouricangas; Palmeiras; Paripiranga; Pedrão; Piatã; Pindobaçu; Piraí do Norte; Pirituba; Presidente Dutra; Queimadas; Rafael Jambeiro; Remanso; Riachão do Jacuípe; Riacho de Santana; Rio Real; Ruy Barbosa; Salvador; Santa Bárbara; Santa Inês; Santa Rita de Cássia; Santa Teresinha; Santo Amaro; Santo Antônio de Jesus; Santo Estêvão; São Felipe; São Félix; São Francisco do Conde; São Gonçalo dos Campos; São Miguel das Matas; São Sebastião do Passé; Sapeaçu; Saúde; Seabra; Senhor do Bonfim; Serra Preta; Serrinha; Serrolândia; Simões Filho; Sobradinho; Souto Soares; Tanque Novo; Tanquinho; Tapiramutá; Teodoro Sampaio; Terra Nova; Tucano; Ubaira; Urandi; Utinga; Valença; Vera Cruz; Wagner.

Psamolestes tertius

Anagé; Cabaceiras do Paraguaçu; Caetitê; Castro Alves; Iaçú; Jaguaquara; Maragogipe; São Sebastião do Passé; Sento Sé; Tabocas do Brejo Velho.

Rhodnius neglectus

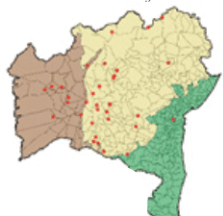
América Dourada; Barra; Campo Alegre de Lourdes; Casa Nova; Central; Curaçá; Guanambi; Igaporã; Irecê; Juazeiro; Lagoa Real; Morpará; Nordestina; Pindaí; Queimadas; Remanso; Santana; São Félix; Sento Sé; Sobradinho; Urandi.

Triatoma brasiliensis

Abaré; América Dourada; Andorinha; Angical; Araças; Aracatu; Araci; Barra; Barra do Mendes; Bom Jesus da Lapa; Campo Alegre de Lourdes; Campo Formoso; Candeal; Candiba; Cansanção; Canudos; Capela do Alto Alegre; Casa Nova; Chorochó; Conceição do Coité; Coribe; Coronel João Sá; Correntina; Curaçá; Euclides da Cunha; Feira de Santana; Gentio do Ouro; Glória; Governador Lomanto Júnior; Guanambi; Iaçú; Ibipeba; Ibititá; Igaporã; Iramaia; Itaberaba; Itaguaçu da Bahia; Itiúba; Ituaçu; Iuiú; Jaborandi; Jacobina; Jaguarari; Jequié; Jeremoabo; João Dourado; Juazeiro; Jussara; Jussiape; Lamarão; Macajuba; Macururé; Malhada; Matina; Mirangaba; Monte Santo; Nordestina; Nova Redenção; Ourolândia; Palmas de Monte Alto; Paulo Afonso; Pé de Serra; Pedro Alexandre; Pilão Arcado; Pindaí; Piritiba; Presidente Dutra; Queimadas; Quijingue; Rafael Jambeiro; Remanso; Retirolândia; Riachão do acuípe; Riacho de Santana; Rio de Contas; Rodelas; Santa Brígida; Santa Rita de Cássia; Santa Luz; São Domingos; São Gabriel; São José o Jacuípe; São Sebastião do Passé; Senhor do Bonfim; Sento Sé; Serra do Ramalho; Serra Preta; Sobradinho; Tanhaçu; Tanque Novo; Tucano; Uauá; Uibaí; Umburanas; Urandi; Valente; Xique-Xique.

Triatoma costalimai

Canudos; São Desidério.

**Triatoma infestans*

Angical; Barra; Barreiras; Bom Jesus da Lapa; Boquira; Brejolândia; Brotas de Macaúbas; Candiba; Contendas do Sincorá; Correntina; Cotegipe; Curaçá; Guanambi; Ibipeba; Ibititá; Iramaia; Irecê; Itaguaçu da Bahia; Jacaraci; Juazeiro; Macaúbas; Novo Horizonte; Oliveira dos Brejinhos; Paramirim; Paratinga; Pindaí; Presidente Tancredo Neves; Queimadas; Remanso; Riacho de Santana; Rio do Pires; Tabocas do Brejo Velho; Tremedal.

*Atualmente a espécie só é encontrada em foco residual no município de Novo Horizonte.

Triatoma juazeirensis

Campo Formoso; Casa Nova; Curaçá; Ibipeba; Ibititá; Itiúba; Jaguarari; Juazeiro.

Triatoma lenti

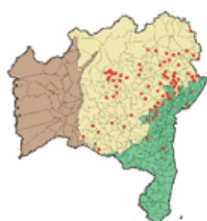
Brotas de Macaúbas; Ipupiara; Macaúbas; Novo Horizonte.

Triatoma babiensis

Seabra ⁽¹⁰⁷⁾, Caturama, Ibipitanga, Macaubas ⁽¹¹⁾

Triatoma melanica

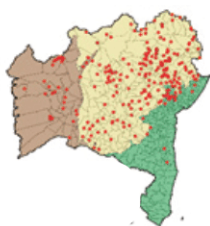
Matina; São Gabriel.

Triatoma melanocephala

Ajustina; América Dourada; Andorinha; Aporá; Araças; Araci; Baixa Grande; Biritinga; Boa Nova; Brejões; Cabaceiras do Paraguaçu; Caetitê; Cafarnaum; Canarana; Candeal; Conceição do Coité; Crisópolis; Euclides da Cunha; Feira de Santana; Governador Lomanto Júnior; Ibipeba; Ibititá; Igaporã; Ipecaetá; Ipirá; Iramaia; Iraquara; Irará; Irecê; Itaberaba; Itaquara; Itatim; Ituaçu; Jacaraci; Jacobina; Jaguarari; João Dourado; Lajedo do Tabocal; Lamarão; Lapão; Livramento de Nossa Senhora; Miguel Calmon; Milagres; Mirangaba; Monte Santo; Morro do Chapéu; Mulungu do Morro; Nordestina; Nova Itarana; Novo Horizonte; Novo Triunfo; Olindina; Palmas de Monte Alto; Paripiranga; Paulo Afonso; Pé de Serra; Presidente Dutra; Queimadas; Quijingue; Rafael Jambeiro; Retirolândia; Rio Real; Ruy Barbosa; Santa Bárbara; Santa Inês; Santa Teresinha; Santaluz; Santo Estêvão; São Gabriel; Seabra; Serrinha; Simões Filho; Teofilândia; Tucano; Uibaí; Valente.

Triatoma petrocobiae

Curaçá; Paulo Afonso.

Triatoma pseudomaculata

Abaré; Acajutiba; Água Fria; América Dourada; Andaraí; Andorinha; Angical; Anguera; Antônio Cardoso; Antônio Gonçalves; Aporá; Araças; Araci; Baianópolis; Baixa Grande; Barra; Barra da Estiva; Barra do Mendes; Barro Alto; Biritinga; Boa Vista do Tupim; Bom Jesus da Lapa; Brejolândia; Brotas de Macaúbas; Cabaceiras do Paraguaçu; Caetitê; Cafarnaum; Caldeirão Grande; Campo Alegre de Lourdes; Campo Formoso; Canarana; Candeal; Candiba; Cansanção; Canudos; Carinhanha; Casa nova; Castro Alves; Catolândia; Central; Chorrochó; Cipó; Conceição da Feira; Conceição do Coité; Contendas do Sincorá; Coração de Maria; Coronel João Sá; Correntina; Cotegipe; Crisópolis; Cristópolis; Curaçá; Entre Rios; Euclides da Cunha; Feira de santana; Filadélfia; Gentio do Ouro; Glória; Governador Lomanto Júnior; Guanambi; Iaçú; Ibipecta; Ibiquera; Ibititá; Ichu; Igaporã; Ipecaetá; Ipirá; Iramaia; Iraquara; Irará; Irecê; Itaberaba; Itaeté; Itaguaçu da Bahia; Itapicuru; Itatim; Itiúba; Ituaçu; Jacaraci; Jacobina; Jaguarari; Jequié; Jeremoabo; João Dourado; Juazeiro; Jussara; Jussiape; Lagoa Real; Lamarão; Lapão; Livramento de Nossa Senhora; Luis Eduardo Magalhães; Macajuba; Mairi; Malhada de Pedras; Manoel Vitorino; Mansidão; Maracás; Marcionílio Souza; Matina; Miguel Calmon; Mirangaba; Mirante; Monte Santo; Morpará; Morro do Chapéu; Mulungu do Morro; Nordestina; Nova Fátima; Nova Redenção; Nova Soure; Novo Horizonte; Novo Triunfo; Olindina; Orolândia; Palmas de Monte Alto; Paramirim; Paripiranga; Paulo Afonso; Pé de Serra; Piatã; Pilão Arcado; Pindobaçu; Pintadas; Ponto Novo; Presidente Dutra; Queimadas; Quijingue; Quixabeira; Rafael Jambeiro; Remanso; Retirolândia; Riachão das Neves; Riachão do Jacuípe; Riacho de Santana; Rio de Contas; Rio Real; Rodelas; Ruy Barbosa; Santa Bárbara; Santa Brígida; Santa Luzia; Santa Rita de Cássia; Santa Teresinha; Santa luz; Santana; Santo Estêvão; São Domingos; São Félix; São Gabriel; São Gonçalo dos Campos; Saúde; Seabra; Senhor do Bonfim; Sento Sé; Serra do Ramalho; Serra Preta; Serrinha; Serrolândia; Sítio do Mato; Sobradinho; Tabocas do Brejo Velho; Tanque Novo; Tanquinho; Teofilândia; Tucano; Uauá; Uibaí; Umbranas; Urandi; Utinga; Valente; Várzea do Poço; Várzea Nova; Wanderley; Xique-Xique.

Triatoma rubrofasciata

Glória; Salvador; São Félix

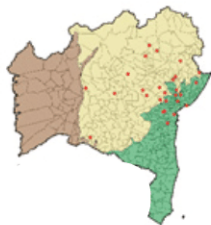
Triatoma sberlocki

Gentio do Ouro.

Triatoma sordida

Abaira; América Dourada; Anagé; Andaraí; Andorinha; Angical; Araças; Aracatu; Baianópolis; Barra; Barra da Estiva; Barra do Mendes; Barreiras; Barro Alto; Belo Campo; Boa Vista do Tupim; Bom Jesus da Lapa; Boninal; Boquira; Botuporã; Brejolândia; Brotas de Macaúbas; Brumado; Buritirama; Cabaceiras do Paraguaçu; Caculé; Caetanos; Caetité; Cafarnaum; Campo Alegre de Lourdes; Campo Formoso; Canápolis; Canarana; Candiba; Cansanção; Canudos; Caraíbas; Carinhonha; Casa Nova; Castro Alves; Catolândia; Caturama; Central; Cocos; Condeúba; Contendas do Sincorá; Coribe; Correntina; Cotegipe; Crisópolis; Cristópolis; Curaçá; Dom Basílio; Feira da Mata; Gentio do Ouro; Guajeru; Guanambi; Ibicuí; Ibipeba; Ibipitanga; Ibiquera; Ibitiara; Ibititá; Ibotirama; Igaporã; Ipupiara; Iramaia; Iraquara; Irará; Irecê; Itaberaba; Itacé; Itagi; Itaguaçu da Bahia; Itambé; Itapicuru; Itiúba; Ituaçu; Iuiú; Jaborandi; Jacaraci; Jacobina; Jaguarari; João Dourado; Juazeiro; Jucuruçu; Jussara; Jussiape; Lagoa Real; Lapão; Lençóis; Livramento de Nossa Senhora; Luis Eduardo Magalhães; Macajuba; Macaúbas; Maetinga; Malhada; Malhada de Pedras; Manoel Vitorino; Mansidão; Maracás; Marcionílio Souza; Matina; Miguel Calmon; Mirangaba; Mirante; Morpará; Morro do Chapéu; Mortugaba; Mucugê; Mulungu do Morro; Nova Fátima; Nova Redenção; Novo Horizonte; Olindina; Oliveira dos Brejinhos; Ouroândia; Palmas de Monte Alto; Palmeiras; Paramirim; Paratinga; Piatã; Pilão Arcado; Pindaf; Pindobaçu; Piraí do Norte; Poções; Potiraguá; Presidente Dutra; Presidente Jânio Quadros; Queimadas; Remanso; Riachão das Neves; Riacho de Santana; Rio de Contas; Rio do Antônio; Rio do Pires; Rio Real; Ruy Barbosa; Santa Maria da Vitória; Santa Rita de Cássia; Santa Teresinha; Santaluz; Santana; Santo Amaro; São Desidério; São Félix do Coribe; São Gabriel; Seabra; Sebastião aranjeiras; Sento Sé; Serra do Ramalho; Serra Dourada; Serrinha; Sítio do Mato; Souto Soares; Tabocas do Brejo Velho; Tanhaçu; Tanque Novo; Taperoá; Tucano; Uibaí; Umburanas; Urandi; Utinga; Vitória da Conquista; Wagner; Wanderley; Xique-Xique.

| TRIATOMÍNEOS da Bahia: manual de identificação e orientações para o serviço

Triatoma tibiamaculata

Candeal; Candiba; Castro Alves; Conceição da Feira; Entre Rios; Feira de Santana; Ibotirama; Ipirá; Itaberaba; Itatim; Itiúba; Jaguarari; Jiquiriçá; Maragogipe; Morro do Chapéu; Mutuípe; Pindobaçu; Pirai do Norte; Rafael Jambeiro; Rio Real; Ruy Barbosa; Salvador; Santo Amaro; Seabra; Serrinha; Tucano; Valença.

Triatoma vitticeps

Jacaraci; Mortugaba.

9.8. Apêndice 8 – Coautoria de artigo publicado: Natural Infection by *Trypanosoma cruzi* in a Dog from Bahia State - Northeast of Brazil.



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CASE REPORT
Pub. 559

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Natural Infection by *Trypanosoma cruzi* in a Dog from Bahia State - Northeast of Brazil

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ABSTRACT

Background: Chagas Disease (CD) is a parasitic anthroponosis caused by the *Trypanosoma cruzi* (*T. cruzi*), a protozoan transmitted by insects from the Reduviidae family. Several species of wild and domestic animals, humans included, are susceptible, developing acute clinical signals (myocarditis and cardiac arrhythmias) or chronic signals (drop on the performance and syncope) of the disease. In Brazil, the disease in dogs shows variable indexes of occurrence. The present paper describes the natural infection by *T. cruzi* in a canine living in the city of Barra, in the state of Bahia, Brazil, a region classified as high risk of vulnerability for the CD in the northeast of Brazil.

Case: A 9-year-old male dog, non-specific breed, was referred to the clinic the Veterinary Clinic for Small Animals, at the Veterinary Medical Teaching Hospital, at the Federal University of the West of Bahia. Its owner reported the presence of bloody secretion in the penile region. During the physical exam it was possible to observe an increase in the volume of the penis base. All of the animal's physiological parameters were within normal levels. Blood samples and cytological laminae from the lesioned area were collected and sent to the Laboratory of Veterinary Clinical Pathology in the same institution. The cytological exam showed monomorphic population of great round cells with a round nucleus, condensed chromatin and one to two prominent nucleolus, abundant and slightly basophilic cytoplasm, with multiple stippled vacuoles, thus confirming a Transmissible venereal tumor (TVT) case. With the blood exam one observed a thrombocytopenia, neutrophilia with a deviation on the left and the presence of a trypomastigote form of *Trypanosoma* sp. in the blood smears. A complete blood sample was sent to the Laboratory of Pathology and Molecular Biology from the Fiocruz-BA (Oswaldo Cruz Foundation in the State of Bahia), being identified by the PCR technique, *T. cruzi*.

Discussion: During the complete blood count, specifically using the blood smear, the microscopic visualization of the trypomastigote form of the *Trypanosoma* sp., a hemoparasite of filiform aspect, with undulating membrane over the whole body and free flagellum, confirming a case of trypanosomiasis. Such diagnostics had not been considered up to this moment, as the animal did not present any clinic symptomatology that indicated the pathological condition. The high levels of parasitemia presented by the animal, confirmed by the presence of the protozoan in the blood smear, probably indicate the acute phase of the infection, period in which the multiplication and development of the agent is higher, consequently allowing the visualization of the hemoparasite in the lamina. Although the TVT had proved to be the main derogatory factor of sanity and wellbeing of the animal, one calls the attention in this case for the trypanosomiasis, a zoonosis that causes great risk to the sole health, highlighting that the canine species is taken as an important reservoir of *T. cruzi*, relevant for the maintenance and interaction among the domestic and wild cycles of the Chagas Disease. This is the first report of a canine naturally infected by *T. cruzi* in the west Region of the State of Bahia. It is, thus, important to emphasize its significance once dogs can be reservoirs of the protozoan, acting in their life cycle and epidemiological chain of the Chagas Disease, enabling the infection of triatomines and humans. Future studies should be done with the purpose of determining the predominance of the agent in the region of the city of Barra, in the State of Bahia.

Keywords: Chagas Disease, dogs, protozoan, reservoir.

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INTRODUCTION

The Chagas Disease (CD) is a parasite anthrozoosis, caused by *Trypanosoma cruzi* (*T. cruzi*) [17]. The CD, or American trypanosomiasis, affects approximately 7 million people around the planet [32]. Approximately 1.2 million documented people are infected in Brazil, which occupies the second place in the ranking of the countries with high levels of infected people, behind only Argentina [31]. The *Trypanosoma cruzi* can infect a wide range of mammal hosts, including domestic dogs. The natural infection of dogs living in the endemic areas, whether it is urban or rural, has been reported in Louisiana [7], Costa Rica [2] in regions of Colombia [15] and in the northeast of Brazil [18]. Papers have shown status indexes that vary from 0.3% to 22.7% all over Brazil [16,25,27,28]. The clinical course of the infection by *T. cruzi* in canines is like the pattern of cardiologic disease in humans. Young dogs may present an acute phase with signs of myocarditis, cardiac arrhythmias and respiratory difficulty. In the chronic phase, on the other hand, intolerance to exercises and syncope are found [26].

The diagnostic of the infection by *T. cruzi* in dogs may be by direct microscopy, making it possible to identify the flagellum in the blood, depending on the parasitisms [14], serological tests (isotopes detection of IgG and IgM) and molecular (polymerase chain reaction - PCR) [1,8,26].

The present study describes the natural infection by *T. cruzi* in a canine (*Canis lupus familiaris*) living in a region classified as in high vulnerability risk for CD in the northeast of Brazil [23,24].

CASE

A 9-year-old male dog, non-specific breed, and weighing 29 kg was attended at the Veterinary Medical Clinic for Small Animals (CMPA), at the University Veterinary Hospital (HVU), from the Federal University of the West of Bahia (UFOB). According to his owner, the animal presented a bloody secretion originating from the penis. When a physical examination was performed, an increase in the volume of the penis base was observed, which suggested a venereal transmissible tumor (TVT), a common affection among intact dogs which mate or have contact with contaminated animals. All of the physiological parameters of the animal were within the normality indexes – lymph nodes without reactivity, pink mucous membranes,

temperature, normal cardiac and respiratory frequency. Blood samples and cytological laminas from the lesioned area were collected and sent to the Laboratory of Veterinary Clinic Pathology in the same institution.

The cytological exam showed monomorphic population of large round cells with a round nuclei, condensed chromatin and between one and two prominent nucleolus, abundant and slightly basophilic cytoplasm with multiple stippled vacuoles, confirming thus a TVT case (Figure 1).

In the hematological test the following was observed: thrombocytopenia, neutrophilia with a deviation to the left and the presence of a flagellum in its trypanomastigote evolutionary form in the direct microscopy (Figure 2). Taking into consideration the epidemiologic history in the region, a complete blood sample was sent to the Laboratory of Pathology and Molecular Biology from the Gonçalo Muniz Institute (LPBM - Fiocruz, BA) for the *T. cruzi* molecular diagnostic.

The extraction of the genetic material was carried out with 200 µL of total blood using the PureLink¹ Genomic DNA Mini Kit, following the manufacturers' instructions. A conventional PCR was conducted according to the protocol established by Ribeiro Jr. et al. [21], using specific primers for the region of Mini-exons (Mini-exon gene intergenic spacer, F: 5'- AAG GTG CGT CGA CAG TGT GG -3'; R: 5'- TTT TCA GAA TGG CCG AAC AGT -3', 150-200 pb) and the COII gene (Cytochrome oxidase subunit II, F: 5'- CCA TAT ATT GTT GCA TTA TT -3'; R: 5'- TTG TAA TAG GAG TCA TGT TT -3', 375 pb). The reagents used for the amplification were Qiagen²

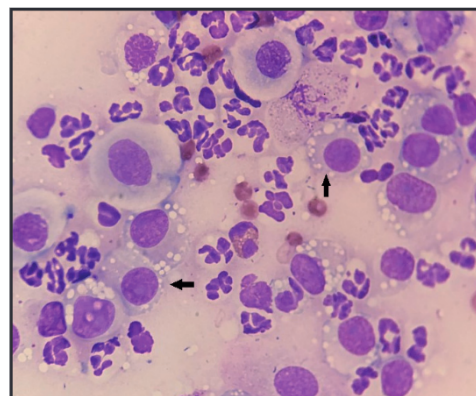


Figure 1. Cytology. Transmissible Venereal Tumor (TVT). One can observe the presence of round cells with round nuclei, abundant and slightly basophilic cytoplasm with multiple stippled vacuoles (black arrows) [Fast panoptic; Obj.100x].

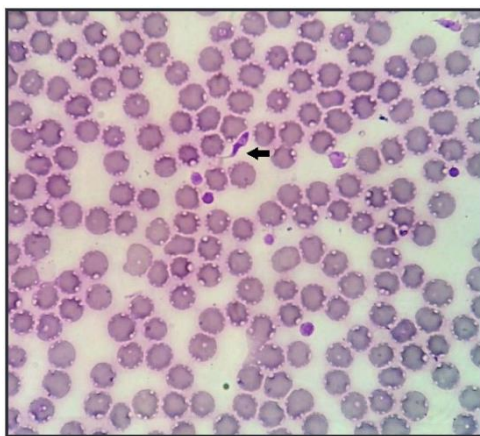


Figure 2. Blood Swab. One can observe the presence of the trypomastigote form of *Trypanosoma cruzi* (black arrow) [Fast panoptic; Obj.100x].

Master mix, 1X; primers, 0.1 μ M; H₂O (Qiagen²); DNA, 1 μ L (~100 ng/ μ L), in a final reaction volume of 25 μ L. The thermocycling occurred in the MasterCycle Gradient³, with initial denaturation at 94° C for 3 min; 40 cycles of 94° C for 30 s, 60° C for 30 s and 72° C for 1 min; with final elongation of 72° C for 10 min. The amplicons produced were visualized in agarose gel 1.5%, through the conventional electrophoresis according to Ribeiro Jr. et al. [21]. A scale of 100 bp DNA (Invitrogen⁴) was used as a molecular size pattern. Through molecular technique, the identification of the *T. cruzi* was confirmed.

No compatible clinical sign with the Chagas Disease was detected. The execution of complementary image and electrocardiogram exams was not possible due to lack of resources. The guidance regarding the condition of the canine bearer of *T. cruzi* was given to his owner, as well as the explanations about the zoonotic potential of the disease. The subject did not return for the conduction of the medical therapy for the TVT regression.

DISCUSSION

Through the present report it is significant the importance of complementary diagnostic tests in the routine of veterinary clinics and hospitals. One could observe that the anamnesis and the physical state of the evaluated animal indicated signs of genital affection which was confirmed by cytological exams. Although the TVT constituted the main derogatory fact in the sanity and well-being of the animal, with the conduction of the hematologic exam, based on the

blood swab technique, the microscopic visualization of the trypomastigote form of the *Trypanosoma* sp. was possible. That is a hemoparasite with a filiform aspect, with a rippling membrane all over its body and free flagellum [3], thus confirming a case of trypanosomiasis. This diagnostic which had not been thought of up to that moment once the animal did not present any symptomatology which might have indicated the pathological frame, which corroborates with already proven studies, where dogs are seen as asymptomatic bearer of the protozoan [22].

One must emphasize that the canine species is seen as an important vessel of *T. cruzi*, with highlighted role in the maintenance and interaction between the domestic and wild cycles of the Chagas Disease [4,27]. Thus, one must call attention to this case of risk to the One Health, since the contact of this dog with triatomines, enables the perpetuation and transmission of the disease to other susceptible animals and humans.

In this case, similar to what had been mentioned by other authors, it was not possible through blood swab exam to differentiate between the species *Trypanosoma* sp. This is due to the great morphological similarity, which proves the necessity for the molecular diagnostic [5], the former carried out through PCR technique (which is as sensitive as the xenodiagnosis) [8], and in this case one confirmed the infection by *T. cruzi*. The high sensitivity of the molecular test is a strong complementary diagnostic tool to the serological test, providing the substitution of the xenodiagnosis or the blood culture in the laboratory analyses [8].

The hemogram reading revealed thrombocytopenia, associated in the literature to the reduction of the platelets survival in the animal organism, these lysed in the presence of protozoan in the blood circulation [5,22]. Neutrophilia with a when facing infectious processes [30].

The high levels of parasitemia presented by the animal, confirmed by the presence of the protozoan in the blood swab probably indicate the acute phase of the infection [17,3], period in which the multiplication and development of the agent is greater [11,27], consequently allowing the visualization of the hemiparasite more easily. One hypothesis that is raised is that due to the genital affection and the stress levels (chronic insinuation caused by the initial illness) a high release of cortisol, inflammatory mediators and catecholamines (among other stressful factors) provides conditions for immunosuppression of the organism defense cells [19,20,30] which

resulted in small and constant failures in the decrease of the illness (parasitosis) [13,19], even though the animal presented normal physiological parameters.

The TVT neof ormation cytological exam [9,10,29] corroborates with the cytological findings in the present report.

The epidemiologically TVT is associated with some predisposing factors (free access to the street without the owner's presence, agglomeration lack of control of the population of stray dogs in the cities) [9,10,12], which, according to the owner's report, were present in the animal's routine.

According to the State Health Department from the State of Bahia (SESAB), Brazil and the informal report from dwellers of the urban and rural areas of the municipality of Barra, region the West of the State of Bahia, there are frequent visualization of triatomines in coops and houses (both around in inside the houses). The macro-region of the West of the State of Bahia has high indexes of CD [23,24], presenting elevated risk of transmission of the vectorial infection, for susceptible humans and animals [22], which is possibly a major factor for the occurrence of the canine infection in the present report.

Taking into consideration the absence of clinical symptomatology of trypanosomiasis in the animal, one proposed that the owner of the canine should receive TVT treatment. Additionally, measures for the containment of the diseases were recommended having in mind its infectious potential either venereal (TVT) [12,29] or parasitic (Chagas Disease) [3,18].

This is the first report of a canine infected naturally by *T. cruzi* in the West region of the State of Bahia. It is important to highlight that these dogs can be reservoirs for the protozoan, acting in the life cycle and the epidemiological chain of the Chagas Disease, enabling the infection of triatomines, both by contact with animals and humans. Further studies should be carried out in order to determine the prevalence of the agent in the region of Barra, State of Bahia, Brazil.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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9.9. Apêndice 9 – Coautoria de artigo publicado: Eco-epidemiology of vectorial *Trypanosoma cruzi* transmission in a region of northeast Brazil.

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Eco-epidemiology of vectorial *Trypanosoma cruzi* transmission in a region of northeast Brazil

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ABSTRACT

Chagas disease (CD) is a parasitic zoonosis endemic in Brazil. Despite virtual control of *Triatoma infestans*, the main domesticated vector of *Trypanosoma cruzi*, vectorial transmission by other triatomine species persists in some rural communities. This study aims to characterize triatomines role in transmitting *T. cruzi* to dogs and humans in the district of Santo Inácio, located in the northwest region of the state of Bahia, Brazil. It also describes environmental factors in housings associated with insect occurrence and assesses the perception, knowledge, and preventive practices adopted by the population regarding CD. Blood samples of humans and dogs, and biological samples of triatomines, were collected between November 2018 and February 2019 and subjected to the detection of *T. cruzi* by serological and molecular biology tests. Also, we applied a questionnaire to research the perception, knowledge, and local practices of people related to CD. The capture of triatomines in households was associated with exploratory variables of the questionnaires using multivariate logistic regression ($p < 0.05$). The 155 triatomines captured in the wild and domestic environment were of the species *Triatoma sherlocki* ($n = 151$), *Panstrongylus sherlocki* ($n = 1$) and *Triatoma sordida* ($n = 3$), and had a natural infection rate for *T. cruzi* by PCR of 18.5%, 100% and 0%, respectively. District residents ($n = 126$) were seronegative for *T. cruzi*, while 17.5% (7/40) of the dogs were seropositive. The fact that residents are aware that triatomines can “cause” CD was configured as a protection factor for residents according to the fitted logistic regression model ($p = 0.04$). However, respondents have limited perception and knowledge about the CD, prevention and control practices for triatomines in a household. The results suggest the existence of a domestic cycle of transmission of *T. cruzi* between triatomines and dogs, configuring a latent risk of infection to the human population of Santo Inácio. Studies that clarify the potential for the establishing of intrusive triatomines in households, surveillance actions for triatomines, and health education in rural communities are indispensable to prevent the reemergence of CD in vulnerable regions of Brazil and other American countries with similar epidemiological characteristics.

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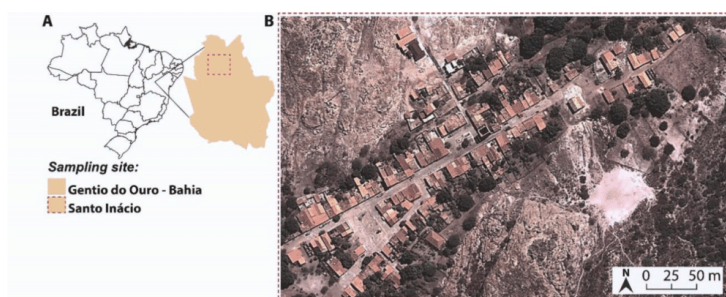


Fig. 1. Characterization of the sampling site. (A) Brazil, state of Bahia, municipality of Gentio do Ouro with the highlight on the district of Santo Inácio (QGIS 3.12). (B) Headquarters of the district of Santo Inácio, Gentio do Ouro, Bahia, surrounded by rocky-outcrops (Google Earth®, 2020).

1. Introduction

Chagas disease (CD) is a parasitic zoonosis caused by *Trypanosoma cruzi* and is usually transmitted between vertebrate hosts by triatomine insects popularly known as "the kissing bug" (Sousa et al., 2020). The disease is endemic in Latin America, especially in Brazil (WHO, 2015), with cases recorded in poor rural areas with triatomines frequent occurrence (Daflon-Teixeira et al., 2019; Lima et al., 2012; Vinhas et al., 2014).

The implementation of the National Chagas Disease Control Program contributed to reducing the population of *Triatoma infestans* (Dias, 2016b), one of the main domiciled vectors in the region, currently with records of residual foci in the state of Bahia (Ribeiro et al., 2019). However, given the diversity of *T. cruzi* vectors, there is a risk of transmission by other intrusive triatomine species (Lima et al., 2012; Ribeiro et al., 2015). Therefore, strengthening the population participation in the notification of vectors in their households in regions vulnerable to infection is essential. This depends on permanent health education actions on the epidemiology of CD, focusing especially on the young population (Yevstigneyeva et al., 2014), seeking to reduce eventual underreporting of cases (Dias et al., 2016a).

According to other authors, vectorial transmission of *T. cruzi* is associated with household invasion by wild triatomines in several countries in the Americas (Waleckx et al., 2015). In the Yucatán Peninsula, Mexico, seasonal invasion of *Triatoma dimidiata* occurs, leading to active infection by *T. cruzi* in its inhabitants (Gamboa-León et al., 2014). In Colombia, the wild triatomine *Triatoma maculata* invades houses, transmitting *T. cruzi* to dogs and humans in a region with the occurrence of synanthropic animals infected by this protozoan, such as *Didelphis marsupialis* (Cantillo-Barraza et al., 2015). The population of the municipality of Apolo, Bolivia, complains of triatomine bites, with the wild species *Eratyrus mucronatus* being the most frequently found in the area (Depickère et al., 2012). Therefore, the presence in households of other triatomine species, previously of less epidemiological importance, infected with *T. cruzi*, represents an important risk of CD re-emergence in regions where the main vector species was controlled (Cantillo-Barraza et al., 2020; Carrasco et al., 2014; Waleckx et al., 2015).

In the study area - district of Santo Inácio, municipality of Gentio do Ouro (Bahia) - there are records of triatomine species *Triatoma sherlocki* (Papa et al., 2002), *Panstrongylus sherlocki* (Jurberg et al., 2001), and *Triatoma sordida* (Ribeiro et al., 2014). There are few reports on the latter two species in the region, with *P. sherlocki* being mentioned in a morphological characterization study, without data on *T. cruzi* infection (Jurberg et al., 2001), and *T. sordida* evaluated for the presence of different strains of *T. cruzi* (Ribeiro et al., 2014). *T. sherlocki* species is

sylvatic and the most frequent in the region (Costa et al., 2014; Mendonça et al., 2009). Studies report *T. cruzi* infection in these triatomines (Alameida et al., 2009; Ribeiro et al., 2019). Birds were the most prevalent dietary pattern among the targets assessed for sylvatic populations of *T. sherlocki* (Ribeiro et al., 2019), but the high frequency of *T. cruzi* infection in this species suggests the involvement of wild mammals in maintaining the cycle transmission of the parasite.

The occurrence of *T. sherlocki* in households and its possible involvement with domestic animals and humans in the transmission cycle of *T. cruzi* causes concern in the region and constitutes a threat to public health (Alameida et al., 2009). Therefore, this study aims to characterize the vector role of triatomines found in the district of Santo Inácio (Bahia), to estimate the frequency of infection by *T. cruzi* in vectors, dogs, and humans, as well as describing characteristics of the households associated with the occurrence of these insects and evaluate the perception, knowledge and preventive practices adopted by the population regarding CD.

2. Material and methods

2.1. Study area and sample population

The study was carried out in the district of Santo Inácio (Fig. 1), belonging to the municipality of Gentio do Ouro (11°22'49.4"S 42°38'05.5"W), located in the northwest region of the state of Bahia, Brazil. It has an estimated population of 255 inhabitants, living in houses surrounded by large rocky-outcrops with significant fractures. Vegetation and fauna are characteristic of the Caatinga biome (semi-arid), which houses, among others, *Thrichomys* sp. and *Kerodon rupestris*, wild mammals of frequent occurrence in the region and possibly involved as blood-meal sources for triatomines (Papa et al., 2002). The region has an average temperature of 25°C, and precipitation around 750 mm, with rains concentrated in the spring (September to December) and summer (December to March) (SEI-Superintendencia de Estudos Econômicos e Sociais da Bahia, 2018).

Between November 2018 and February 2019, visits were made to households in Santo Inácio to invite residents to volunteer in the study. Those who agreed to participate signed an Informed Consent Form. Volunteers were submitted to a questionnaire, blood sample collection, triatomines were captured in their houses and, after data collection, they were informed about the CD. Also, those who owned dogs had their animals sampled for blood. The study was approved by the Research Ethics Committee of the Federal University of West Bahia (protocol 2965700) and by the Ethics Committee for Animal Use of the School of Veterinary Medicine and Zootechnics of the Federal University Bahia (protocol 75/2017).

2.2. Serological survey of *T. cruzi* in humans and dogs

Blood collection was carried out in the volunteers' households by a nursing technician on previously scheduled days. Five ml of blood were collected in aseptic conditions, with dry tubes. The samples were immediately refrigerated and transported to the Laboratory of the Multidisciplinary Center of the *Campus* de Barra (CMB) of the Federal University of West Bahia (UFOB) for centrifugation and serum collection. Two ml serum aliquots were packed in microtubes and kept at -20°C , being sent to the Advanced Laboratory of Public Health (LASP) of the Gonalo Moniz Institute (IGM, Fiocruz-BA) under refrigeration, subjected to two commercial indirect ELISA tests for detection of anti-*T. cruzi* antibodies: ELISA Chagas III (BIOSChile, Ingenieria Genética S. A, Santiago, Chile) and Gold ELISA Chagas (batch CHA132A; Rem, São Paulo, Brazil). All results were analyzed in duplicate, and the cutoff values were determined according to the manufacturers.

The consent for sampling the dogs was requested to the tutors through signing of an authorization term. Animals were submitted to physical restraint, clinical evaluation and demographic data collection. Blood samples were collected through the jugular or cephalic veins in aseptic conditions, with dry tubes, in a volume of 3 to 5 ml. They were immediately refrigerated and transported to the CMB/UFOB laboratory. After separation and storage at -20°C , the serum samples were sent to LASP (IGM, Fiocruz-BA). They were analyzed by the modified Gold ELISA Chagas commercial test (batch CHA132A; Rem, São Paulo, Brazil) and *in-house* ELISA, using four *T. cruzi* chimeric recombinant antigens (IBMP-8.1, IBMP-8.2, IBMP-8.3, and IBMP -8.4) according to Leony et al. (2019). Dogs were considered seropositive for *T. cruzi* when reactive in both tests. The samples were also tested for anti-*Leishmania* antibodies, using the protocol recommended by the Ministry of Health: rapid test DPP© and ELISA/EIE [Bio-Manguinhos/Fundação Oswaldo Cruz (FIOCRUZ)]. Dogs were considered seropositive for *Leishmania* sp. when reactive in both tests.

2.3. Analysis of households and triatomines

The manual capture of triatomines in the households took place during the day, with the use of flashlights, gloves and tweezers, being carried out in all places of the house such as under the mattresses, behind furniture and in cracks that could house the vectors, in a clockwise direction. In the peridomicile, the search covered: the annexes, such as chicken coops and sheep corrals; accumulation of construction waste; piles of tiles and bricks; and places of garbage accumulation. The collected triatomines were placed in plastic tubes with a lid, allowing aeration and containing filter paper (Galvão, 2014). The triatomines capture activities in the domestic environment was executed by two people, with an average of 12.5 min/person/home. The research members also filled in a list of household characteristics associated with triatomines, such as roof and wall type, and presence of annexes for raising animals.

The capture of triatomines in the wild took place at dusk, with the aid of flashlights and frequent checking of the surface of rocks that had fissures, hollows of tree trunks and rock burrows of small wild mammals (example *Thrichomys* sp. and *K. rupestres*). The triatomines were collected with tweezers and placed in plastic tubes with aeration. The capture activity involved three to four people per collection in the wild environment, with an average duration of two hours/person. The capture of triatomines was authorized by the Brazilian Environmental Agency (IBAMA/SISBIO protocol 61011-2).

The insects were sent to the CMB/UFOB laboratory for taxonomic identification by optical microscopy of the characters of the head, chest, and abdomen, according to the identification keys of Lent and Wydzinsky (1979) and Galvão (2014). The insects were also classified into nymphs, males, and females. Entomological indicators were evaluated: (i) the natural infection index, which is the number of triatomines infected by *T. cruzi* divided by the total examined x 100; (ii) the

infestation index, which is the number of positive households for triatomines divided by the households surveyed x 100; (iii) the colonization index, which is the number of households with nymphs divided by the number of households with triatomines x 100 and (iv) overall triatomine abundance, which is the number of triatomines captured in the households divided by the number of households surveyed.

2.4. Molecular diagnosis of mammalian hosts and triatomine

The samples obtained from triatomines, humans, and dogs were examined by conventional PCR and sequenced for detection and confirmation of *T. cruzi* at the Laboratory of Pathology and Molecular Biology (LPBM) of the Gonalo Moniz Institute (IGM/Fiocruz-BA).

DNA extraction from triatomines was adapted from Ribeiro et al. (2019), using abdomen samples from each insect and the DNAzol standard protocol. The purified DNA was eluted in 50 μl of the elution buffer. We used the commercial kit (PureLink Genomic DNA Mini Kit, Invitrogen®) to extract DNA from whole blood samples with EDTA anticoagulant (dogs and humans), following the manufacturer recommendations, and eluted in 30 μl of elution buffer. All DNA concentration of the samples was determined on a NanoDrop™ spectrophotometer, being adjusted to $\sim 100\text{ ng}/\mu\text{l}$, and the samples were then stored at -80°C . The entire extraction process was carried out in a security booth, and the material used was sterilized to avoid contamination of the extracted DNA.

Conventional PCR aimed to amplify the histone H2A gene, corresponding to the 16-248 nucleotides of a sequence called SIRE (Pavia et al., 2007), a small interleaved repetitive element presents in *T. cruzi*. Primers Tch2AF (5'- GAGAGTGATCGTGGGAGAGC-3') and Tch2AR (5'-AGTGGCAGACTTTGGGGTC-3') were used, specific for *T. cruzi*, and which produce a 234 bp amplicon (Lilioso et al., 2020). In 25 μl volumes, PCR reactions were performed with 11 μl of the Qiagen PCR Master Mix commercial kit (QIAamp, Qiagen, Hilden, Germany), 0.5 μM of each primer, 2 μl of genomic DNA, and DNase free water until the volume is complete. The reaction was performed on the Mastercycler Gradient thermocycler (Eppendorf, Foster City, California, USA), with 94°C programming for 3 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s, with a final extension of 72°C for 10 min.

The PCR products were stained with SYBR Safe solution (Invitrogen, CA, USA) and submitted to a horizontal electrophoretic run (Micro SSPTM Gel System, One Lambda, USA) in 1.5% agarose gel, in Tris borate EDTA buffer (TBE), together with the 100 bp molecular weight marker (Invitrogen, USA). The results were visualized on a blue light transilluminator and photographed with the MultiDocit Photodocumenter (UVP, Imaging Systems, Upland, CA, USA). The positive control was a triatomine sample positive for *T. cruzi* previously sequenced and with 99% identity with the sequence deposited in GenBank (CP015671.1) and the negative control was ultrapure water. PCR products were purified with a commercial QIAquick PCR Purification kit (Qiagen, Germany) and sent for bidirectional sequencing (Sanger et al., 1977).

2.5. Epidemiological questionnaire

The semi-structured epidemiological questionnaires had objective and subjective questions and were applied according to the profile of the interviewees: (i) addressed to the household provider (adult responsible for the home), containing questions about triatomines, CD, and demographic data (biological, socioeconomic, and cultural); and (ii) addressed to other residents of the households, with questions about demographic data, being applied to individuals over ten years of age.

The interviews were conducted in the interviewees' households by four trained interviewers. The questions were asked without inducing the answer and without reading the alternatives. In cases where respondents were unable to answer, this option was checked in the

Table 1Natural infection index for *T. cruzi* in triatomines collected in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, from 2018 to 2019.

	<i>Triatoma sherlocki</i>					<i>Triatoma sordida</i>					<i>Panstrongylus sherlocki</i>				
	F	M	N	P/A	I%	F	M	N	P/A	I%	F	M	N	P/A	I%
INTRA	1 ^a	1 ^a	1 ^b	0/3	0.0	1 ^a	0	0	0/1	0	0	0	0	0	0.0
PERI	2 ^c	0	1 ^d	1/3	33.3	0	0	2 ^{e,f}	0/2	0	0	1 ^g	0	1/1	100.0
WILD	21	24	100	27/145	18.6	0	0	0	0/0	0	0	0	0	0.0	
TOTAL	24	25	102	28/151	18.5	1	0	2	0/3	0	0	1	0	1/1	100.0

F: female; M: male; N: nymph; P: positive triatomines; A: analyzed triatomines; I%: Natural Infection Index - the percentage of positive triatomines for *T. cruzi*; INTRA: inside the house; PERI: peridomicile; WILD: wild environment

^a captured in the bathroom

^b captured in the bedroom

^c captured in the backyard

^d captured on the balcony

^e captured in a pile of tiles in the backyard

^f captured in the backyard where a *T. cruzi* seronegative dog was sleeping; ^g captured in the backyard where a *T. cruzi* seropositive dog was sleeping.



Fig. 2. Characteristics of households and proximity to a wild environment. (A) Rocky-outcrops surrounding the dwellings and considered major ecotopes of *T. sherlocki*. (B) Peridomicile with rock formations serving as a shelter for a dog and triatomines. (C) Dog in the peridomicile, with accumulation of diverse materials in the background, serving as a hiding place for triatomines.

questionnaire.

2.6. Data analysis and geoprocessing

Data from qualitative variables were categorized and their absolute and relative frequencies described. For each analysis, tables were created highlighting the outcome variables and the independent variables, namely: i) demographic and clinical data of the dogs that were associated with the outcome variable “Seropositivity for *T. cruzi* in dogs”; ii) data on the perception, knowledge and practices of providers that were associated with the outcome variable “Infestation rate of triatomines in households”. In addition, “Structural and environmental characteristics of households” were associated with the outcome variable “Index of triatomine infestation in households”. To analyze the association between the outcome variables and the independent variables, non-fitted model and multivariate analyses were performed using logistic regression. Prevalence ratios (PR) and 95% confidence intervals (95%CI) were estimated. The *forward* variable selection method was

used to include the variables in the multiple model. The inclusion criterion was $p < 0.20$ in the fitted model analysis. The criterion for maintaining the variables in the final model was $p < 0.05$. Data analysis was performed using Stata®14 statistical software.

The map that determines the studied area’s location was made using the software Qgis 3.12 and Adobe Illustrator CS6.

3. Results

3.1. Demographic data, serological and molecular diagnosis in humans and dogs

In this study, 255 residents were recruited but only 126 individuals agreed to participate. The demographic data of the volunteers are shown in Supplementary Table 1. All volunteers sampled were negative for *T. cruzi* in serological and molecular tests, which is equivalent to 49.5% (126/255) of the population in the district of Santo Inácio.

In the study area, 40 dog samples were collected. Demographic and clinical data of dogs are shown in Supplementary Table 2. 17.5% of the dogs sampled (7/40) were reactive for *T. cruzi* in both the Gold ELISA Chagas and *in-house* ELISA tests (chimeric antigens), and 7.5% (3/40) were reactive to both the DPP© and ELISA/EIE for anti-*Leishmania* sp. A single dog was seropositive for *T. cruzi* (Gold ELISA Chagas and *in-house* ELISA tests) and *Leishmania* sp. (rapid test DPP© and ELISA/EIE), suggesting possible co-infection by both pathogens. Of the dogs sampled, 75% (30/40) were male and 25% (10/40) female. 85.71% (6/7) of males and 14.29% (1/7) of females were seropositive for *T. cruzi*. In addition, dogs older than one year of age were the most seroreactive for *T. cruzi* (85.71%, 6/7). No dog was positive for *T. cruzi* by the PCR technique.

3.2. Analysis of triatomines

The 155 triatomines were captured in the following environments: inside houses ($n = 4$); peridomicile ($n = 6$); and wild environment ($n = 145$). The ten triatomines captured in households ($n = 7$) were of the following species: *Triatoma sherlocki* ($n = 6$), *Triatoma sordida* ($n = 3$), and *Panstrongylus sherlocki* ($n = 1$). All specimens found in the wild ($n = 145$) were of the species *T. sherlocki* and most were captured on the surface of rock formations ($n = 131$). Only one colony was found in a rock burrow ($n = 14$). In this colony, all triatomines were engorged and 12 of them were positive for *T. cruzi*. Twenty-nine (18.7%) triatomines were positive for *T. cruzi* according to PCR testing (Table 1) and confirmed by sequencing.

3.3. Analysis of households

Of the 106 existing households in the district of Santo Inácio, 48 were sampled (45%). Among the information about the infrastructure and organization of the households, it is noteworthy that the 94% (45/48) had ceramic roofs; 88% (42/48) masonry walls with plaster; 88% (42/48) peridomicile materials such as tiles and blocks, 60% (29/48) construction debris and 46% (22/48) accumulated garbage. Forty households (38%) were not inspected, as they were vacant, and in 18 households (17%) there was no permission from the resident.

In 34 households sampled, 351 domestic animals were registered, in the following proportions: 62% (217/351) chickens, 14% (50/351) sheep, 12% (43/351) dogs, 10% (36/351) cats, and 1% (5/351) birds. The presence of annexes for raising domestic animals was verified in 21% (10/48) of the households, of which nine were chicken coops, and one had a chicken coop and a sheep corral. Entomological indicators calculated resulted in an infestation index of ~15% (7/48), triatomine abundance of 0.2 (10/48) and colonization index of 57% (4/7). Fig. 2 shows some households characteristics and their proximity to the rocky-outcrops of the wild environment. Supplementary Table 3 shows the environmental conditions of households positive for triatomines.

3.4. Epidemiological questionnaire

Of the 112 questionnaires applied, 42% (47/112) were answered by the household provider and 58% (65/112) by other residents. The questionnaire was not applied to one specific provider and thirteen other residents for various reasons beyond our control ($n = 14$). The results and frequencies of perception, knowledge, and practices about triatomines and CD are presented in Table 2, and demographic data of the population are in Supplementary Table 1.

3.5. Data analysis

The fitted logistic regression model between "Provider perception, knowledge and practice data" and the "Triatomine infestation index in households" suggests that the fact that the provider has knowledge that the triatomine can cause CD may be configured as a factor of protection for it ($p = 0.04$). Thus, providers with this knowledge may adopt preventive measures to avoid contact with the triatomine (Table 2).

The non-fitted model of the analysis between "Demographic and clinical data of dogs" and "Seropositivity for *T. cruzi* in dogs" was not significant (Supplementary Table 2). Thus, a fitted model was not carried out. Therefore, the exploratory variables do not sufficiently explain

Table 2

Descriptive and statistical analysis of questionnaires on perception, knowledge, and practices in relation to triatomines and Chagas disease obtained from 47 providers and their association with the infestation index of triatomine in households in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, from 2018 to 2019.

Independent variables	n/N (%)	^a Frequency (%)	PR (95% CI)Non-fitted Model	PR (95% CI)Fitted Model
Have you found triatomines at your house? (N = 47)				
No/Do not know	14 (29.79)	1/7 (14.29)	1	
Yes	33 (70.21)	6/7 (85.71)	3.21 (0.11-92.79)	
What place in the house did you find the triatomine? (N = 33)				
Peridomicile	3 (9.09)	1/6 (16.67)	*	
Inside house	22 (66.67)	3/6 (50.00)		
Both	8 (24.24)	2/6 (33.33)		
^a What did you do when you found the triatomine? (N = 33)				
Suitable destination	2 (6.06)	0	*	
Inappropriate destination	31 (93.94)	6/6 (100.00)		
What is the delivery location for the triatomine? (N = 47)				
Health Service (person or institution)	13 (27.66)	0	*	
^b Others/Do not know	34 (72.34)	7/7 (100.00)		
^c Which of the insects is a triatomine? (N = 47)				
Incorrectly pointed/ Cannot answer	8 (17.02)	2/7 (28.57)	1	1
Correctly pointed	39 (82.98)	5/7 (71.43)	0.09 (0.00-1.39)**	0.23 (0.03-1.82)
Do you do anything to avoid the triatomine? (N = 47)				
No	36 (76.60)	6/7 (85.71)	1	
Yes	11 (23.40)	1/7 (14.29)	0.42 (0.02-7.67)	
Do triatomines cause any disease? (N = 47)				
No/Do not know	3 (6.38)	2/7 (28.57)	1	1
Yes	44 (93.62)	5/7 (71.43)	0.01 (0.00-0.74) **	0.04 (0.00-0.66)***
What disease does the triatomine cause? (N = 44)				
Chagas disease	36 (81.82)	5/5 (100.00)	*	
Others	8 (18.18)	0		
Which organ does Chagas disease affect? (N = 47)				
Others	10 (21.28)	1/7 (14.29)	1	
Heart	37 (78.72)	6/7 (85.71)	0.12 (0.00-4.91)	
Do you know someone who has Chagas disease? (N = 47)				
No/Do not know	24 (51.06)	3/7 (42.86)	1	
Yes	23 (48.94)	4/7 (57.14)	2.54 (0.18-34.23)	
Is Chagas disease curable? (N = 44)				
No/Do not know	21 (47.73)	6/7 (85.71)	*	
Yes	23 (52.17)	1/7 (14.29)		
Is there a Chagas disease control service in the region? (N = 45)				
No/Do not know	38 (84.44)	6/7 (85.71)	1	
Yes	7 (15.56)	1/7 (14.29)	0.82 (0.04-16.53)	

The name "the kissing bug" was replaced in the text by triatomine; PR = Prevalence ratios; CI = confidence intervals

^a Frequency of triatomine infestation rate in households in relation to independent variables; * Not included in the non-fitted model due to lack of sufficient data to perform the analysis; ** Criterion used in the non-fitted model was p-value <0.20; *** The criterion for maintaining the variables in the fitted model was p<0.05

^b Suitable destination (delivered to the university researcher) and Inappropriate destination (killed immediately/ threw in the garbage)

^c Do not know ($n = 27$), there is no location ($n = 5$), dumping ground ($n = 2$)

^d Showcase with the presence of three adult hemipteral insects (predator, phytophagous and *T. sherlocki*) was shown to the provider.

Supplementary Table 1

Descriptive analysis of demographic data of residents of the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, in the period 2018 to 2019.

Variables	n/112* (%)
Sex	
Men	44 (39.29)
Women	68 (60.71)
^a Age	
≤18 years-old	17 (15.8)
>18≤29 years-old	18 (16.07)
>29≤45 years-old	23 (20.54)
>45≤65 years-old	35 (31.25)
>65 years-old	19 (16.96)
Self-reported race/color	
Black	27 (24.11)
Brown	62 (55.36)
Others	21 (18.75)
Do not know	2 (1.79)
Education	
^b Illiterate and incomplete basic level	39 (34.82)
Complete basic level and incomplete elementary school	24 (21.43)
Complete elementary school and incomplete university graduation	30 (26.79)
Complete university graduation	17 (15.18)
Does not know	2 (1.79)
Do you have a health complaint?	
No	66 (58.93)
Yes	46 (41.07)
Did you have a blood transfusion?	
No	104 (92.86)
Yes	8 (7.14)
Are you in the habit of eating açai and/or drinking sugarcane juice?	
No	87 (77.68)
Yes	25 (22.32)
Do you eat game meat?	
No	37 (33.04)
Yes	75 (66.96)
Have you ever been stung by triatomines?	
No	57 (50.89)
Yes	41 (36.61)
Does not know	14 (12.50)
Do you use mosquito net?	
No	18 (16.07)
Yes	94 (83.93)

*Information is missing from one provider and thirteen residents ($n = 14$), because the questionnaire was not applied. ^a Age ranged from 10 to 96 years-old, with an average of 45 years-old and 85% of respondents over 19 years-old ^b 9% (10/112) of the respondents were illiterate.

the result of serology for *T. cruzi* in dogs in the studied population. The fitted logistic regression model between “Structural and environmental characteristics of households” and the “Infestation index of triatomines in households” was also not significant (Supplementary Table 3). Therefore, the exploratory variables do not sufficiently explain the rate of triatomine infestation in the sampled households. The two statistical analyses mentioned above were purposely not presented here (for more information see Supplemental Tables 2 and 3).

4. Discussion

Three species of triatomines were identified, *T. sherlocki*, *T. sordida*, and *P. sherlocki*, occurring in different numbers in the researched environments, with 18.7% of the investigated triatomines being infected with *T. cruzi*. It was also observed the presence of seropositive dogs and all human samples were negative for *T. cruzi*.

Adults and nymphs of the *T. sherlocki* species were the most found in households, and the only ones detected in the wild environment. They were positive for *T. cruzi* in both environments. *T. sherlocki* is considered a species of the wild environment (Papa et al., 2002). However, it is believed that at some point, *T. sherlocki*, which cannot fly (Almeida et al., 2012), colonizes households through walking (Almeida et al.,

2009), where it can lay its eggs, with the potential for colonization. The adaptability of the *T. sherlocki* species to the domestic environment was tested in the laboratory, being registered a complete development cycle (egg to adult), low mortality rate, high frequency of blood meal, and resistance to hunger, which can make it possible to remain in households (Lima-Neiva et al., 2017). The infection rate of *T. sherlocki* in this study was 18.5%. Previous studies, also carried out in the district of Santo Inácio, such as Almeida et al. (2009) and Ribeiro et al. (2019), showed infection rates in *T. sherlocki* by *T. cruzi* of 11% and 43%, respectively. Demonstrating that the occurrence of the parasite infecting this species of triatomine is expected in the region, and imposes the need for entomological investigations on the possible colonization probabilities of this vector in households.

Species *T. sordida* and *P. sherlocki* were also captured in households, and the only adult specimen captured of the species *P. sherlocki* in the peridomicile was infected with *T. cruzi*. There is no previous record of infection by *T. cruzi* for this species in the literature consulted. Also, *P. sherlocki* behavioral and occurrence data are scarce (Jurberg et al., 2001), with *P. sherlocki* being considered a chromatic variation of the species *Panstrongylus lutzii* (Garcia et al., 2005). Although the species *T. sordida* is more frequent in the state of Bahia, mostly found in the peridomicile of households, with an infection rate for *T. cruzi* of 4% (Ribeiro et al., 2019), in the present study, few specimens of this species were captured inside households ($n = 1$; in the bathroom) and peridomicile ($n = 2$; in the backyard), the same being negative for *T. cruzi*. Species of the genus *Panstrongylus* occupy natural ecotopes associated with hosts (armadillo burrow, rocks with cavy, bird nests) and are found among large tree roots (Sousa et al., 2020), while the natural ecotope for *T. sordida* is the hollow trunk of trees and bird nests (Sousa et al., 2020). Although studies show the occurrence of *T. sordida* (Ribeiro et al., 2014) and *P. sherlocki* (Jurberg et al., 2001) in natural ecotopes of Santo Inácio, both triatomines were not captured in the wild during our study.

Even with a low rate of infestation and low density of triatomines in households, a significant number of the interviewed providers reported finding triatomines in their households (Table 2). Household places with the highest occurrence of insects were the living room (72%), the bedroom (39%), and the kitchen (30%). According to the interviewees, the backyard ($n = 6$) and chicken coop ($n = 1$) presented less occurrence of triatomines, perhaps due to the short time that people stay in these areas or, also, because insects find more hiding places in this type of environment. Detection of triatomines is generally low and may vary depending on insect abundance, ecotope characteristics, and capture method (Abad-Franch et al. 2014). We used the manual capture of triatomines as recommended by entomological surveillance as a method. However, capture in households during the day, when the vector hides, where hiding places are diverse, and where the abundance of invasive triatomines is generally smaller, made it difficult to detect the insect. Therefore, we recommend that future studies in this district use dislodging insecticides in households and count on the participation of the residents to capture the insects. In the wild, the capture activity was carried out at night, when the insects are most active and in the natural ecotope of triatomines, allowing for greater capture of vectors (Table 1).

Household invasions and colonization by wild triatomines in Santo Inácio occur for several reasons. Some of them are: i) proximity of the rocky-outcrops to the households; ii) presence of shelters for triatomines [ex: peridomiciles with an accumulation of several materials], and annexes of domestic animals very close to the households; iii) the presence of several domestic animals; iv) artificial light attraction of insects and, finally, v) passive transport by domestic animals and human due to work activities, such as hunting and mining in the wild environment, being able to carry triatomines in their utensils (Almeida et al., 2009). Regarding phototropism, during the stay of the research team in this district, three adult specimens of *T. sherlocki* were found at night inside households, one of which was positive for *T. cruzi* (data not counted). These triatomines are attracted by artificial light (Sousa et al., 2020), which favors the nocturnal invasion of households.

Supplementary Table 2

Descriptive and statistical analysis of the demographic and clinical data of 40 dogs and their association with seropositivity for *T. cruzi* in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil in 2019.

Independent variables	n/40 (%)	# Frequency (%)	PR (95% CI) Non-fitted Model
Sex			
Female	10 (25.00)	1/7 (14.29)	1
Male	30 (75.00)	6/7 (85.71)	0.51 (0.00-39.64)
Age			
≤5 years-old	29 (72.50)	3/7 (42.86)	1
>5 years-old	10 (25.00)	3/7 (42.86)	3.95 (0.27-57.38)
Does not know	1 (2.50)	1/7 (14.29)	-
Breed			
Undefined race	38 (95.00)	7/7 (100.00)	*
Others	2 (5.00)	0	
Is the dog a hunter?			
No	34 (85.00)	6/7 (85.71)	1
Yes	6 (15.00)	1/7 (14.29)	0.60 (0.02-16.89)
Is the dog fed with game meat?			
No	29 (72.50)	5/7 (71.43)	1
Yes	11 (27.50)	2/7 (28.57)	0.56 (0.03-8.41)
Where does the dog sleep?			
Inside house	3 (7.50)	0	*
Backyard	37 (92.50)	7/7 (100.00)	
Anemic mucous membranes			
No	28 (70.00)	5/7 (71.43)	1
Yes	12 (30.00)	2/7 (28.57)	0.68 (0.04-10.12)
Cardiac arrhythmia			
No	33 (82.50)	7/7 (100.00)	*
Yes	7 (17.50)	0	
Crusts on the ears			
No	24 (60.00)	4/7 (57.14)	1
Yes	16 (40.00)	3/7 (42.86)	2.99 (0.07-124.86)
Hyperkeratosis of the snout			
No	31 (77.50)	6/7 (85.71)	1
Yes	9 (22.50)	1/7 (14.29)	0.51 (0.04-5.97)
Alopecia			
No	26 (65.00)	3/7 (42.86)	1
Yes	14 (35.00)	4/7 (57.14)	2.28 (0.24-21.41)
Lymphadenomegaly			
No	10 (25.00)	0	*
Yes	30 (75.00)	7/7 (100.00)	
Splenomegaly			
No	36 (90.00)	6/7 (85.71)	1
Yes	4 (10.00)	1/7 (14.29)	2.56 (0.11-55.33)
Onychogryphosis			
No	32 (80.00)	5/7 (71.43)	1
Yes	8 (20.00)	2/7 (28.57)	1.26 (0.08-19.04)
Tick			
No	1 (2.50)	0	*
Yes	39 (97.50)	7/7 (100.00)	
Outcome variable: Serology for <i>T. cruzi</i>			
Negative	33 (82.50)		
Positive	7 (17.50)		

#Frequency of seropositive dogs for *T. cruzi* in relation to independent variables); PR = Prevalence ratios; CI = confidence intervals; * Not included in the non-fitted model due to lack of sufficient data to perform the statistical analysis ($p < 0.20$).

The presence of domestic animals in households may be more attractive for triatomines to feed on than human presence (Daflon-Teixeira et al., 2019; Gürtler et al., 2007). Among the animals raised in the district, the presence of chickens stands out, which are the most accessible blood-meal sources for triatomines (Daflon-Teixeira et al., 2019; Ribeiro et al., 2015). Dogs come in second place, being considered a link in the circulation of *T. cruzi* between the wild and domestic environment (Porfirio et al., 2018). This fact was reinforced in the present study by registering 17% of dogs seropositive for *T. cruzi*.

Dogs generally develop the chronic form of CD, with no apparent clinical signs (Santana et al., 2012) with low parasitemia at this disease stage (Araújo et al., 2002). However, some naturally infected dogs may have detectable parasitemia, depending on the genotype of the parasite circulating in the region and the health status of the animals, as detected by xenodiagnosis, conventional PCR (Enriquez et al., 2013), and qPCR (Enriquez et al., 2014) in other countries (Gürtler and Cardinal, 2015).

In this study, dogs infected with *T. cruzi* had a varied clinical picture, compatible with other diseases such as visceral leishmaniasis, making the clinical diagnosis of CD difficult. Only one dog showed seropositivity for *Leishmania* sp. and *T. cruzi*, indicating coinfection. The diagnosis of these diseases in co-endemic regions is a challenge due to the possibility of cross-reaction (Leony et al., 2019; Porfirio et al., 2018), which was discarded in our study because we used two different serological techniques to detect both parasites. In addition, co-infected dogs remain parasitized for long periods, which represents a significant risk in the spread of these zoonoses (Cruz et al., 2020; Porfirio et al., 2018).

Most of the dogs sampled in this study are not used for hunting wild animals and are not fed game meat, even though game meat intake is cultural among people in the region. The two wild animal species most cited as a food source by the interviewees were the rock cavy [*K. rupestris* (87%)] and the armadillo [Dasypodidae (55%)], both being *T. cruzi* reservoirs (Jansen et al., 2020). It is assumed that, during the

Supplementary Table 3

Descriptive and statistical analysis of the structural and environmental characteristics of the 48 households and their association with the rate of triatomine infestation in households in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, in the period 2018 to 2019.

Independent variables	n/48 (%)	# Frequency (%)	PR (95% CI)Non-fitted Model	PR (95% CI)Fitted Model
Roof tiles				
Ceramic	45 (93.75)	6/7 (85.71)	1	
Others	3 (6.25)	1/7 (14.29)	29.32 (0.51–1683.04)**	***
Walls				
Masonry with plaster	42 (87.50)	7/7 (100.00)	*	
Masonry without plaster	5 (10.42)	0		
Clay without plaster	1 (2.08)	0		
Construction debris in the peridomicile				
No	19 (39.58)	4/7 (57.14)	1	1
Yes	29 (60.42)	3/7 (42.86)	0.11 (0.01–1.24)**	0.18 (0.02–1.39)
Piles of tiles and bricks in the peridomicile				
No	6 (12.50)	1/7 (14.29)	1	
Yes	42 (87.50)	6/7 (85.71)	1.91 (0.10–33.41)	
Garbage accumulation				
No	26 (54.17)	2/7 (28.57)	1	1
Yes	22 (45.83)	5/7 (71.43)	28.98 (1.21–694.11)**	6.49 (0.81–51.65)
Annexes for domestic animals				
No	38 (79.17)	6/7 (85.71)	*	
Yes	10 (20.83)	1/7 (14.29)		
Presence of dogs				
No	25 (52.08)	2/7 (28.57)	1	1
Yes	23 (47.92)	5/7 (71.43)	4.66 (0.49–44.30) **	4.62 (0.63–33.39)
Presence of cats				
No	41 (85.42)	6/7 (85.71)	1	
Yes	7 (14.58)	1/7 (14.29)	1.55 (0.09–25.14)	
Presence of chickens				
No	37 (77.08)	5/7 (71.43)	1	
Yes	11 (22.92)	2/7 (28.57)	4.58 (0.38–54.21)	
Outcome variable: Households with triatomine capture				
No	41 (85.42)			
Yes	7 (14.58)			

Frequency of triatomine infestation rate in households in relation to independent variables; PR = Prevalence ratios; CI = confidence intervals; * Not included in the non-fitted model due to lack of sufficient data and/or collinear variable with other variables to perform the analysis; ** Criterion used in the non-fitted model was p-value <0.20; *** Removed from the fitted model as it is a confounding variable that modifies the effect of the other variables; The criterion for maintaining the variables in the fitted model was p<0.05.

preparation of the game meat, the remains of these animals are given to the dogs. Two dogs seropositive for *T. cruzi* fed on wild animals, which suggests that oral transmission of the parasite could occur, as reported by Porfirio et al. (2018), who detected 76% of dogs in a rural community in the state of Mato Grosso do Sul infected with *T. cruzi*, 40% of which were fed with wild mammals.

All *T. cruzi* seropositive dogs slept in backyards. However, there was no association between the serological result and the variable "Where does the dog sleep?" (Supplementary Table 2), even knowing that the overnight stay of dogs in the peridomicile increases exposure to triatomines because of the more significant number of vectors in these places (Walter et al., 2005). We recorded an adult triatomine positive for *T. cruzi* living with a seropositive dog in the same peridomicile, and a negative nymph captured in the place where a seronegative dog slept. This scenario shows the severity of CD in this region, where triatomines and dogs share the same space (Fig. 2.B,C).

However, we did not find any PCR-positive dog for *T. cruzi*, which would confirm the infection in tested animals. Some points can be raised in this regard: i) the tested aliquot does not contain parasite DNA (*T. cruzi* is not present in the peripheral circulation of the animal); ii) the fact that the primer does not detect some *T. cruzi* strains (Barrera et al., 2008); and iii) the difference in the objectivity of serological tests in relation to molecular testing (antibodies/immunological memory x DNA/infection). The sensitivity of the PCR reaction using TcH2AF/TcH2AR primers was 0.1 ng DNA/ μ L, which was determined using *T. cruzi* positive control DNA in 10x serial dilutions, ruling out the possibility of negative results due to problems of PCR sensitivity. Regarding possible reaction inhibitors present in the DNA sample, this problem was ruled out by satisfactorily amplifying all negative samples

after adding 1.0 μ l (50 ng) of parasite DNA (positive control) to them, following the methodology of Gil et al. (2007). The sensitivity of PCR with other primers (for example kinetoplast target) in naturally infected dogs ranged between 40-90% (Araújo-Neto et al., 2019; Enriquez et al., 2013; Porfirio et al., 2018). However, the study by Cantillo-Barraza et al. (2020) showed PCR negativity in *T. cruzi* seropositive dogs, even using more sensitive primers than those used here, which shows the importance of using different diagnostic tests to expand the possibilities of reactive and/or detecting the parasite (antibodies and/or DNA). The PCR test has high sensitivity and specificity to detect infected dogs (Araújo-Neto et al., 2019), being used as a complementary diagnosis to serology and allowing the detection of circulating *T. cruzi* lineages (DTUs TcI-TcVI) and other species of *Trypanosoma* (Malavazi et al., 2020; Porfirio et al., 2018).

Diverse studies deal with human seroprevalence of *T. cruzi* in several regions considered endemic for triatomines in northeast Brazil (Araújo et al., 2002; Borges-Pereira et al., 2006; Borges-Pereira et al., 2008; Brito et al., 2012; Lima et al., 2012; Santos et al., 2015). In the present study, all people tested negative for *T. cruzi*. According to Cerqueira et al. (1998), previous studies carried out in 1975 and 1995, in this same study area, registered 3% and 4% of seropositivity for *T. cruzi*, respectively. The low prevalence of people infected in these two periods was attributed to better conditions in the infrastructure of households, not being attractive for colonization of triatomines, and preservation of the wild environment (Cerqueira et al., 1998). These conditions were also observed, which may have contributed to the absence of human cases of *T. cruzi* infection in the present study. Furthermore, this seronegativity may be associated with the significant elimination of *Triatoma infestans* in the 1990s through the frequent application of insecticides in

households, which reduced the occurrence of new cases of CD in several regions of Brazil (Dias, 2016b).

The species *T. sherlocki* is known and called by the population of Santo Inácio as “bicudo”, and it is a vector of the CD protozoan (Almeida et al., 2009), which was identified in the hemipteral insects showcase by 83% of the providers. The correct identification of triatomines by the population allows the notification of these insects to the government agencies responsible for disease control and prevention programs (Dias et al., 2016a). However, there was a lack of information from the population regarding the correct procedure for capturing and disposing of triatomines captured in their households, with most providers immediately killing the insect when they found it and 57% of providers not knowing where to forward them. Similar results have also been reported in other studies (Dias et al., 2016a; Rosecrans et al., 2014).

To avoid triatomines in their households, 77% of providers use some preventive measures. Of these, 50% are insecticide spraying and 42% seek to improve hygiene aspects to avoid an accumulation of garbage and materials (data not shown). These precautions are also the most cited in other studies (Rosecrans et al., 2014; Urioste-Stone et al., 2015). Spraying households with insecticides was suggested by 31% of providers to improve the region's CD control service. However, for this to happen, the resident needs to notify entomological surveillance of the occurrence of triatomines in their household (Dias et al., 2016b), which does not happen in this population as previously reported, even though they have a health post in the district that receives the insects and sends them to the responsible sector.

In the present study, most individuals used a mosquito net attached to the mattress to sleep (Supplementary Table 1). This palliative action protects people from the blood meal of triatomines when they are sleeping. However, 37% of people reported having been bitten by insects, 29% with a sting frequency greater than ten times (data not shown), demonstrating that this occurs when individuals are awake, thus being able to protect themselves from insects. In general, the questionnaire analysis revealed several gaps in respondent knowledge about CD and about the set of practices associated with reducing exposure to triatomines. However, logistic regression analysis showed a protective factor for providers who know that triatomines “cause” CD, as they avoid favorable conditions for invasion and colonization of these insects in their homes (Table 2). Thus, the questionnaire can help to produce educational materials that reinforce aspects still deficient about CD in this population.

The epidemiological scenario revealed is worrying and also occurs in other regions of Brazil (Araújo-Neto et al., 2019; Lima et al., 2012; Sangenis et al., 2015) and in other countries in the Americas (Waleckx et al., 2015), with the aggravation of detecting active cases of *T. cruzi* in humans in the context of invasions by the triatomines *Triatoma vitticeps*, *Triatoma maculata*, *Triatoma dimidiata* and *Eratyrus mucronatus* in Brazil (Sangenis et al., 2015), in Colombia (Cantillo-Barraza et al., 2015), in Mexico (Gamboa-León et al. 2014) and in Bolivia (Depickère et al., 2012), respectively. These studies generally show that people have socioeconomic and cultural characteristics that favor the invasion of these insects in their houses (Daflon-Teixeira et al., 2019). In Bolivia, for example, the population of the municipality of Apolo has the habit of building walls with clay to delimit the peridomicile of their dwellings, an ideal hiding place for triatomines (Depickère et al., 2012). While in a certain region of Colombia, the transmission of *T. cruzi* to humans and dogs is related to the occurrence of synanthropic animals such as *Didelphis marsupialis* (Cantillo-Barraza et al., 2015) that finds shelters in the peridomicile of households. Thus, the control of transmission of *T. cruzi*, in the current scenario, must be analyzed from a holistic perspective, engaging the residents of these regions in the recognition and notification of triatomines (Rosecrans et al., 2014; Yevstigejeva et al., 2014), as well as the use of prevention and control methods for these insects inside residents households (Urioste-Stone et al., 2015).

5. Conclusion

Based on previous reports and the detection of anti-*T. cruzi* antibodies in dogs and *T. cruzi* infection in specimens of *T. sherlocki* collected in the wild and domestic environment, the presence of the etiological agent of CD in the district of Santo Inácio is verified. Although uncommon in the studied area, the species *T. sordida* and *P. sherlocki* can contribute to the maintenance of the *T. cruzi* transmission cycle. We also report the first record of the infection of *P. sherlocki* by *T. cruzi*. Though limited, the knowledge about CD and the adoption of some preventive measures against the vector practiced by a portion of the population seems to have contributed temporarily to prevent the transmission of *T. cruzi* to humans. However, the data reveal a latent risk of infection and spread of CD in the population studied. The epidemiological scenario observed has already been reported in other regions of Brazil and in other countries with similar characteristics. This situation can only be overcome with frequent actions of entomological surveillance, active search for cases, and the inclusion of health education content in school materials, with further dissemination of these contents to the general population.

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CRedit authorship contribution statement

Flavia Santos: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Visualization. **Jairo Torres Magalhães-Junior:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Ianei de Oliveira Carneiro:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Fred Luciano Neves Santos:** Methodology, Supervision, Writing – review & editing. **Angelo Antônio Oliveira Silva:** Methodology, Validation, Investigation. **Joane Maíra Cavalcante Braga Novais:** Methodology, Investigation. **Jessica Samile Sousa Santos:** Methodology, Investigation. **Gilmar Ribeiro-Jr:** Methodology, Supervision, Writing – review & editing. **Mitermayer G. Reis:** Methodology, Supervision, Writing – review & editing. **Carlos Roberto Franke:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary Table 1, Supplementary Table 2, Supplementary Table 3.

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9.10. Apêndice 10 – A autoria de manuscrito em produção: Sometimes, the size matters: wing geometric morphometrics as a tool to assess house invasion and colonization by *Triatoma sordida* (Stål 1859).

Sometimes, the size matters: wing geometric morphometrics as a tool to assess house invasion and colonization by *Triatoma sordida* (Stål 1859)

As vezes, o tamanho importa: morfometria geométrica da asa como uma ferramenta para avaliar a invasão de casas e colonização por *Triatoma sordida* (Stål 1859)

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RESUMO

Background: *Triatoma sordida* é um triatomíneo com ampla distribuição no Brasil. Na Bahia é a espécie mais frequente no ambiente domiciliar, onde já foi associada a surtos de transmissão oral de *Trypanosoma cruzi*. Durante o processo de colonização, os triatomíneos passam por alterações morfológicas, e redução de tamanho, quando comparados às populações silvestres. Considerando a progressiva colonização de *T. sordida* no ambiente domiciliar e o seu potencial na transmissão de *T. cruzi* no Brasil, nosso objetivo foi avaliar a existência de alterações morfológicas em populações intra e peridomiciliares desta espécie; nós hipotetizamos que: 1) o tamanho das asas de populações capturadas dentro das casas é menor que o de populações do peridomicílio e 2) o dimorfismo sexual relacionado ao tamanho é menor nas populações das casas. **Methodology:** Analisamos triatomíneos capturados em regiões da Bahia, entre 2013 e 2014. Para as análises morfológicas selecionamos os exemplares do município de Barra, onde a colonização intradomiciliar foi mais frequente. As asas foram montadas entre lâminas, digitalizadas e editadas para análises de morfometria geométrica através dos softwares TPSdig e TPSrelw. Analisamos as variáveis de tamanho de acordo com o sexo e hábitat dos indivíduos usando o teste t. A análise de componentes principais (PCA) foi aplicada para explorar a variação de forma das asas. **Principal Findings:** Capturamos 410 espécimes no intra e peridomicílio, de onde selecionamos 120 indivíduos para as análises de morfometria. Observamos significativa variação de tamanho (teste t = -4.04, p<0.01), sendo os espécimes capturados nas casas menores que os dos peridomicílios. Além disso, machos e fêmeas das casas apresentam tamanho mais similar que os do peridomicílio, em que as fêmeas foram claramente maiores indicando que o efeito de diminuição de tamanho é maior nas fêmeas. A PCA não revelou diferenças evidentes de forma das asas entre as populações das casas e peridomicílios. **Conclusions/Significance:** Os resultados revelam que a colonização de casas por *T. sordida* é frequente no município de Barra, Bahia, e que a morfometria geométrica das asas pode ser aplicada para discriminar as populações de dentro e fora das casas, e quantificar diferenças de tamanho de machos e fêmeas (dimorfismo sexual) nesses ambientes. Nossos resultados reforçam a ideia de analisar o dimorfismo sexual para indicar processos de adaptação ao ambiente domiciliar e a hipótese de que *T. sordida* obteve sucesso na colonização de casas nesse município. Sugerimos que a morfometria geométrica seja aplicada para auxiliar a detecção de populações intradomiciliares de *T. sordida* e dessa forma aprimorar a vigilância e controle vetorial da doença de Chagas.

Key words: *Triatoma sordida*, domiciliação, morfometria, doença de Chagas, *T. cruzi*.

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INTRODUÇÃO

A doença de Chagas (DC) é uma doença tropical negligenciada transmitida por insetos hematófagos popularmente conhecidos como barbeiros (Hotez et al., 2012; Stevens and Dorn, 2017). Atualmente, são conhecidas +150 espécies de triatomíneos, das quais ~70 ocorrem no Brasil, sendo algumas mais relevantes na transmissão do *T. cruzi* ao homem e seus animais domésticos devido a capacidade de colonizar o ambiente intradomiciliar (Galvão, 2014; Gurgel-Gonçalves et al., 2012). O estudo de indicadores de invasão, infestação e colonização de casas por triatomíneos é um componente-chave para a vigilância e estratificação do risco de transmissão da doença de Chagas (Ribeiro et al., 2021).

Triatoma sordida tem ampla distribuição geográfica e é a espécie mais capturada no Brasil (Almeida et al., 2008; Cominetti et al., 2014; da Silva et al., 2003; de Oliveira and da Silva, 2007; Forattini, 2006; Silistino-Souza et al., 2013). No ambiente domiciliar, *T. sordida* é frequentemente associada a aves e galinheiros (Da Silva et al., 2011) e apesar de ter sido considerada de baixa competência vetorial pelos seus hábitos naturalmente ornitofílicos, há evidências de que adultos e ninfas de *T. sordida* dispersaram ativamente no ambiente peridomiciliar (Dantas et al. 2018, 2022), e alcançaram as casas, originando casos agudos da DC na Bahia a partir de transmissão vetorial-oral (Barrett et al., 1979; Bastos et al., 2010; Dias et al., 2008). Dessa forma, é relevante saber as áreas onde há invasão e colonização de casas por *T. sordida* para auxiliar estratégias de prevenção.

Triatomíneos desenvolvem adaptações morfológicas para se adequar aos seus habitats, por exemplo, variações fenotípicas intraespecíficas marcantes (tamanho, forma, coloração) têm sido observadas em triatomíneos como respostas adaptativas a microhabitats distintos (Abad-Franch et al., 2021). Outros estudos mostram que a diminuição de tamanho corporal e do dimorfismo sexual seriam marcadores importantes nos processos de domiciliação em espécies de triatomíneos (Dujardin, 2008; Dujardin et al., 1999; Garcia et al., 2021; Gurgel-Goncalves et al., 2011; Nattero et al., 2017). Essas evidências sugerem que populações peridomiciliares de

triatomíneos podem ser diferenciadas de populações do interior das casas e que o tamanho dos adultos detectados pela vigilância poderia ser um indicador adicional de colonização das casas quando ninfas não são encontradas.

Um dos métodos mais aplicados para estudar populações de triatomíneos é a morfometria geométrica, que utiliza marcos anatômicos e métodos de superposição de imagens para analisar a variação de tamanho forma entre indivíduos (James Rohlf and Marcus, 1993). Essa técnica foi aplicada para diferenciar *T. sordida* de uma espécie muito similar, *T. garciabesi* (Gurgel-Gonçalves et al. 2011) e para analisar a variação morfométrica de *T. sordida* no Brasil (Vendrami et al. 2017). Considerando as evidências de adaptação de *T. sordida* ao ambiente domiciliar no Brasil, particularmente no Estado da Bahia, e o seu potencial na transmissão de *T. cruzi* (Batista et al., 2013), nosso objetivo foi avaliar a existência de alterações morfométricas em populações intra e peridomiciliares desta espécie em um município onde a espécie foi detectada dentro das casas. Nossas hipóteses são: 1) o tamanho das asas de populações de *T. sordida* capturadas dentro das casas é menor que o de populações peridomiciliares e 2) o dimorfismo sexual relacionado ao tamanho é menor nas populações das casas.

METODOLOGIA

Amostragem de triatomíneos

As amostragens de triatomíneos foram realizadas entre janeiro de 2013 e dezembro de 2014 conforme metodologia previamente descrita (Ribeiro et al., 2019) em parceria com a SESAB e realizada por agentes de saúde do Programa Estadual de Endemias Rurais das Diretorias Regionais de Saúde, DIRES-BA, que procederam conforme o estabelecido pelo Programa Nacional de Controle da doença de Chagas (Brasil, 1980, 1996, 2001) (Figura 1A). Os triatomíneos coletados nas DIRES-BA foram encaminhados para o Laboratório de Entomologia do LACEN-BA e insetário do Laboratório de Patologia e Biologia Molecular

(LPBM), Instituto Gonçalo Moniz (IGM)/Fiocruz-BA, quando vivos, em frascos coletores, e os mortos conservados em álcool 70%.

Identificação, dissecação, estocagem e morfometria geométrica dos triatomíneos

Os triatomíneos foram identificados por de chaves dicotômicas de identificação (Galvão, 2014; Gurgel-Gonçalves et al., 2012) em processo de dupla verificação, no insetário do Laboratório de Patologia e Biologia Molecular (LPBM), e no lab. de entomologia, LACEN-BA. As identificações discordantes foram discutidas caso a caso.

Fotografamos os triatomíneos na posição dorsal e ventral com uma câmera MOTIC 3.0 acoplada a um estereoscópio Olympus SZX7, e todas as imagens estão publicamente disponíveis ([LINK](#)). Posteriormente, separamos a cabeça e asas, pernas e abdômen dos insetos. Estocamos as asas em tubos para microcentrifuga com 1,5mL de volume, a seco e à temperatura ambiente (~25°C), no insetário do LPBM. Codificamos os insetos com número e QRcode usando impressora Zebra TLP 2844 e o software Zebradesign V.2.

As amostras das asas dos insetos foram selecionadas para análises de morfometria geométrica. Nós montamos os hemiélitros dos triatomíneos entre lâminas e fixamos com auxílio de fita dupla face, posteriormente digitalizamos em alta resolução (900dpi). Após a marcação dos pontos anatômicos (Figura 2), as imagens foram editadas com software Photoscape® e organizadas por sexo e ambiente de coleta. Nós digitalizamos sete pontos anatômicos em cada asa (Tipo I e II) usando o software Tpsdig (Rohlf, 2006) para determinação das coordenadas (x e y). O tamanho e a forma das asas foram obtidos por meio de um estimador de tamanho (tamanho do centróide - TC) calculado a partir das coordenadas de cada um dos pontos anatômicos das asas através do software TPSrelw e em seguida, o alinhamento das coordenadas das asas e a determinação das variáveis de forma (Rohlf, 2007).

Análise estatística

Nós comparamos a média de tamanho (valores de CS das asas de cada grupo) entre as populações por meio do teste t ($\alpha = 0.01$). Além disso, realizamos uma Análise de Variância (ANOVA) fatorial para saber o efeito do sexo e do habitat (casa ou peridomicílio) no tamanho dos espécimes de *T. sordida* capturados. Nós utilizamos variáveis de forma, derivadas das deformações parciais do TPSrelw, para a Análise de Componentes Principais (PCA). Um mapa fatorial dos dois primeiros componentes principais foi utilizado para ilustrar os principais resultados. O teste t, ANOVA e a PCA foram calculados com o software Statistica® (StatSoft, Inc., Tulsa, OK, EUA).

RESULTADOS

Nós capturamos 410 espécimes de *T. sordida* no município de Barra (Figura 1.B), colonizando os ambientes intra 53,9% (n=221) e peridomiciliar 46,1% (n=189), oriundos de dez localidades e 33 colônias, com média de 12 (SD=7.6) triatomíneos por colônia, entre indivíduos adultos e ninfas (Tabela 1). No ambiente peridomiciliar todas as colônias foram observadas em galinheiros. Já no ambiente intradomiciliar, as colônias foram observadas na cozinha, quarto e sala.

Nós selecionamos 120 espécimes adultos, 64 do peridomicílio (30 machos e 33 fêmeas) e 57 das casas (30 machos e 27 fêmeas) para as análises de morfometria geométrica de asas. Houve significativa variação de tamanho das asas entre as populações de *T. sordida* nos ambientes de coleta (teste t = -4.04, $p < 0.01$) sendo os espécimes capturados no intradomicílio menores que os do peridomicílio. Ao analisarmos o tamanho entre habitats separado por sexo observamos que as fêmeas foram claramente diferentes (teste t = -4.43, $p < 0.01$) sendo as de dentro das casas muito menores que as do peridomicílio. Entretanto, os machos das casas apresentaram um tamanho similar aos do peridomicílio (teste t = -1.54, $p = 0.12$) (Figura 3). Conforme esperado, observamos dimorfismo sexual, com fêmeas significativamente maiores do que os machos no peridomicílio (teste t = 6.68, $p < 0.01$). Entretanto, dentro das casas não

encontramos diferença significativa de tamanho entre machos e fêmeas considerando $\alpha = 0.01$ (teste $t = 2.22$, $p=0.03$). Machos e fêmeas do intradomicílio apresentaram tamanho mais similar que os do peridomicílio, em que as fêmeas foram claramente maiores sugerindo que o efeito de diminuição de tamanho entre os habitats (ANOVA $F_{1,116} = 5,86$, $p=0,017$) é maior nas fêmeas (Figura 4). Dessa forma, o dimorfismo sexual relacionado ao tamanho é menor nas populações de *T. sordida* do interior das casas. O PCA não revelou diferenças evidentes de forma das asas entre as populações das casas e peridomicílios (Figura 5).

DISCUSSÃO

Nosso trabalho mostrou que as asas das populações de *T. sordida* do interior das casas do município de Barra, Bahia, são menores do que as encontradas no peridomicílio, que o dimorfismo sexual relacionado ao tamanho é menor nas populações das casas e que não houve diferença marcante de forma de asa entre as populações. A morfometria geométrica em triatomíneos tem sido utilizada para a diferenciação de táxons fenotipicamente semelhantes (de la Fuente et al., 2011; Oliveira et al., 2017), para demonstrar diferenças entre as populações da mesma espécie em diferentes regiões (Nattero et al., 2017), e para esclarecer questões relacionadas à reinfestação local a partir de focos silvestres ou focos residuais de populações sinantrópicas (Dujardin et al., 1997; Hernandez et al., 2013). Tais fatos reforçam o papel da morfometria geométrica para a vigilância dos vetores da doença de Chagas (Dumonteil et al., 2007; Vargas et al., 2006).

Com base na análise por morfometria geométrica das asas das populações de *T. sordida* analisadas observamos diferenças entre as populações estudadas quando estratificamos os dados por sexos, bem como entre os ambientes domiciliares, intra e peridomiciliar estudados. Como esperado, observamos um menor tamanho das populações de dentro das casas, concordando com resultados obtidos para outras espécies como *T. infestans* (de la Fuente et al., 2011). Além disso, observamos que a diferença de tamanho das populações entre esses

ambientes foi mais marcante entre as fêmeas. A diferença de tamanho entre machos e fêmeas (dimorfismo sexual) foi maior na população peridomiciliar. Fenômeno similar já foi demonstrado por Dujardin et al. (1999) em *T. infestans* e *Rhodnius domesticus* comparando populações silvestres e de laboratório. Durante a transição de ambientes naturais para artificiais existem mudanças populacionais que influenciam a quantidade e qualidade da alimentação dos triatomíneos, o que estaria relacionado com o tamanho de machos e fêmeas das populações. Dujardin et al. (1999) recomendou que o dimorfismo sexual baseado no tamanho é uma característica importante para estudar triatomíneos em processo de adaptação a ambientes domésticos. Nossos resultados baseados em dados de campo reforçam a ideia de analisar o dimorfismo sexual para indicar processos de adaptação ao ambiente domiciliar e a hipótese de que *T. sordida* obteve sucesso na colonização de casas no município de Barra, Bahia.

O processo de colonização de casas por *T. sordida* pode ser influenciado pelo manejo inadequado de aves de criação, em especial da galinha doméstica (*Gallus gallus domesticus* L.). A forte associação entre *T. sordida* e aves é bastante conhecida, o que explica a alta frequência dessa espécie em galinheiros no peridomicílio (Rossi et al. 2014). A entrada de *T. sordida* nas casas pode ser explicada pela boa capacidade de movimentação dessa espécie no peridomicílio (Dantas et al. 2018, 2022) e facilitada por comportamentos dos moradores de criar aves dentro de casas, principalmente quando elas estão colocando ovos. Dessa forma, espécimes de *T. sordida* poderiam entrar de forma passiva (ninfas pequenas) ou ativa (adultos e ninfas caminhando ativamente atraídas pelas aves no interior das casas). No ambiente peridomiciliar, todas as colônias foram coletadas em galinheiros. Já no ambiente intradomiciliar, as colônias foram encontradas na cozinha, quarto e sala, geralmente associadas a ninhos de aves mantidos pelos moradores com objetivo de proteger as aves de predadores durante a noite.

A possibilidade de colonização de ambientes artificiais por *T. sordida* já foi alvo de ampla discussão e avaliação nas décadas finais do século XX (Forattini et al., 1973) e de relatos

no início do século XXI (Diotaiuti et al., 1994), demonstrando uma progressiva adaptação desta espécie ao território nacional, inclusive na Bahia (Mendonca et al., 2015; Ribeiro-Jr et al., 2022). Entre janeiro de 2013 e dezembro de 2014 foram amostrados 127 municípios no Estado da Bahia (29.7%), distribuídos em todas as regiões do Estado, quando foram capturados 5906 triatomíneos distribuídos em 15 espécies. A espécie *T. sordida* correspondeu a 3576 exemplares (60.54%) de toda amostragem e foi encontrada infestando 70 (59.91%) dos municípios amostrados (Ribeiro et al., 2019). Esses dados mostram a relevância de investigar os fatores que influenciam a domiciliação de *T. sordida*.

Além da análise por morfometria geométrica, outras estratégias podem ser utilizadas para estudar a domiciliação de populações de triatomíneos, entre elas podemos citar a análise por microssatélites, que já foi utilizada para avaliar as fontes de reinsufestação por *Triatoma infestans* após tratamento com inseticidas químicos na região do Gran Chaco, onde a espécie é endêmica, silvestre e sinantrópica (Rojas de Arias et al., 2022). Estudos similares podem ser realizados com populações de *T. sordida* no Brasil comparando a estrutura genética de populações dentro e ao redor das casas. As evidências existentes mostram que há baixa variação genética entre populações peridomiciliares de *T. sordida* no Brasil baseado em variação de aloenzimas (Monteiro et al. 2009), porém novos estudos devem ser realizados com outros marcadores e incluindo populações do interior das casas. Estes estudos são necessários considerando que *T. sordida* é a espécie de triatomíneo mais capturado no Brasil, as evidências de colonização das casas descritas em nosso estudo e a associação dessa espécie com surtos de transmissão pela via vetorial-oral na Bahia (Bastos et al., 2010; Dias et al., 2008).

Outras estratégias moleculares mais recentes exploraram a modulação do transcriptoma em triatomíneos, e utilizam o RNA-Seq para avaliar a resposta transcriptômica precoce após exposição à inseticidas em populações de *T. infestans*, e evidenciaram alguns genes diferencialmente expressos, identificando diversas famílias de proteínas como as

quimiossensoriais e de ligação, transportadores ABC e proteínas do tipo heat shock (Traverso et al., 2022). Essa estratégia é ainda mais versátil, pois permite identificar quais os genes e famílias de proteínas podem estar associadas à exposição por um determinado agente químico ou inseticida, estresse físico, privação alimentar e até mesmo frente à infecção ou não por *T. cruzi*. Em perspectiva semelhante, e com o intuito de avaliar o efeito da domiciliação, e analisando as antenas (chemiosensory transcriptome) de *T. brasiliensis* oriundos dos ambientes silvestre e doméstico (dentro das casas e no peridomicílio) pesquisadores observaram contigs diferencialmente expressos entre os grupos de amostras analisadas, tais como as Odorant Binding Proteins (OBP), Chemosensory Proteins (CSP), Takeout proteins, dentre outras, sendo as CSP e cuticle proteins genes considerados bons candidatos para contribuir no processo de domiciliação desta espécie (Marchant, 2016). Apesar dessas informações, novas análises, de novos tecidos e órgãos dos triatomíneos, poderão complementar o conhecimento sobre o processo de domiciliação e fornecer novas informações para o desenvolvimento de ferramentas para o controle vetorial transmissão de *T. cruzi*. Sugerimos ainda a realização de novas pesquisas, em outras regiões da Bahia com histórico de colonização domiciliar, intra e peridomiciliar, por *T. sordida*, para confirmar o processo colonização domiciliar por essa espécie no Estado, reforçando o seu papel como vetor de *T. cruzi*. Tais informações são relevantes, pois possibilitam a tomada de decisões baseada em evidências, e de políticas regionais direcionadas às questões locais.

CONCLUSÃO

Descrevemos a colonização de casas por *T. sordida* na Bahia e observamos diferenças de tamanho entre os sexos, e das populações capturadas dentro e fora das casas aplicando morfometria geométrica de asas. Nós mostramos que os espécimes capturados nas casas são muito menores que os presentes do peridomicílio e que a diferença de tamanho entre sexos também diminui, principalmente devido a um menor tamanho das fêmeas de *T. sordida* nas

casas. Nossos resultados reforçam a ideia de analisar o dimorfismo sexual para indicar processos de adaptação ao ambiente domiciliar e a hipótese de que *T. sordida* obteve sucesso na colonização de casas nesse município. Sugerimos que a morfometria geométrica seja aplicada para auxiliar a detecção de populações intradomiciliares de *T. sordida* e dessa forma aprimorar a vigilância e controle vetorial da doença de Chagas. A capacidade de colonização do intradomicílio por *T. sordida* impõe desafios para o controle vetorial, pois ainda que as populações sejam susceptíveis aos métodos de tratamento químico convencional, há a possibilidade de reinfestação a partir de populações silvestres do vetor, sendo necessárias políticas regionais direcionadas às questões locais e de fomento a pesquisas relacionadas ao controle e monitoramento dos vetores de *T. cruzi*.

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HIGHLIGHTS

- Triatomines are sylvatic vectors, and some populations of them can colonize human domicile.
- During the house colonization phenotypical modifications occurs on triatomines.
- *Triatoma sordida* specimens from houses were short and sexual dimorphism was reduced.
- Geometric morphometrics of triatomine wings could be a useful tool to evaluate house colonization

AUTHOR CONTRIBUTIONS

Conceptualization: GRJr; RGG **Data curation:** GRJr; **Formal analysis;** RGG; **Funding acquisition;** MGR; **Investigation:** GRJr, JR, FV, ACOS, FCL; **Methodology:** GRJr, JR, FV, ACOS, FCL; **Project administration:** GRJr, MGR; **Resources;** MGR; **Software;** GRJr, RGG; **Supervision;** GRJr, RGG, MGR; **Validation;** GRJr, RGG, MGR; **Visualization;** GRJr, RGG, MGR; **Roles/Writing - original draft:** GRJr, JR, FV, DM, RGG; **Writing - review & editing:** GRJr, RGG, DM, MGR.

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TABELAS

Tabela 1 – Informações de colonização dos ambientes intra e peridomiciliar por *T. sordida* no município de Barra – BA, por localidade avaliada.

Locality	Houses				Peridomiciles				Total
	♀	♂	Nymph	Subtotal	♀	♂	Nymph	Subtotal	
Barro Vermelho	18	12	13	53 (14)					53
Lagoa do canto	26	45	34	105 (24)					105
Sambaíba	18	26	18	62 (19)					62
Boca do saco					8	8	5	21	21
Brejinho					14	10	20	44 (19)	44
Extrema					15	16	15	46 (11)	46
Quixaba					18	16	12	46 (33)	46
-9 (non informed)					14	6	13	33	33
Total	62	83	75	220 (57)	69	56	65	190 (63)	410

Legenda: Valores entre parênteses () representam as amostras selecionadas para as análises por morfometria geométrica.

FIGURAS

Figure 1 – Study area. **A.** municipal spatial distribution as weight “presence” of *Triatoma sordida* in Bahia. **B.** Google earth satellite image of the domiciliary units where triatomines were captured.

Legend: * shows the municipality of Barra and overall, the sampled area; the yellow markers show the sample locations of the *T. sordida* in 2013-2014, margin on the São Francisco River.

Figure 2 – **(A)** Exemplar adulto macho de *Triatoma sordida* e **(B)** Asa direita de um espécime dissecado demonstrando os sete pontos anatômicos aferidos. Foto: Exemplar de *Triatoma sordida* (GURGEL-GONÇALVES, 2011).

Figure 3 – Análise de tamanho das asas de *T. sordida* adultos machos e fêmeas coletados nas casas **(H)** e peridomicílio **(P)** de unidades domiciliares no município de Barra, Bahia.

Figure 4 – Análise de componente principal (PCA) da análise da forma das asas de *T. sordida* adultos coletados dentro e no peridomicílio de unidades domiciliares no município de Barra, Bahia.

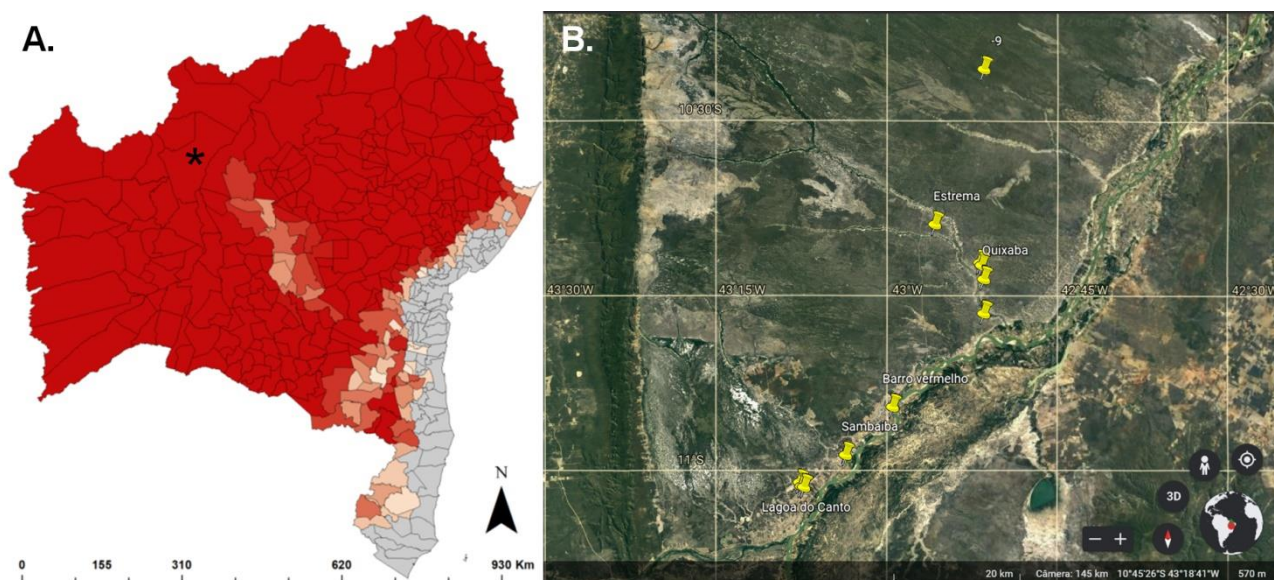


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Legend: (A) * shows the municipality of Barra and overall, the sampled area; (B) the yellow markers show the sample locations of the *T. sordida* in 2013-2014, margin on the São Francisco River.



Figure 2 – (A) Exemplar adulto macho de *Triatoma sordida* e (B) Asa direita de um espécime dissecado demonstrando os sete pontos anatômicos aferidos. Foto: Exemplar da asa de *Triatoma sordida* (GURGEL-GONÇALVES, 2011).

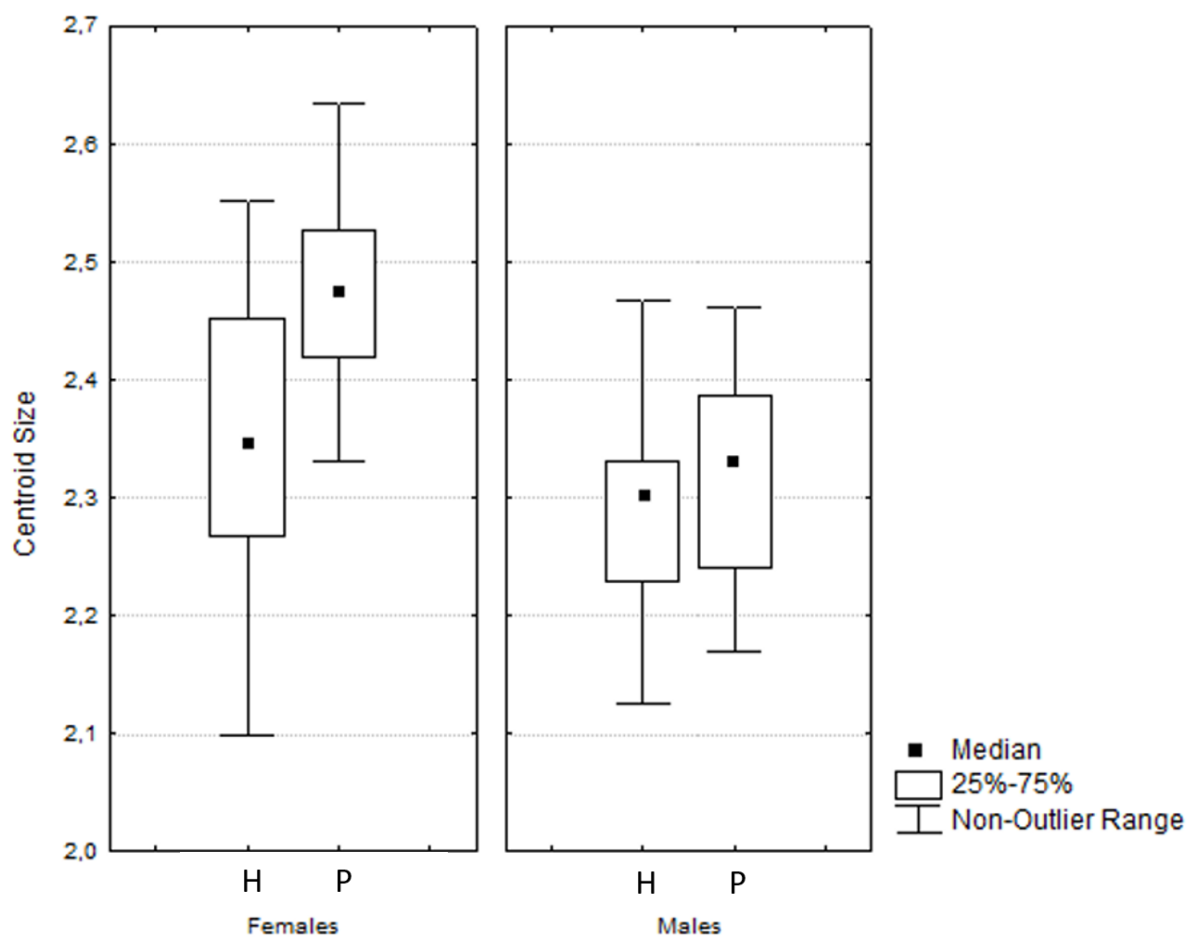


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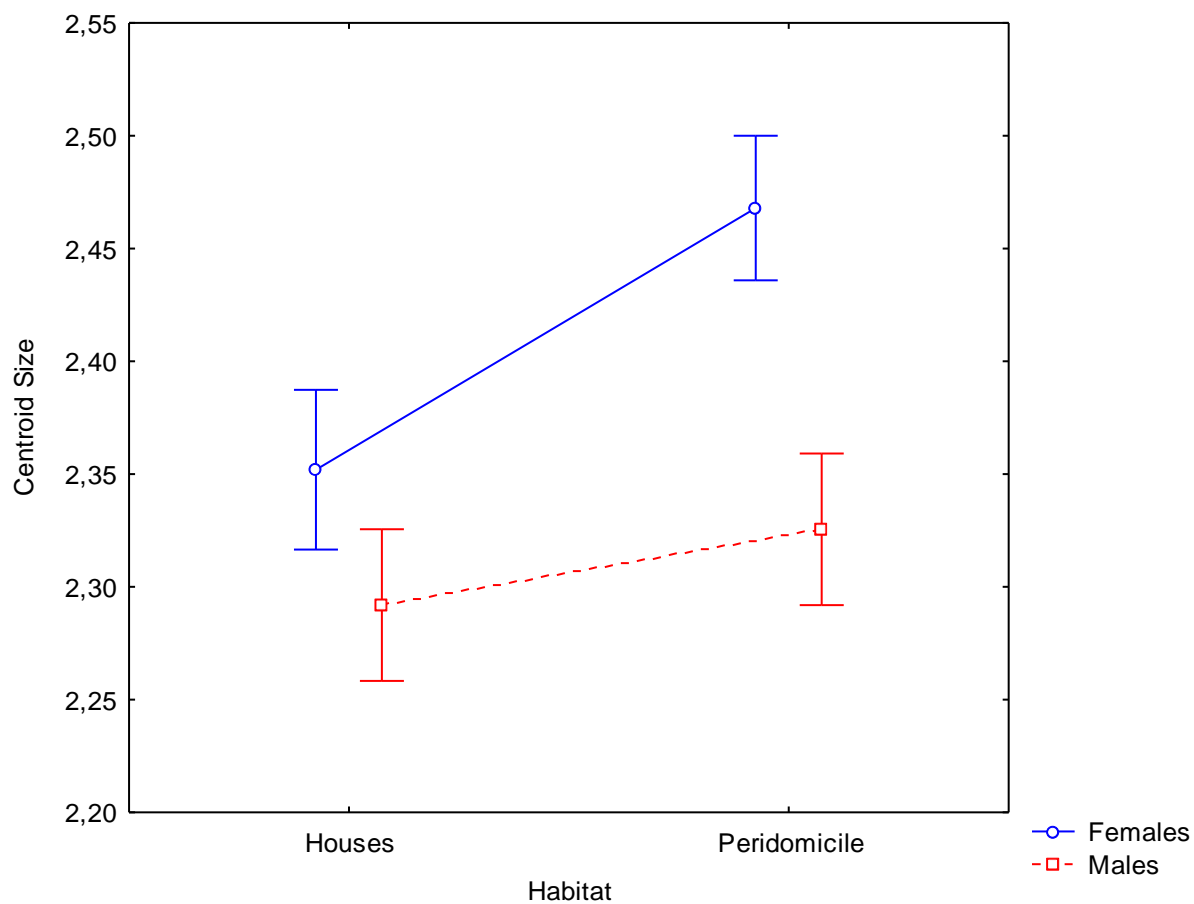


Figura 4. Resultado da Análise de Variação (ANOVA) fatorial do tamanho do centroide de machos e fêmeas de *Triatoma sordida* acordo com o habitat de coleta. Os dados mostram que o tamanho das asas é menor na população das casas e que a diferença de tamanho é maior para fêmeas, o que também leva a uma diminuição do dimorfismo sexual após a colonização das casas.

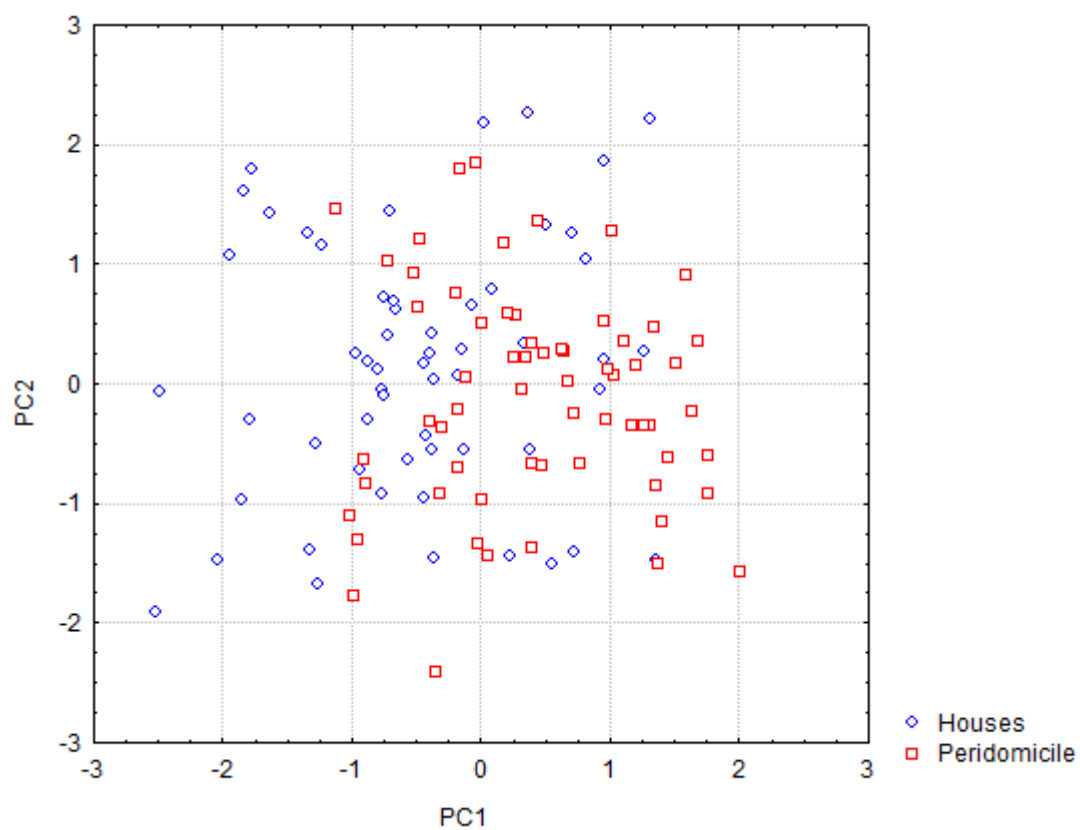


Figure 5 – Análise de componente principal (PCA) da análise da forma das asas de *T. sordida* adultos coletados dentro e no peridomicílio de unidades domiciliares no município de Barra, Bahia, Brasil.