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Triatoma costalimai Naturally Infected by *Trypanosoma cruzi*: A Public Health Concern

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Abstract. The rupestrian *Triatoma costalimai* species has been found infected by *Trypanosoma cruzi* in wild, peridomicile, and intradomicile environments in the municipality of Aurora do Tocantins, Tocantins, Brazil. Proximity between rock outcrops increases the risk of vector transmission of Chagas disease via this species. This work describes a focus of colonization by *T. costalimai* specimens infected by *T. cruzi* in rock outcrops located in an urban area in this municipality. Parasitological examination of feces from the collected specimens, axenic cultivation of *T. cruzi*–positive samples, and genetic characterization of the isolates were performed. Nymph and adult specimens were collected with a high infection prevalence (64.5%) for *T. cruzi* discrete type unit (DTU I). Participation of the *T. costalimai* species in the wild cycle of *T. cruzi* in rock outcrops located in an urban area demonstrates the need for entomological surveillance and control of vector transmission of Chagas disease in the municipality of Aurora do Tocantins, Tocantins.

The state of Tocantins presents the Amazonian biomes predominating in the north and northwest regions and in the Cerrado in the northeast, southwest, and southeast. A total of 16 species of triatomines were caught inside or around dwellings in this state, among these *Triatoma costalimai* Verano and Galvão, 1959, rarely found in the home. However, the prevalence of *Trypanosoma cruzi* infection in *T. costalimai* was 13.5%.[1] This specie was described from specimens collected in rocky outcrops in the municipality of Taguatinga, State of Tocantins, and has since been found in the regions of Cerrado Biome in the States of Goiás, Tocantins, and Bahia.[2] Housing conditions, enhanced by human impact on the environment, favor the domiciliation of triatomines,[3] and, combined with oral transmission, exacerbate the epidemiological profile of Chagas disease in the state of Tocantins. According to the Health Surveillance Secretary of the Brazilian Ministry of Health in the State of Tocantins, 23 cases of acute Chagas disease were confirmed for the period of 2000–2014, of which 19 were associated with oral transmission, two with vector transmission, and two with no information on the type of transmission.[4] In the period of 2010–2013, 59% of the deaths caused by Chagas disease in the northern region of Brazil occurred in the state of Tocantins, where the average number of reports was 54 deaths/year, southeast health regions with 25%, Capim Dourado with 23%, and Cerrado Tocantins Araguaia with 13% (Oliveira et al., Secretary of Health of the State of Tocantins (SESAU), Tocantins 2016, unpublished data). The Ministry of Health included the State of Tocantins in two surveillance models created for the Legal Amazon region, one aimed at preventing vector transmission and the other aimed at early detection of acute cases. In Tocantins, and particularly in Aurora do Tocantins, proximity between dwellings and rock outcrops increases the risk of vector transmission by *T. costalimai*. This municipality was classified in 2006 as being at high risk for vector transmission based on the following indicators: morbidity (acute or chronic autochthonous cases of the disease), entomological factors (vector species, infestation, and dispersion), and environmental factors (domicile and extra-domicile).[5] These, in turn, would be associated with anthropogenic effects on ecosystems, where this species naturally participates in the life cycle of *T. cruzi*. Studies have shown that there is an association between *T. cruzi* genotypes and the clinical forms of Chagas disease,[6–8] reinforcing the importance of knowing the discrete typing units (DTUs) circulating in endemic areas. Thus, the objective of this work was to perform the following: 1) describe a focus of infected *T. costalimai* in an urban area of the municipality of Aurora do Tocantins, 2) determine its prevalence of infection by *T. cruzi*, and 3) characterize the genotypes of *T. cruzi* in the isolates.

Captures were performed in the municipality of Aurora do Tocantins (12°42′32.06″S 46°24′21.24″W), State of Tocantins, Brazil, in a sylvatic environment of rock outcrops, with approximately 30 m of height, located 10 m far from the house, with the presence of annexes of the peridomicile where domestic animals are kept (dog, chicken, and pig) (Figure 1). Sixteen Noireau traps[9] were used for two nights in a period of 14 hours each one (IBAMA SISBIO License 43393-1 of 05/27/2014).

Taxonomic identification of adults was performed according to the morphological criteria described by Lent and Wygodzinsky[10] and a dichotomous key for species of the subcomplex Matogrossensis by Gonçalves et al.[11] Nymphs were kept in the laboratory to confirm their species classification as they reached adulthood.

The presence of trypanosomatids was investigated through the analysis of the intestinal contents using optical microscopy and the results were sent to the SESAU-Tocantins. Samples positive for epimastigote and metacyclic trypomastigote forms similar to *T. cruzi* and also negatives ones were

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FIGURE 1. General aspect of the studied environment: domicile, peridomestic, and wild environment in the background. This figure appears in color at www.ajtmh.org.

cultured at 28°C in biphasic axenic media of Neal, Mc Novy and Nicolle and LIT (liver infusion tryptose), containing 20% fetal bovine serum, penicillin, and fluorocytosine, for isolation of parasites. After 15 days, positive samples were cultured at 28°C for 20 days in LIT medium, with five times the volume of the isolation medium, to obtain the mass of the parasites. The cultures were washed in 0.01 M phosphate-buffered saline at pH 7.4, through centrifugations at 2,500 rpm, and the pellet containing the parasites was stored at –20°C until further use.

DNA extraction was performed using the phenol–chloroform method.[12] DNA quantification was performed using a Denovix spectophotometer DS-11 at a wavelength of 260 nm.

Genotyping, for the determination of DTUs of these samples, was performed through amplification and analysis of three gene regions: Subunit 2 of the mitochondrial cytochrome oxidase (COII) enzyme followed digested with the enzyme *Alu* I (Promega), D7 divergent domain of the 24S alpha rRNA gene, and intergenic region of the spliced leader (SL-IRac), according to Martins et al.[13] PCR and digestion products were analyzed on a 6% acrylamide gel stained with ethidium bromide. As controls, the strains Col1.7G2 (TcI), JG (TcII), RN19 (TcIII), AM64 (TcIV), 3253 (TcV), and CL-Brener (TcVI) were used.

A total of 53 specimens of *T. costalimai* (female = 8; male = 4; N_2 = 9; N_3 = 12; N_4 = 9; and N_5 = 11) were captured. Of these, adults and nymphal instar (N = 34, 64.15%) were infected by *T. cruzi* (Table 1). A total of 21 parasite samples were isolated in cultures in axenic medium, and molecular analyses confirmed the samples belonged to the species *T. cruzi* based on DTU TCI profile. It was not possible to isolate 13 samples probably because of an incompatibility of the sylvatic strains with culture medium or low infections rates.

The high *T. cruzi* infection prevalence in adults and nymphs of stages IV and V of *T. costalimai* suggests that this species is maintaining the wild cycle of the parasite in a peridomicile environment in the municipality of Aurora do Tocantins, constituting a risk of vector transmission of Chagas disease in this area. These results corroborate a study performed by Gonçalves et al. (unpublished data) in nine municipalities in Southeastern Tocantins, including Aurora do Tocantins, that reported the presence of *T. costalimai* with high infection prevalence in wild and peridomicile ecotypes but did not determine the circulating DTU. Likewise, Brito et al.[14] also observed that *T. costalimai* may contribute to the transmission of *T. cruzi* in the peridomicile and intradomicile environments, with high infection prevalence in triatomines adults, of municipalities of Southeastern Tocantins, including Aurora do Tocantins, after analyzing data obtained in the SESAU-Tocantins. The occurrence of this natural infection of *T. costalimai* by *T. cruzi* was initially evidenced by Mello and Borges[15] and Mello,[16] in Mambaí - Goiás State (GO), but with infection prevalences of 13.5% and 0.4%, respectively. More recently, also in Mambaí-GO, Machiner et al.[17] reported a higher occurrence of *T. costalimai* in rock outcrops located in the peridomicile, near hens and pigs, comparable to a wild environment, indicating the synanthropic potential of the species, without the presence of *T. cruzi* infection. In the present study, a similar environment was observed with the high infection prevalence, suggesting the transmission cycle of the parasite among the wild animals that inhabit the rock outcrops where the triatomines were captured. Notably, there are patients with Chagas disease in this area. Although the association of *T. costalimai* and rodents of *Kerodon rupestris* species have been described,[10] the association of these triatomines with this reservoir at the study site is not proven. In specimens captured in the North of Goiás, Lorosa et al.[18] observed that *T. costalimai* presented eclectic feeding habits (rodents, possums, lizards, horses, armadillos, and birds). The *T. cruzi* genotype TcI, identified in this work for the first time in *T. costalimai*, was also reported in a study with wild TCI isolates obtained from different reservoirs in the Cerrado Biome,[19] as well as from bats captured in Tocantins.[20] Jansen et al.[19] in 2015 observed a greater predominance of TcI isolates in wild reservoirs and stated that *Didelphis* spp. infected with TcI can maintain high parasitemia for a long period of time. The high prevalence of infection in nymphs and adults of *T. costalimai* by TcI shows the importance of constant entomological and epidemiological surveillance actions in the

TABLE 1

Prevalence of *Trypanosoma cruzi* infection in *Triatoma costalimai* collected in rock outcrops in Aurora do Tocantins, State of Tocantis, Brazil

Stage	Total	Infected (*N*/%)
N_2	9	1 (11.1%)
N_3	12	5 (41.6%)
N_4	9	7 (77.7%)
N_5	11	10 (90.9%)
Female	8	8 (100%)
Male	4	3 (75%)
Total	53	34 (64.15%)

N_2, N_3, N_4, N_5 = nymph of second, third, fourth and fifth stage, respectively.

municipality of Aurora do Tocantins because of the proximity of man to the wild cycle of the parasite.

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REFERENCES

1. Brito RN, Gorla DE, Diotaiuti L, Gomes ACF, Souza RCM, Abad-Franch F, 2017. Drivers of house invasion by sylvatic Chagas disease vectors in the Amazon-Cerrado transition: a multi-year, state wide assessment of municipality aggregated surveillance data. *PLoS Negl Trop Dis 11:* e0006035.
2. Gurgel-Gonçalves R, Galvão C, Costa J, Peterson AT, 2012. Geographic distribution of Chagas disease vectors in Brazil based on ecological Niche modeling. *J Trop Med 2012:* 1–15.
3. Vaz VC, D'Andrea PS, Jansen AM, 2007. Effects of habitat fragmentation on wild mammal infection by *Trypanosoma cruzi*. *Parasitology 134:* 1785–1793.
4. MS, 2015. Ministério da Saúde – Secretaria de Vigilância em Saúde. Boletim epidemiológico. Doença de Chagas aguda no Brasil: série histórica de 2000 a 2013. *46:* 1–9. Available at: http://portalsaude.saude.gov.br/images/pdf/2015/agosto/03/2014-020.pdf.
5. Oliveira IAS, Maia AAS, Dantas EC, 2008. Avaliação do controle de qualidade na identificação taxonômica e exame parasitológico de triatomíneos, indicadores de resultados discordantes e positividade, nos anos de 2004 a 2006. *Boletim Epidemiológico Superintendência de Vigilância e Proteção a Saúde do Tocantins 5:* 1–3.
6. Miles MA, Llewellyn MS, Lewis MD, Yeo M, Baleela R, Fitzpatrick S, Gaunt MW, Mauricio IL, 2009. The molecular epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*: looking back and to the future. *Parasitology 136:* 1509–1528.
7. Sturm NR, Campbell DA, 2010. Alternative lifestyles: the population structure of *Trypanosoma cruzi*. *Acta Trop 115:* 35–43.
8. Zingales B et al., 2012. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. *Infect Genet Evol 12:* 240–253.
9. Noireau F, Flores R, Vargas F, 1999. Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Trans R Soc Trop Med Hyg 93:* 13–14.
10. Lent H, Wygodzinsky P, 1979. Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas disease. *Bull Am Mus Nat Hist 163:* 127–520.
11. Gonçalves TC, Teves-Neves SC, Santos-Mallet JR, Carbajal de la Fuente AL, Lopes CM, 2013. *Triatoma jatai* sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae). *Mem Inst Oswaldo Cruz 108:* 429–437.
12. Sambrook J, Fritsch EF, Maniatis T, 1989. *Molecular Cloning: A Laboratory Manual*. New York, NY: Cold Spring Harbor Laboratory Press.
13. Martins K, Andrade CM, Barbosa-Silva AN, Nascimento GB, Chiari E, Galvão LMC, Câmara ACJ, 2015. *Trypanosoma cruzi* III causing the indeterminate form of Chagas disease in a semiarid region of Brazil. *Int J Infect Dis 39:* 68–75.
14. Brito RN, Diotaiute L, Gomes ACF, De Souza RCM, Abad-Franch F, 2017. *Triatoma costalimai* (Hemiptera: Reduviidae) in and around houses of Tocantins State, Brazil, 2005–2014. *J Med Entomol 54:* 1771–1774.
15. Mello DA, Borges MM, 1981. Initial discovery of *Triatoma costalimai* naturally infected with *Trypanosoma cruzi*: study of the biological aspects of an isolated sample [article in Portuguese]. *Mem Inst Oswaldo Cruz 76:* 61–69.
16. Mello DA, 1982. Roedores, marsupiais e triatomíneos silvestres capturados no município de Mabaí-Goiás. Infecção natural pelo *Trypanosoma cruzi*. *Rev Saúde Publ 16:* 282–291.
17. Machiner F, Cardoso RM, Castro C, Gurgel-Gonçalves R, 2012. Ocurrence of *Triatoma costalimai* (Hemiptera: Reduviidae) in diferrent environments and climatic seasons: a field study in the Brazilian savanna. *Rev Soc Bras Med Trop 45:* 567–571.
18. Lorosa ES, Andrade RE, Santos SM, Pereira CA, Vinhaes MC, Jurberg J, 1999. Estudo da infecção natural e fontes alimentares de *Triatoma costalimai* Verano & Galvão. 1959. *Rhodnius neglectus* Lent, 1954 e *Psammolestes tertius* Lent & Jurberg, 1965 do estado de Goiás, Brasil, através da técnica de precipitina. *Entomol Vectores 6:* 405–414.
19. Jansen AM, Xavier SCC, Roque ALR, 2015. The multiple and complex and changeable scenarios of the *Trypanosoma cruzi* transmission cycle in the sylvatic environment. *Acta Trop 151:* 1–15.
20. Lima L, Espinosa- Álvarez O, Ortiz PA, Trejo-Varon JA, Carranza JC, Pinto CM, Serrano MG, Buck GA, Camargo EP, Teixeira MM, 2015. Genetic diversity of *Trypanosoma cruzi* in bats, and multilocus phylogenetic and phylogeographical analyses supporting Tcbat as an independent DTU (discrete typing unit). *Acta Trop 151:* 166–177.