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Short Communication

Occurrence of triatomines in an urban residential complex in the municipality of Rio Branco, Acre, South-Western Amazon

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Abstract

Introduction: This study describes the occurrence of triatomines, and their positivity for trypanosomatids, in a residential complex in Rio Branco, Acre, Brazil. **Methods:** Triatomines were collected through direct capture in a home environment. Positivity analysis for trypanosomatids was performed by PCR assays. **Results**: Collected insects consisted of 31 *Rhodnius robustus*, 4 *Rhodnius montenegrensis*, and 1 *Panstrongylus geniculatus* specimens. All were adults, with no presence of domiciliation, and with an infection rate of 30.6%. **Conclusions**: Future studies are recommended in other locations of Rio Branco in order to develop a georeference database of the occurrence of triatomines in urban areas.

Keywords: Chagas disease. Vector control. Intradomiciliary. Triatominae.

American trypanosomiasis or Chagas disease is a public health problem in America. This disease is caused by *Trypanosoma cruzi*, a protozoan transmitted to humans through the feces or urine of insect vectors from the family Reduviidae, subfamily Triatominae[1]. The Triatominae subfamily comprises 154 species (151 living and 3 fossil species) distributed in 19 genera, from which *Triatoma*, *Panstrongylus*, and *Rhodnius* (with 74, 15, and 21 species, respectively) stand out due to their potential domiciliation and transmission[2-5].

In the Amazon region, domiciliation is not common, but the number of triatomine intrusion records is increasing among households in urban and peri-urban areas[6]. Therefore, this study attempted to describe the occurrence of triatomine species and their positivity for trypanosomatids in an urban residential complex in the municipality of Rio Branco, Acre, Brazil.

Triatomines were captured from November 2015 to October 2016 from direct catches carried out in an in-home environment at a private condominium (**Figure 1**) in Rio Branco (latitude 9° 58' 8.394" S, longitude 67° 48' 41.747" W). Insects were kept in cool boxes at room temperature (28 to 32 °C) and sent

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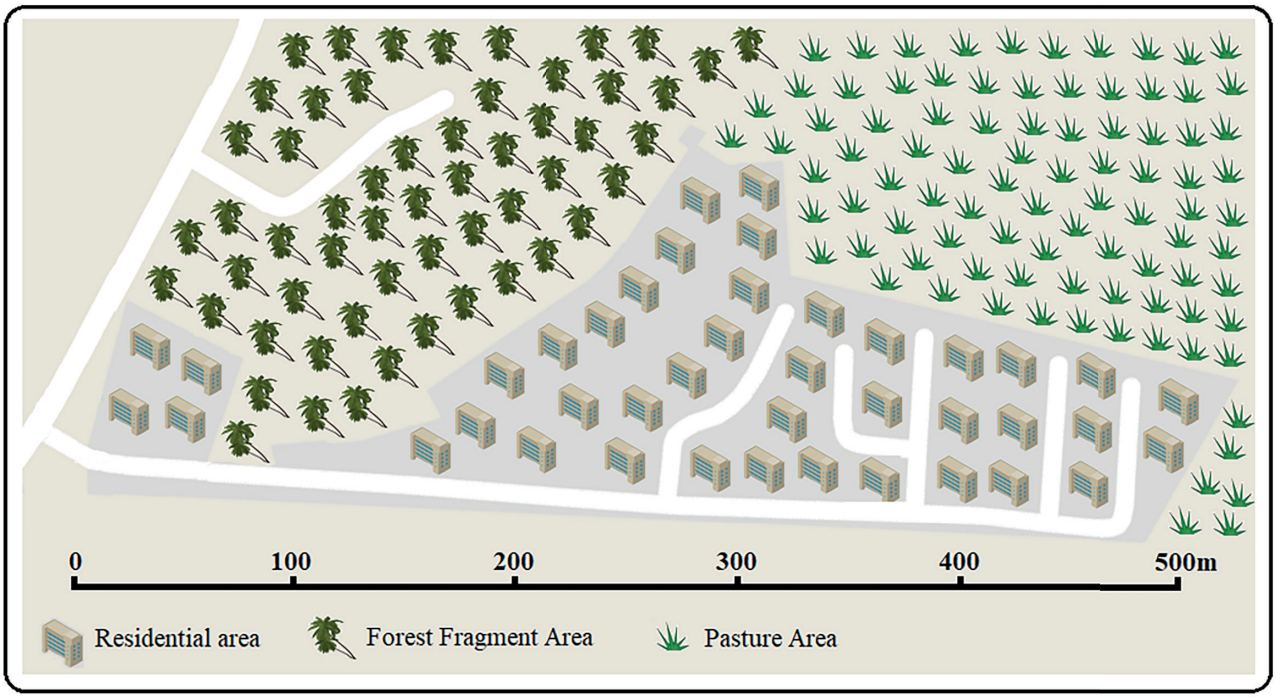

FIGURE 1: Characteristics of the residential condominium where this study was conducted, Rio Branco, Acre, Brazil, 2016.

to the Tropical Medicine Laboratory (Laboratório de Medicina Tropical - LABMEDT) of the Federal University of Acre (UFAC), where the identification of the species was done taking into account the morphological characteristics described by Lent & Wygodzinsky[7] and Rosa et al[8].

Analysis of triatomine infection by trypanosomatids was initially done by optical microscopy (1600×): part of the content of the rectal ampulla was analyzed on slides stained with a quick panoptic kit (0.1% triarylmethane, 0.1% xatenes, and tiazines at 0.1%). Then, a molecular analysis was performed. Briefly, DNA was extracted from triatomine rectal samples using a Qiagen DNA extraction kit. Identification and genotyping of the isolates were performed by PCR-multiplex assays based on the method described by Fernandes[9]. This method specifically amplifies a part of the non-transcribed spacer of the mini-exon gene, which varies with the *Trypanosoma* species (*T. cruzi* and *T. rangeli*) and with the *T. cruzi* lineage (TCI and TCII). Generated fragments ranged from 100 to 250 base pairs. The oligonucleotide primers used for PCR were: TCI (200 pb), 5′-ACACTTTCTGGCGCTGATCG-3′; TCII (250 pb), 5′-TTGCTCGCACACTCGGCTGCAT-3′; Z3 (150 pb), 5′-CCGCGCACAACCCCTATAAAAATG-3′; TR (100 pb), 5′-CCTATTGTGATCCCCATCTTCG-3′; and EXON, 5′-TACCAATATAGTACAGAACTG-3′.

Every reaction mix consisted of 100 pmol of each primer and 150 µM dNTPs in a swab with 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 25 mM KCl, 0.1 mg/mL of bovine albumin, and 2.5 U of TaqDNA polymerase. Approximately 10 ng of genomic DNA were added and Type I water was used to make up the reaction volume to 50 µL. The PCR cycling thermal program consisted of an initial step of 5 minutes at 95° C, followed by 34 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C, with a final extension time of 10 min at 72 °C. For each PCR reaction, the following reference strains were used as control treatments: TC1 X10 Clone 1, TC2 Strain Y, Z3 Esmeraldo Clone 1, and *T. rangeli* R1625. Amplified products underwent electrophoresis in a 2% agarose gel at 100 volts for 1 hour. After electrophoresis, DNA fragments were stained with ethidium bromide and visualized under ultraviolet light. A 50 bp DNA ladder was used to calculate the size of amplified fragments[10]. Results obtained both from the identification of the triatomines collected in the residential complex and their positivity for trypanosomatids are summarized in **Table 1**.

A similar study was conducted in Cochabamba, Bolivia, a region near the state of Acre, Brazil, where 21 triatomines from the species *Panstrongylus geniculatus* Latreille, 1811 and *Rhodnius robustus* Larrousse, 1927 were captured, 63% of which were infected by *T. cruzi*[11]. This percentage of infection is higher than that found in this study, showing the potential of these species as vectors for Chagas disease. Moreover, *R. robustus* and *P. geniculatus* were found invading residences in other locations of the Brazilian Amazon[6,12], but in this research no domiciliation evidence was found. In addition, *Rhodnius montenegrensis* Rosa et al., 2012 has also been found in houses in a rural area[13], and its natural infection by *T. rangeli*, which was observed in this study, has also been confirmed[10]. These results are worrying, since the occurrence of these parasites and the mixed infection by both *T. cruzi* and *T. rangeli* may hinder isolation and differential diagnosis, leading to possible misdiagnoses of Chagas disease[14].

TABLE 1: Collected species of triatomines and their positivity to trypanosomatid infection in a residential area from November 2015 to October 2016

Month/Year	Species	Quantity	Trypanosomatid positivity	*T. cruzi* positivity	*T. rangeli* positivity
11/2015	*R. robustus*	2	0	0	0
12/2015	*R. robustus*	2	1	1	0
01/2016	*R. robustus*	2	2	2	0
	R. montenegrensis	1	1	0	1
02/2016	*R. robustus*	1	1	1	0
	R. montenegrensis	1	1	0	1
03/2016	*R. robustus*	6	3	3	0
04/2016	*R. robustus*	11	2	2	0
	R. montenegrensis	1	0	0	0
05/2016	*R. robustus*	2	0	0	0
	R. montenegrensis	1	0	0	0
	P. geniculatus	1	0	0	0
06/2016	*R. robustus*	2	0	0	0
07/2016	*R. robustus*	2	0	0	0
08/2016	-	0	0	0	0
09/2016	-	0	0	0	0
10/2016	*R. robustus*	1	0	0	0
Total	**3**	**36**	**11 (30.6%)**	**9 (25%)**	**2 (5.6%)**

All the insects collected in the study were at the adult stage and showed no domiciliation. It is believed that the occurrence of these triatomines may be related to their attraction for lights of the apartments, which are close to a forest fragment and a pasture area that suffered deforestation for the construction of the residential complex (**Figure 1**).

Interestingly, in this forest fragment there are several species of palm trees, such as *Attalea* sp., *Euterpe oleracea*, and *Bactris gasipaes*. These species are already related to occurrences of triatomines[8,10,15]. This forest fragment also serves as a shelter for some species of small mammals, such as agoutis, monkeys, bats, gray four-eyed opossums, and opossums, that may be serving as reservoirs for *T. cruzi* and *T. rangeli* in this location, justifying the need for prophylaxis measures, since the mechanism for the transmission of the Chagas disease is completed when a reservoir, a vector, an etiological agent, and a host are all present in the same place.

It is worth highlighting that the presented data are from a single residential condominium, and future studies are recommended in other locations of Rio Branco, in order to develop a georeferenced database of the occurrence of triatomines in urban areas as well as the risk areas for vector-borne transmission of the Chagas disease, and from this, to develop vector control measures and surveillance for this disease.

Ethical considerations

The specimens were collected with permission from the Brazilian Institute of Environment and Renewable Natural Resources [*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA)], permanent license Nr. 52260-1.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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