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# *Trypanosoma cruzi* strains from triatomine collected in Bahia and Rio Grande do Sul, Brazil

## *Trypanosoma cruzi* isolado de triatomíneos coletados na Bahia e Rio Grande do Sul

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### ABSTRACT

**OBJECTIVE:** Collection of triatomines in domestic, peridomestic and sylvatic environments in states of Bahia and Rio Grande do Sul, Northeastern and Southern Brazil respectively, and isolation of *Trypanosoma cruzi* strains.

**METHODS:** First, the captured triatomines were identified using insect identification keys, then their intestinal content was examined by abdominal compression, and the samples containing trypanosomatid forms were inoculated in LIT medium and *Swiss* mice.

**RESULTS:** Six triatomine species were collected in cities in Bahia, namely *Panstrongylus geniculatus* (01), *Triatoma melanocephala* (11), *T. lenti* (94), *T. pseudomaculata* (02), *T. sherlocki* (26) and *T. sordida* (460), and two in cities in Rio Grande do Sul, namely *T. circummaculata* (11) and *T. rubrovaria* (115). Out of the specimens examined, *T. cruzi* was isolated from 28 triatomine divided into four different species: *T. melanocephala* (one), *T. lenti* (one), *T. rubrovaria* (16) and *T. sordida* (10). Their index of natural infection by *T. cruzi* was 6.4%.

**CONCLUSIONS:** The isolation of *T. cruzi* strains from triatomines found in domestic and peridomestic areas shows the potential risk of transmission of Chagas disease in the studied cities. The maintenance of those *T. cruzi* strains in laboratory is intended to promote studies that facilitate the understanding of the parasite-vector-host relationship.

**DESCRIPTORS:** Chagas Disease, transmission. Triatominae, *Trypanosoma cruzi*, isolation. Communicable Diseases, epidemiology.

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## RESUMO

**OBJETIVO:** Isolar cepas de *Trypanosoma cruzi* em triatomíneos capturados nos ambientes domiciliar, peridomiciliar e silvestre da Bahia e do Rio Grande do Sul.

**MÉTODOS:** Os triatomíneos capturados nos estados da Bahia e Rio Grande do Sul foram identificados por meio de chaves entomológicas. O conteúdo intestinal foi examinado por compressão abdominal e as amostras que continham formas de *Trypanosomatidae* foram inoculadas em meio de cultura *Liver Infusion Tryptose* e em camundongos Swiss.

**RESULTADOS:** Foram identificadas seis espécies de triatomíneos nas coletas realizadas em municípios do Estado da Bahia: *Panstrongylus geniculatus* (01), *Triatoma melanocephala* (11), *T. lenti* (94), *T. pseudomaculata* (02), *T. sherlocki* (26) e *T. sordida* (460), e duas no Estado do Rio Grande do Sul: *T. circummaculata* (11) e *T. rubrovaria* (115). Dos exemplares examinados, *T. cruzi* foi isolado de 28 triatomíneos pertencentes a quatro espécies: *T. melanocephala* (uma), *T. lenti* (uma), *T. rubrovaria* (16) e *T. sordida* (10). O índice de infecção natural de triatomíneos por *T. cruzi* foi de 6,4%.

**CONCLUSÕES:** O isolamento de cepas de *T. cruzi* em triatomíneos encontrados no intra e peridomicílio reflete o potencial risco de transmissão da doença de Chagas nos municípios estudados.

**DESCRITORES:** Doença de Chagas, transmissão. Triatominae, *Trypanosoma cruzi*, isolamento. Doenças Transmissíveis, epidemiologia.

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## INTRODUCTION

Chagas disease is estimated to affect around 10 million people worldwide and 25 million people in Latin America are at risk of infection.<sup>7</sup>

In relation to the epidemiology of Chagas disease in Brazil, in 1996 the Chagas Disease Control Program, of the National Foundation for Health/Brazilian Ministry of Health (FNS/MS), collected 290,576 triatomines, of which 201,156 (69.2%) were captured in the Northeast region, where more attention and care are required in view of the presence of sylvatic species that are potentially invasive and difficult to control.<sup>8</sup>

In 1975, the Superintendency for Public Health Campaigns (SUCAM) implemented the Chagas Disease Control Program in the state of Rio Grande do Sul, Southern Brazil. This program was responsible for methodological standardization, supply regulation and rationalization of the activities that provided a basis for vector control.<sup>4</sup> Almeida et al<sup>1</sup> (2009) reported the presence of *Triatoma rubrovaria* naturally infected by *T. cruzi* in domestic and peridomestic areas of Rio Grande do Sul, where Rosa et al<sup>18</sup> (2000) collected both *T. rubrovaria* and *Triatoma circummaculata* also naturally infected by the parasite. Pena et al<sup>16</sup> (2011) isolated *T. cruzi* strains that came from patients, reservoirs and vectors during an outbreak of acute Chagas disease in the city of Navegantes, state of

Santa Catarina, Southern Brazil, which shows the persistence of transmission of the disease in that region.

Silveira & Pinto Dias<sup>20</sup> (2011) reported the control activities exercised on a regular basis in Brazil, using systematic methodology and epidemiological, entomological and serological surveys. These actions led to depletion of the main vector species, *Triatoma infestans*, allochthonous and domestic only, and control of household colonization species that are important in transmission. Transmission is now residual in native species such as *Triatoma brasiliensis* and *Triatoma pseudomaculata*. There is a risk of domestication of species, previously considered of wild behavior, such as *Panstrongylus lutzi* and *T. rubrovaria*, beyond the possibility of human infections, directly related to the zoonotic transmission cycle. Therefore, it is still essential to maintain strict entomological surveillance.<sup>20</sup>

The ecological history of the distribution of the several strains of *T. cruzi* is marked by important changes starting from the migration of mammals in the Americas, habitat expansion, and destruction and urbanization of the environment by humans.<sup>22</sup>

Collecting species, studying geographic distribution and infection of triatomines by *T. cruzi* can contribute to the reporting of bugs and surveillance measures.

This study aimed to isolate *Trypanosoma cruzi* strains from triatomines in domestic, peridomestic and sylvatic environments in states of Bahia and Rio Grande do Sul, Brazil.

## METHODS

In Rio Grande do Sul, triatomines were collected in sylvatic environments in fourteen sites in six cities (Figure) in the period between 2002 and 2003. In the state of Bahia, triatomines were captured in sylvatic, peridomestic and domestic areas in nine sites of five cities in 2003, 2006, 2008 and 2009 (Table 1). The Bahia and Rio Grande do Sul State Departments of Health cooperated in the collection activities. The cities were chosen according to the report against triatomine by the health departments of Bahia and Rio Grande do Sul.

Environments of domestic units closed by doors, i.e., houses and annexes, were considered domestic ecotopes.

Triatomines were found sheltering in cracks in those environments, under beds or behind objects placed along the walls. The studied peridomestic ecotopes were in a radius of 50 m from the houses, where domestic animals sleep or are bred. In the active researches, triatomines were collected manually with tweezers and placed in individual flasks labeled with information on the place of collection.<sup>12</sup>

The captured triatomines were taken to the Triatominae Insectarium of the *Faculdade de Ciências Farmacêuticas* of the *Universidade Estadual Paulista "Júlio de Mesquita Filho"* (FCF/UNESP) at Araraquara, SP, Southeastern Brazil, where they were identified according to morphological characters present in insect identification keys<sup>13</sup> and kept under controlled temperature and humidity ( $27\pm 2^{\circ}\text{C}$  and  $60\pm 3\%$  RH). This species are maintained in the colony with controlled conditions in the Laboratory of Parasitology. The capture was conducted by a group containing guides, researchers, and department of health employees and lasted



**Figure.** Cities in Bahia and Rio Grande do Sul where triatomines were collected, 2002-2009.

**Table 1.** Locality, collected species, year and environment of collection. Brazil, 2002-2009.

State	Locality	Triatominae	Year	Ecotope
Rio Grande do Sul	Sao Sepé	<i>T. rubrovaria</i>	2002	S
	Caçapava do Sul	<i>T. rubrovaria/T. circummaculata</i>	2002	S
	Lavras	<i>T. rubrovaria/T. circummaculata</i>	2002	S
	Alegrete	<i>T. rubrovaria</i>	2002	S
	Quaraí	<i>T. rubrovaria</i>	2002	S
Bahia	Sta. Livramento	<i>T. rubrovaria</i>	2003	S
	Santo Inácio	<i>T. sordida/T. sherlocki</i>	2003	P/S
	Santo Inácio	<i>T. sordida/T. sherlocki/T. pseudomaculata</i>	2006	P/D
	Macaúbas	<i>T. lenti/T. sordida/T. pseudomaculata</i>	2008	P/D
	Macaúbas	<i>T. lenti/T. sordida/T. pseudomaculata/P. geniculatus</i>	2009	P/D
	Poçoões/Jequié/Bom Jesus da Serra	<i>T. melanocephala</i>	2009	P

S: Sylvatic; P: Peridomestic; D: Domestic

around ten days. The study took place during the day, but there were nocturnal collections that stayed in the environment overnight and used white cloth to attract the insects. Triatominae samples were sought in trees, stones and their attachments.

Natural infection of triatomines by trypanosomatid forms was analyzed in feces obtained by abdominal compression. The natural infection index was obtained from the ratio between the number of infected triatomines and the number of examined triatomines.<sup>12</sup>

In order to isolate and confirm the parasite, samples tested positive for trypanosomatids were diluted with 0.9% saline and examined between slide and coverslip under optical microscopy. Then 0.3 mL was intraperitoneally injected into Swiss mice aged 23 and 35 days, and another 0.3 mL was inoculated in Liver Infusion Tryptose medium. Around 15 days following the inoculation, the peripheral blood of the mice was checked for flagellate forms and the regular growth of the parasite in LIT medium was verified.<sup>17</sup> The 28 strains of *T. cruzi* are

**Table 2.** Natural ecotopes investigated in cities in the states of Bahia and Rio Grande do Sul, indicating Triatominae foci, 2002-2009.

Species	Locality	Triatominae examined	Positive	%
<i>T. circummaculata</i>	Caçapava do Sul, RS	8	0	0
<i>T. circummaculata</i>	Lavras, RS	3	0	0
<i>T. sherlocki</i>	Santo Inácio, BA	26	0	0
<i>T. pseudomaculata</i>	Santo Inácio, BA	1	0	0
<i>T. pseudomaculata</i>	Macaúbas, BA	1	0	0
<i>P. geniculatus</i>	Macaúbas, BA	1	0	0
<i>T. sordida</i>	Santo Inácio, BA	1	0	0
<i>T. sordida</i>	Macaúbas, BA	324	0	0
<i>T. rubrovaria</i>	Lavras, RS	36	0	0
<i>T. rubrovaria</i>	Quaraí, RS	53	2	3.7
<i>T. lenti</i>	Macaúbas, BA	90	4	4.4
<i>T. sordida</i>	Macaúbas, BA	100	6	6.1
<i>T. melanocephala</i>	Poçoões, Jequié, Bom Jesus da Serra, BA	11	1	9.0
<i>T. sordida</i>	Santo Inácio, BA	35	12	34.0
<i>T. rubrovaria</i>	Santana do Livramento, RS	5	3	60.0
<i>T. rubrovaria</i>	Quaraí, RS	21	15	71.0
<i>T. lenti</i>	Macaúbas, BA	4	3	75.0
Total	–	720	46	6.4

in study by molecular techniques and biological assays such as cellular infection, growth kinetics in LIT medium and parasitemia curve in BALB/c mice. In the present work these data were not included because some trials are ongoing in the laboratory and will be published later.

All the procedures were reported to and approved by the Ethical Committee for Animal Experimentation of the *Universidade Estadual Paulista "Júlio de Mesquita Filho"* – Araraquara (Protocol 13/2012).

## RESULTS

In Rio Grande do Sul, two triatomine species were collected: *Triatoma circummaculata*, which tested negative for *T. cruzi* in all 11 samples taken, and *Triatoma rubrovaria*, with 20 specimens (of the 115) testing positive for *T. cruzi* (Table 2, Figure).

In Bahia, six species were collected: *Panstrongylus geniculatus*, *Triatoma melanocephala*, *Triatoma lenti*, *Triatoma pseudomaculata*, *Triatoma sherlocki* and *Triatoma sordida*, summing up 594 captured specimens, 4.4% of them naturally infected (Table 2, Figure).

From the examined specimens (720) a total of 28 *T. cruzi* strains were isolated: one from the vector *T. melanocephala*, one from *T. lenti*, 16 from *T. rubrovaria*, and 10 from *T. sordida* with the SIGR<sub>3</sub> strain was isolated in 2006 by cat xenodiagnosis in a peridomestic area of Santo Inácio, Bahia.<sup>17</sup> (Table 3).

*Triatoma melanocephala* specimens were collected in the cities of Jequié, Poções and Bom Jesus da Serra, BA, and only one specimen provided a *T. cruzi* strain, named "Tm".

## DISCUSSION

Epidemiological studies on Chagas disease and vector capture have been conducted in both endemic and non-endemic areas.<sup>1,7,8,18</sup> In areas where vector-borne transmission was controlled by measures against the main vector of the disease, namely *Triatoma infestans*, invasion and colonization by secondary vectors can be observed, and they were sometimes captured inside the houses. Species like *T. sordida* and *P. megistus* in Sao Paulo (Southeastern Brazil), *T. rubrovaria* in Rio Grande do Sul,<sup>2</sup> Southern Brazil, and *T. brasiliensis* in Northeastern Brazil<sup>2</sup> are examples of that situation.

Originally, sylvatic specimens of triatomine can adapt to peridomestic and domestic environments occupying the ecological niche of primary species. In this sense, the gathering of the *T. sherlocki* in sylvatic and domestic environment may indicate that sylvatic populations have invaded and colonized localities, thus the importance of entomological surveys. The presence of triatomines

**Table 3.** Strains of *Trypanosoma cruzi* isolated and maintained in laboratory.

Species	Strain	Year of collection
<i>T. rubrovaria</i>	QG1	2002
	QG2	2002
	J1	2003
	J111	2003
	QB1	2003
	QMM1	2008
	QMM2	2008
	QMM3	2008
	QMM4	2008
	QMM5	2008
	QMM6	2008
	QMM7	2008
<i>T. sordida</i>	QMM9	2008
	QMM10	2008
	QMM11	2008
	QMM12	2008
	SI1	2003
	SI2	2003
	SI3	2003
	SI5	2003
	SI7	2003
	SI8	2003
	SI9	2003
<i>T. lenti</i>	SI11	2003
	SIGR3 <sup>a</sup>	2006
<i>T. melanocephala</i>	SIGR5 <sup>a</sup>	2006
	T. lenti	2008
Total	Tm	2009
	28	–

<sup>a</sup> Strains isolated from cats by xenodiagnosis.

inside houses represents a risk of vector-borne transmission of Chagas disease to humans.<sup>3</sup> In Brazil, there are 52 species, but five of them – *Panstrongylus megistus*, *Triatoma brasiliensis*, *T. infestans*, *T. pseudomaculata* and *T. sordida* – have considerable epidemiological importance.<sup>6</sup> *Triatoma sordida* is one of the most captured species in Brazil and is regarded as mostly peridomestic, whereupon it can be found naturally infected by *T. cruzi*. Therefore, that species has an important role in the maintenance and transmission of Chagas disease.<sup>3</sup>

In order to have a better understanding of the response of that triatomine to the control activities, research was carried out in 12 rural localities in the city of Porteirinha, MG, Southeastern Brazil.<sup>9</sup> A total of 772 specimens of *T. sordida* were captured, of which 3.6% were infected by *T. cruzi*. Of the 406 domiciliary units researched, 34.9% were

infested. Of the 695 peridomestic ecotopes, 27.6% had *T. sordida*. *Triatoma rubrovaria* is distributed in a wide area comprehending Argentina, Uruguay and Southern Brazil<sup>13,18</sup> and it lives in holes and cracks in rocky places in fields. It can be captured in domiciles in rural areas next to sylvatic ecotopes, which explains its epidemiological importance.<sup>18</sup> Almeida et al<sup>1</sup> (2000) reported the presence of that species in a domiciliary environment, confirming its ability to invade and colonize houses.<sup>2</sup>

*Trypanosoma cruzi* has genetically different strains for which more than 100 mammalian species can serve as reservoirs.<sup>7</sup> Geographic distribution in the Americas depends on the interaction of the parasite with the vector, and natural infection can only take place when the trypanosomatid-triatomine system is balanced.<sup>11</sup> So far, there are six groups of parasite – Discrete Typing Units (DTU): TcI, TcII, TcIII, TcIV, TcV and TcVI<sup>14</sup> and biological differences among strains are pointed out by researchers.<sup>5,16,21</sup> Martins et al<sup>15</sup> (2008) performed the molecular and biological characterization of five isolates obtained from *T. rubrovaria* collected in Rio Grande do Sul wherein 1.6% of the collected specimens were positive for *T. cruzi*. Rimoldi et al<sup>17</sup> (2012) showed the biological diversity of strains isolated from *T. sordida* and a domestic cat in Santo Inácio, Bahia.

Freitas et al<sup>10</sup> (2005) collected 921 specimens of *T. pseudomaculata* in cities in the state of Ceará, Northeastern Brazil. Even though they tested negative for *T. cruzi*, the presence of that vector in that region is clear. In 1980 Sherlock & Guitton<sup>19</sup> (1980) collected 12 samples of *T. lenti* in Bahia and all of them were positive for *T. cruzi*. The fact that the specimens tested positive shows the

importance of that vector in the transmission of Chagas disease in the state. Few studies related to *T. lenti* and *T. melanocephala* are found in the literature and in this sense this work can contribute.

Chagas disease can be controlled by interruption of the transmission mechanisms, improvement of dwellings and adjacent areas, education, basic sanitation for exposed populations, and treatment. To make control successful, it is essential to completely prevent contact between triatomines and humans in areas surrounding habitations, which could be achieved by the use of residual insecticides, improvement of dwellings, and repair of crevices in walls using plaster, preventing triatomines from having a place to hide.<sup>6</sup>

In view of the 28 strains of *T. cruzi* collected from species regarded as “secondary”, such as *T. sordida* and *T. rubrovaria*, it is necessary to continue entomological surveillance and health education actions in order to minimize the chances of triatomines colonizing domestic and peridomestic areas in cities in the states of Bahia and Rio Grande do Sul. This study continues with biological, morphological and molecular approaches under development.

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## HIGHLIGHTS

The transmission of Chagas disease by vectors was considered to be under control in certain regions of Brazil. This work shows that some species of triatominae, such as *Panstrongylus geniculatus*, *Triatoma melanocephala*, *Triatoma lenti*, *Triatoma pseudomaculata*, *Triatoma sherlocki*, *Triatoma sordida*, *Triatoma circummaculata* and *Triatoma rubrovaria* can be found in municipalities in the states of Bahia and Rio Grande do Sul. Some of the specimens studied tested positive for *Trypanosoma cruzi*, calling for extra attention to be paid, as during the capture period (2002-2009) infected vertebrate reservoirs were found in household, peridomestic and wild environments.

Of the specimens examined, *T. cruzi* was isolated in 28 triatominae from four different species: *T. melanocephala*, *T. lenti*, *T. rubrovaria* and *T. sordida*, presenting a natural infection rate of 6.4%.

Isolating strains of *T. cruzi* from the triatominae captured in the intra and peridomestic environments reflects the potential risk of Chagas disease transmission in the municipalities studied. This work contributes to increasing knowledge of the distribution of triatominae, as well as encouraging effective control of Chagas infection, as it presents data on natural infection and the distribution of vectors in the states of Bahia and Rio Grande do Sul.

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