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Short Communication

# Synanthropic triatomines as potential vectors of *Trypanosoma cruzi* in Central Brazil

***Thaís Tâmara Castro Minuzzi-Souza*[1], *Nadjar Nitz*[2], *César Augusto Cuba Cuba*[1], *Marcelo Santalucia*[3], *Monique Knox*[4], *Luciana Hagström*[2], *Camilla Bernardes Furtado*[1], *Tamires Emanuele Vital*[2], *Marcos Takashi Obara*[1], *Mariana Machado Hecht*[2] *and Rodrigo Gurgel-Gonçalves*[1]**

[1]. Laboratório de Parasitologia Médica e Biologia de Vetores, Faculdade de Medicina, Universidade de Brasília, Brasília, DF, Brasil.
[2]. Laboratório Interdisciplinar de Biociências, Faculdade de Medicina, Universidade de Brasília, Brasília, DF, Brasil.
[3]. Laboratório Central de Saúde Pública, Secretaria Estadual de Saúde de Goiás, Goiânia, GO, Brasil.
[4]. Diretoria de Vigilância Ambiental, Secretaria de Saúde do Distrito Federal, Brasília, DF, Brasil.

## Abstract

**Introduction:** Chagas disease surveillance requires current knowledge on synanthropic triatomines. We analyzed the occurrence and *Trypanosoma cruzi* infection rates of triatomine bugs in central Brazil, during 2012-2014. **Methods:** Triatomines were collected inside or around houses, and *T. cruzi* infection was determined by optical microscopy and conventional/quantitative polymerase chain reaction. **Results:** Of the 2706 triatomines collected, *Triatoma sordida* was the most frequent species in Goiás State, whereas *Panstrongylus megistus* predominated in the Federal District. Parasites identified were *T. cruzi*, *T. rangeli,* and *Blastocrithidia* sp. **Conclusions:** *P. megistus* and *T. sordida* sustained the risk of *T. cruzi* transmission to humans in central Brazil.

**Keywords:** Triatominae. Brazilian Central-West region. Trypanosomatids.

More than 150 triatomine species have so far been described; 68 of them occur in Brazil[1], and at least 27 triatomine species have been recorded in the Central-West region of Brazil[2]. The Brazilian Ministry of Health reported about 3,500 deaths caused by Chagas disease in Goiás between 2007 and 2011; most involved adults over 39 years (chronic cases). This Chagas disease-specific mortality rate (~11 deaths per 100,000 inhabitants per year) is much higher than the Brazilian average (~3.4)[3]. Furthermore, nine confirmed cases of acute Chagas disease were reported from 2006-2012, showing that transmission persists in Goiás[4]. Fourteen triatomine species occur in the state, among which *Triatoma sordida* is particularly common[2].

Vector-borne *Trypanosoma cruzi* transmission to humans has never been documented in the Federal District of Brazil, although infected triatomines invade, and at times colonize, houses and peridomestic structures. *Panstrongylus megistus*, *Triatoma pseudomaculata*, *Triatoma sordida, Rhodnius neglectus*, *Psammolestes tertius*, *Panstrongylus geniculatus*, and *Panstrongylus diasi* have all been recorded in the Federal District[5,6].

***Corresponding author:*** Dr. Rodrigo Gurgel-Gonçalves.
**e-mail:** rgurgel@unb.br
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Chagas disease surveillance requires current knowledge on the distribution and infection of synanthropic triatomines. These data are also crucial for the planning and evaluation of vector control interventions. Our aims were I) to analyze the occurrence of triatomine bugs in and around houses in Goiás State and the Federal District, Brazil, from 2012-2014, and II) to investigate *T. cruzi* infection rates in those bugs.

Triatomines were collected inside houses or in peridomestic structures during routine entomological surveillance between August 2012 and December 2014. They were sent to the University of Brasília [*Universidade de Brasília* (UnB)] from the Central Laboratory (LACEN) of Goiás State Health Department or from the Environmental Surveillance Agency (DIVAL) of the Federal District.

In Goiás, municipality technicians identified the bugs and checked them for *T. cruzi* infection using optical microscopy. All bugs and their Giemsa-stained microscope slides were submitted to local reference laboratories and state-level LACEN for a first and second quality checks, respectively. All bugs and slides received at the Goiás LACEN between 2013 and 2014 were sent to the Medical Parasitology and Vector Biology Laboratory (MPVBL) at the UnB.

In the Federal District, triatomines were collected through either community-based surveillance, whereby residents can notify about the presence of suspected insects in their homes, or through active searches by DIVAL staff in dwellings

where householders had found triatomines. Between 2012 and 2014, all the bugs arriving at the DIVAL were submitted directly to the MPVBL at UnB. Triatomine species were identified at the MPBVL using dichotomic keys[7] and categorized according to developmental stages (first to fifth instar nymphs and adults) and sex. The date, locality, and site of capture (indoors or in peridomestic structures) were also recorded.

Live triatomines submitted by the DIVAL were examined for infection at the MPVBL. *Trypanosoma cruzi* infection was investigated by optical microscopy in live bugs at the laboratory as described by Minuzzi-Souza et al.[8]. Bugs from LACEN in Goiás were brought dead to MPVBL, precluding fresh-slide examination. We re-examined (at 1,000× magnification) all the Giemsa-stained slides of the bugs. A specific parasitological diagnosis based on morphological criteria to differentiate *T. cruzi*, *Trypanosoma rangeli*, and *Blastocrithidia* sp. was performed[9].

We used quantitative real time polymerase chain reaction (qPCR) to detect *T. cruzi* deoxyribonucleic acid (DNA) in bugs for which no Giemsa-stained slides were available and to confirm parasitological diagnosis based on morphological criteria. DNA was extracted using Illustra Tissue and Cells Genomic (GE), the QIAamp DNA Mini Kit (Qiagen), or the Biopur Kit Extraction Mini Spin Plus following the manufacturers' instructions. To confirm the integrity of DNA in each sample, we PCR-amplified a 414-bp fragment of the mitochondrial cytochrome *b* gene[10]. The qPCR was performed with primers TCZ3 and TCZ4, which amplified a 168-bp region of the *T. cruzi* nuclear microsatellite repetitive region[11]. Reactions were run in a final volume of 20µl, with 1X SYBR® Green PCR Master Mix (Applied Biosystems), 0.2µM of each primer, and 2µl of DNA (10ng). All qPCRs were run in duplicate in a StepOnePlus Real Time PCR System (Applied Biosystems) as follows: 50°C for 2 min, 95°C for 10 min, plus 40 cycles of 95°C for 15s, 60°C for 45s, and 72°C for 10s. The results were analyzed with the StepOne 2.3 software (Applied Biosystems). Each run included positive and negative controls, as well as blank controls with no template DNA.

To identify the DNA of *T. rangeli*, we performed a qPCR with primers S36 and S67 antisense, modified after Sturm et al.[12] which amplify approximately 330-pb region of *T. rangeli* kinetoplast DNA minicircle (kDNA). Reactions were run in a final volume of 20µl, using SYBR® Green PCR Master Mix (Applied Biosystems, CA, USA) under the same described conditions of qPCR TCZ. The following amplification conditions were used 50°C for 2 min, 95°C for 10 min and 40 cycles at 95ºC for 15s, 57°C for 1 min, and 72°C for 10s.

A conventional PCR was used to identify *Blastocrithidia* by a *touch-down* PCR nuclear ribosomal deoxyribonucleic acid (rDNA-24sα) with primers D75 and D76 which amplified a 250 region of *Blastocrithidia* nuclear 24sα ribosomal DNA as described by Schijman et al.[13].

In total, 1,812 triatomines of eight species were collected from 95 of the 246 municipalities, in Goiás State. Most of these vectors (89.9%) were found around houses (**Table 1**). *Triatoma sordida* was the most frequently collected species, with records from 63 municipalities.

Of the 1,602 *T. sordida* collected, 74 (4.6%) were recorded as *T. cruzi* infected by Goiás State surveillance staff. However, re-examination of stained slides at the UnB and qPCR confirmed *T. cruzi* infection in only 48 *T. sordida* (3%) (**Table 1**). Trypanosomatids other than *T. cruzi* were detected in two of the slides; one was positive for *Blastocrithidia* sp. and another for *T. rangeli* – also confirmed by conventional PCR and qPCR respectively (**Table 1** and **Figure 1**). Eight *T. sordida* nymphs infected with *T. cruzi*, as confirmed by microscopy and qPCR, were caught indoors.

The second most frequently collected species in Goiás was *R. neglectus* (7% of all bugs), which was recorded in 36 municipalities. Twenty-nine (24.2%) of the 120 *R. neglectus* caught inside or around houses in Goiás were scored as infected with *T. cruzi* by surveillance staff. Most of the infected bugs were found indoors and 18 (15%) were confirmed as infected by microscopy and qPCR (**Table 1**). Some positive slides by surveillance staff were found to be negative at re-examination by UnB researchers and by qPCR. *Panstrongylus geniculatus*, *Panstrongylus diasi*, *Triatoma pseudomaculata*, *Triatoma williami*, and *Triatoma costalimai* were rarely recorded in Goiás (**Table 1**).

Five triatomine species (894 bugs in total) were recorded in 12 of the 30 administrative regions in the Federal District, most frequently in Vicente Pires and Park Way. The majority of these records corresponded to *P. megistus*, with 828 specimens overall, of those 53 were caught indoors (**Table 2**). Thirteen *P. megistus* (1.6%) were confirmed as infected with *T. cruzi* in stained slides and qPCR; four infected *P. megistus* were found inside houses. Moreover, 41 peridomiciliary specimens were infected by *Blastocrithidia* sp. (**Table 2**) in the same locality. The second most common species was *T. pseudomaculata*, but none of the 51 specimens collected (one found indoors) were infected. One *P. geniculatus* caught inside a house was *T. cruzi*-positive by both microscopy and qPCR. *P. diasi* and *R. neglectus* were also recorded in the Federal District (**Table 2**).

The results indicate that *T. sordida*, *P. megistus*, and *R. neglectus* play key roles in sustaining the risk of *T. cruzi* transmission to humans in Goiás State and the Federal District of Brazil. Over 1,800 triatomines were caught inside or around houses in 95 municipalities of Goiás State during the two years of surveillance. About ten years earlier, between 2000 and 2003, intensive entomological surveys recorded 51,570 bugs in Goiás[14]. Although these data might be suggestive of a reduction in triatomine bug presence, the two studies differed in the methods used to ascertain infestation and in dwelling coverage. Oliveira and Silva[14] used data from active bug searches in nearly 250,000 dwellings of 201 municipalities. Our data, in contrast, were generated by a 'passive' surveillance system based on the notification of suspected insects by residents, so that municipality health agents only searched for bugs in houses whose owners report triatomines. These data suggest that synanthropic triatomines are probably much more common in Goiás than the results we report here indicate. *Triatoma sordida* was the species most frequently caught in Goiás, with most records corresponding to peridomestic environments as

**TABLE 1:** Triatomine bugs collected by routine surveillance in Goiás State, Brazil, 2013-2014: bug characteristics, collection sites, and natural infection with *Trypanosoma cruzi*.

| Species | Indoors | | | | | | | Peridomestic | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | female | male | ND | nymph | total | II (%)[a] | II (%)[b] | female | male | ND | nymph | total | II (%)[a] | II (%)[b] |
| *Triatoma sordida* | 86 | 44 | 0 | 43 | 173 | 15 (8.7) | 11 (6.4)[c] | 441 | 265 | 5 | 718 | 1,429 | 59 (4.1) | 37 (2.6)[d,e] |
| *Triatoma williami* | 3 | 2 | 3 | 3 | 11 | 0 (0.0) | 0 (0.0) | 0 | 0 | 0 | 0 | 0 | 0 (0.0) | 0 (0.0 |
| *Triatoma pseudomaculata* | 2 | 2 | 0 | 0 | 4 | 1 (25.0) | 0 (0.0)[e] | 1 | 0 | 0 | 0 | 1 | 0 (0.0) | 0 (0.0) |
| *Triatoma costalimai* | 0 | 3 | 0 | 0 | 3 | 1 (33.3) | 0 (0.0)[e] | 2 | 0 | 0 | 1 | 3 | 0 (0.0) | 0 (0.0) |
| *Panstrongylus geniculatus* | 1 | 12 | 0 | 0 | 13 | 1 (7.7) | 0 (0.0)[e] | 5 | 1 | 0 | 1 | 7 | 0 (0.0) | 0 (0.0) |
| *Panstrongylus megistus* | 4 | 4 | 0 | 2 | 10 | 3 (30.0) | 1 (10.0)[f] | 17 | 3 | 0 | 13 | 33 | 7 (21.2) | 1 (3.0)[g] |
| *Panstrongylus diasi* | 3 | 1 | 0 | 0 | 4 | 0 (0.0) | 0 (0.0) | 0 | 1 | 0 | 0 | 1 | 0 (0.0) | 0 (0.0) |
| *Rhodnius neglectus* | 68 | 20 | 2 | 2 | 92 | 25 (27.2) | 15 (16.3)[h] | 21 | 7 | 0 | 0 | 28 | 4 (14.3) | 3 (10.7)[i] |
| **Total** | **167** | **88** | **5** | **50** | **310** | **46 (14.8)** | **27 (8.7)** | **487** | **277** | **5** | **733** | **1,502** | **70 (4.7)** | **41 (2.7)** |

**ND:** sex not determined; **II:** infection index (number and % of bugs scored as positive for *T. cruzi*); **UnB:** *Universidade de Brasília;* **qPCR**: quantitative polymerase chain reaction; ***T. cruzi:*** *Trypanosoma cruzi*; ***T. rangeli****: Trypanosoma rangeli.* [a]Giemsa-stained slides examined by Goiás State surveillance staff. [b]Giemsa-stained slides examined by UnB researchers; some positives were confirmed by qPCR. [c]Two slides were scored as negative when re-examined at the UnB, and two slides were found positive for, respectively, *Blastocrithidia* sp. and *T. rangeli* (qPCR-confirmed). [d]Twenty bugs were positive by qPCR, and in 17, re-examinations of slides at the UnB revealed infection. [e]The slides were scored as negative when re-examined at the UnB; qPCR was also negative. [f]Two slides were scored as negative when re-examined at the UnB; both qPCRs were also negative. [g]Six slides were scored as negative when re-examined at the UnB; all six qPCRs were also negative. [h]Two bugs were found positive by qPCR; six slides re-examined at UnB were negative, and the corresponding qPCRs were also negative. [i]Three bugs were found positive by qPCR; one slide re-examined at UnB was negative, and the corresponding qPCR was also negative.

previously described[15]. Our data show that 6.4% of *T. sordida* caught indoors were infected –about 10 times larger than the infection index reported by Oliveira and Silva[14] or Rossi et al.[15].

Over two years, 894 triatomines, 14 of which were infected with *T. cruzi*, were captured in or around houses of 12 administrative regions of the Federal District. In 2002-2010, and using methods comparable to ours, 754 bugs were caught in 20 administrative regions[6]. This suggests that sylvatic foci of synanthropic triatomines persisted in the Federal District, with the bugs (especially *P. megistus*) regularly invading and at times colonizing human habitats. Continuous entomological surveillance should therefore be maintained to detect and quickly eliminate domestic and peridomestic infestation foci.

In our dataset, the two administrative regions with the most reported triatomines in the Federal District were Vicente Pires and Park Way, whereas Maeda et al.[6] reported most bugs from the more rural Planaltina and Sobradinho. This suggests that the number of triatomines, particularly *P. megistus*, found in urbanized environments may have increased over the last few years. In Vicente Pires and Park Way, middle-upper class condominiums are often built along gallery forests likely inhabited by wild *P. megistus*. House invasion and colonization by *P. megistus* must hence be closely monitored in areas with gallery forest remnants – and particularly over the early rainy season, when the records of the species in human environments peak[6]. Autochthonous *T. cruzi* transmission mediated by *P. megistus* was recently reported in primate units at the Brasília zoo[8]. In spite of the presence of *T. cruzi*-infected triatomines inside houses, no autochthonous cases of human Chagas disease have so far been reported from the Federal District.

The detection of *T. cruzi* infection in triatomine bugs has traditionally relied on the examination of vector feces through optical microscopy[9]. This inexpensive technique has, however, several important drawbacks. For example, detection may depend on whether the bug is alive or dead, technician microscopy skills and, especially, the quality of slide staining may affect the specificity of the test – possibly leading to confusion between *T. cruzi* and the morphologically similar *T. rangeli* or *Blastocrithidia triatomae*[4]. In this study, all stained

**TABLE 2:** Triatomine bugs collected by routine surveillance in the Federal District, Brazil, 2012-2014: bug characteristics, collection sites, and natural infection with *Trypanosoma cruzi*.

| Species | Indoors | | | | | | Peridomestic | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | female | male | ND | nymph | total | II (%)[a] | female | male | ND | nymph | total | II (%)[a] |
| *Panstrongylus megistus* | 14 | 7 | 0 | 32 | 53 | 4 (7.5) | 165 | 144 | 2 | 464 | 775 | 9 (1.2)[b] |
| *Panstrongylus geniculatus* | 3 | 4 | 0 | 0 | 7 | 1 (14.3) | 0 | 1 | 0 | 0 | 1 | 0 (0.0) |
| *Panstrongylus diasi* | 1 | 1 | 0 | 0 | 2 | 0 (0.0) | 0 | 0 | 0 | 0 | 0 | 0 (0.0) |
| *Triatoma pseudomaculata* | 8 | 7 | 0 | 35 | 50 | 0 (0.0) | 1 | 0 | 0 | 0 | 1 | 0 (0.0) |
| *Rhodnius neglectus* | 0 | 4 | 1 | 0 | 5 | 0 (0.0) | 0 | 0 | 0 | 0 | 0 | 0 (0.0) |
| **Total** | **26** | **23** | **1** | **67** | **117** | **5 (4.0)** | **166** | **145** | **2** | **464** | **777** | **9 (2.2)** |

**ND:** sex not determined; **II:** infection index (number and % of bugs scored as positive for *T. cruzi*); ***T. cruzi:*** *Trypanosoma cruzi*; **UnB:** *Universidade de Brasília;* **PCR**: polymerase chain reaction. [a]Giemsa-stained slides examined at the UnB; positive results were confirmed by quantitative PCR. [b]Forty-one triatomines were infected by *Blastocrithidia* sp.

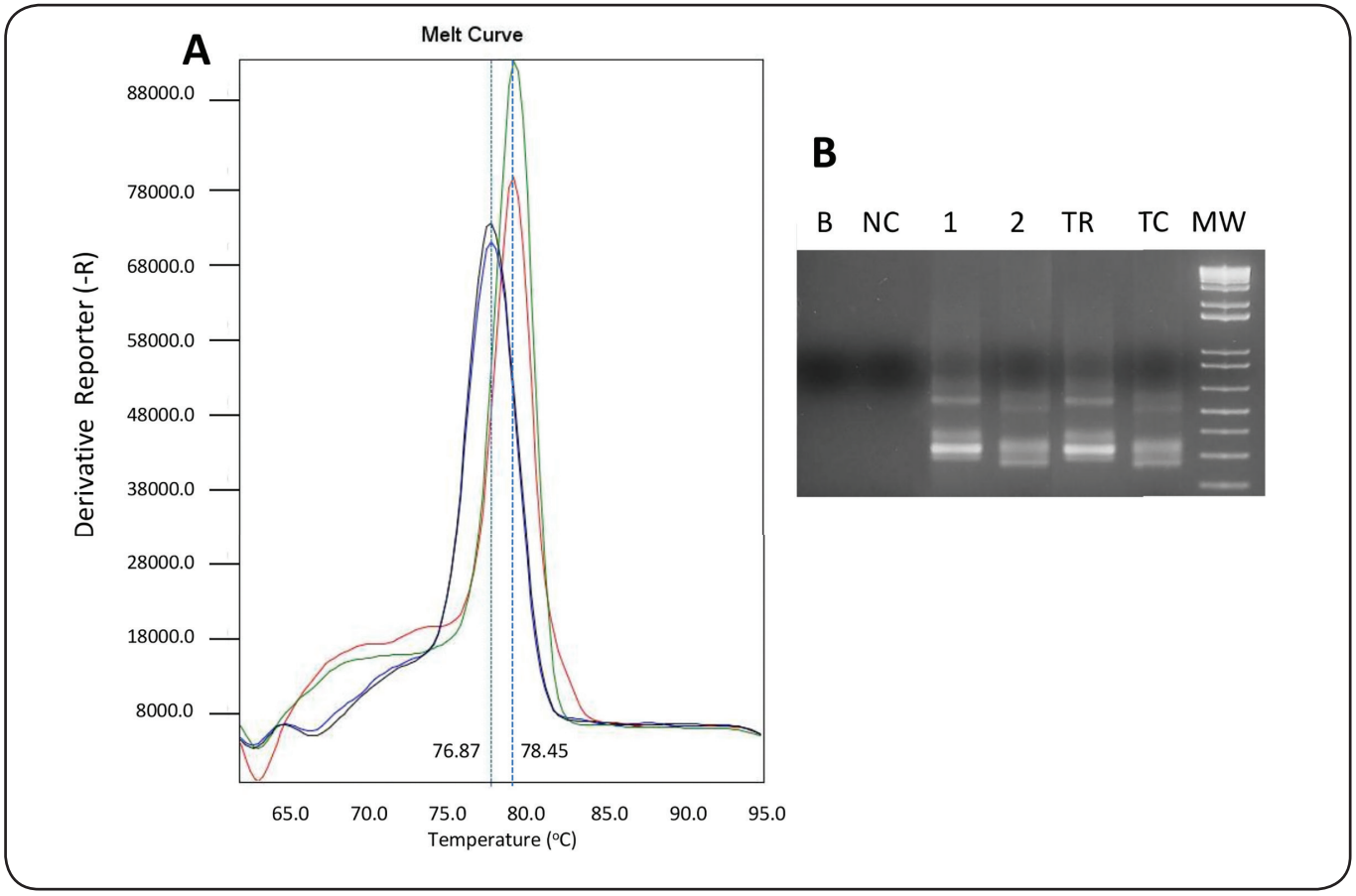


**FIGURE 1 -** Quantitative PCR of kinetoplastid DNA (kDNA qPCR), which amplifies a region of the kDNA minicircle of *Trypanosoma cruzi* and *Trypanosoma rangeli*. **A)** black color 'melt curve' of the *T. cruzi* positive control (Berenice strain); blue color 'melt curve' of a triatomine bug sample (*Panstrongylus megistus*) positive for *T. cruzi*; green color 'melt curve' of the *T. rangeli* positive control (SC-58 strain); red color 'melt curve' of a *Triatoma sordida* sample positive for *T. rangeli*. Dash lines represent the melt temperature of the positive samples of *T. cruzi* and *T. rangeli*. **B**) Agarose gel 1.3% of kDNA qPCR in triatomine samples. B: blank; **NC:** negative control; **1:** *Triatoma sordida* sample positive for *T. rangeli*; **2:** *Panstrongylus megistus* sample positive for *T. cruzi*; **TR:** *T. rangeli* culture (SC-58 strain) and **TC:** *T. cruzi* culture (Berenice´s strain); **MW:** molecular weight; **kDNA qPCR:** kinetoplast deoxyribonucleic acid quantitative polymerase chain reaction; ***T. cruzi:*** *Trypanosoma cruzi*; ***T. rangeli****: Trypanosoma rangeli*.

slides scored as positive by surveillance staff were specifically reported as '*T. cruzi*-positive'. When re-examined at the UnB a few of those slides were scored as positive for *T. rangeli* or *Blastocrithidia* sp., but negative for *T. cruzi* – or simply as negative for any trypanosomatid. This was further confirmed by *T. cruzi*-negative qPCR assays.

Our results show that synanthropic triatomines maintain the risk of *T. cruzi* transmission to humans in Goiás and the Federal District of Brazil. Furthermore, our parasitological examination data highlight the need for continuous training of surveillance staff in charge of *T. cruzi* detection in vectors. We therefore recommend that Chagas disease vector surveillance be strengthened across the Central-West region of Brazil.

The development of health education strategies for Chagas disease prevention should emphasize community-based surveillance to enhance the system's capacity to detect infestation foci. Moreover, triatomine bug surveillance should become an integral part of epidemiological surveillance. Thus, for example, disease surveillance staff could visit dwellings where entomological surveillance detected *T. cruzi*-positive vectors to investigate infection in dwellers and possibly their domestic mammals. Such an integrated strategy would provide public health officials and researchers with a more accurate understanding of how *T. cruzi* circulates in human environments.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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