



## Article/Artigo

# Biological behavior of *Trypanosoma cruzi* stocks obtained from the State of Amazonas, Western Brazilian Amazon, in mice

Comportamento biológico de isolados de *Trypanosoma cruzi* obtidos no Estado do Amazonas, Amazônia Ocidental Brasileira, em camundongos

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### ABSTRACT

**Introduction:** The biological diversity of circulating *Trypanosoma cruzi* stocks in the Amazon region most likely plays an important role in the peculiar clinic-epidemiological features of Chagas disease in this area. **Methods:** Seven stocks of *T. cruzi* were recently isolated in the State of Amazonas, Brazil, from humans, wild mammals, and triatomines. They belonged to the TcI and Z3 genotypes and were biologically characterized in Swiss mice. Parasitological and histopathological parameters were determined. **Results:** Four stocks did not promote patent parasitemia in mice. Three stocks produced low parasitemia, long pre-patent periods, and a patent period of 1 day or oscillating parasitemia. Maximum parasitemia ranged from 1,400 to 2,800 trypomastigotes/0.1mL blood. Mice inoculated with the *T. cruzi* stocks studied showed low positivity during fresh blood examinations, ranging from 0% to 28.6%. In hemoculture, positivity ranged from 0% to 100%. Heart tissue parasitism was observed in mice inoculated with stocks AM49 and AM61. Stock AM49 triggered a moderate inflammatory process in heart tissue. A mild inflammatory process was observed in heart tissue for stocks AM28, AM38, AM61, and AM69. An inflammatory process was frequently observed in skeletal muscle. Examinations of brain tissue revealed inflammatory foci and gliosis in mice inoculated with stock AM49. **Conclusions:** Biological and histopathological characterization allowed us to demonstrate the low infectivity and virulence of *T. cruzi* stocks isolated from the State of Amazonas.

**Keywords:** Chagas disease. *Trypanosoma cruzi*. Virulence. Pathogenicity. Swiss mice. Amazon.

### RESUMO

**Introdução:** A diversidade biológica dos estoques *Trypanosoma cruzi* circulantes na Região Amazônica pode desempenhar importante papel nas características clínico-epidemiológicas peculiares da doença de Chagas nesta área. **Métodos:** Sete isolados de *T. cruzi* do Estado do Amazonas provenientes de humanos, mamíferos silvestres e triatomíneos, pertencentes aos genótipos TcI e Z3, foram biologicamente caracterizados em camundongos Swiss. Foram avaliados parâmetros parasitológicos e histopatológicos. **Resultados:** Quatro isolados não produziram parasitemia patente em camundongos. Três isolados promoveram baixa parasitemia com longos períodos pré-patentes, período patente de um dia ou parasitemia oscilante. A parasitemia máxima variou de 1.400 a 2.800 tripomastigotas/0,1mL de sangue. Os camundongos inoculados com os isolados estudados mostraram baixa positividade no exame a fresco, variando de 0 a 28,6%. Para a hemocultura, a positividade variou de 0 a 100%. Parasitismo cardíaco foi observado em camundongos inoculados com os isolados AM49 e AM61. O isolado AM49 produziu inflamação moderada no tecido cardíaco. Processo inflamatório discreto foi observado no tecido cardíaco de camundongos inoculados com os isolados AM28, AM38, AM61 e AM69. Processo inflamatório em músculo esquelético foi muito frequente. O exame do tecido cerebral revelou focos inflamatórios e gliose em camundongos inoculados com o isolado AM49. **Conclusões:** A caracterização biológica e histopatológica demonstrou baixa infectiosidade e virulência dos estoques de *T. cruzi* isolados no Estado do Amazonas.

**Palavras-chaves:** Doença de Chagas. *Trypanosoma cruzi*. Virulência. Patogenicidade. Camundongos Swiss. Amazônia.

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### INTRODUCTION

Chagas disease is an important parasitic disease that results from an infection by the flagellate protozoan *Trypanosoma cruzi*. Its evolutionary pattern is not fully defined because the factors that determine its considerable spatial variation in morbidity and mortality are unknown<sup>1</sup>. However, the idea that parasite variability and host response play important roles during the course of infection is consensual<sup>2</sup>. In fact, studies show that *T. cruzi* comprises a highly heterogeneous population of organisms, both in terms of genetic and phenotypic points of view, including biological (behavior in vertebrates and triatomines), pathological (tissue tropism, virulence, intensity of inflammatory reactions, and mortality), clinical (indeterminate and cardiac and/or digestive forms), immunological, and biochemical features<sup>2,3</sup>.

In the Amazon region, Chagas disease has been recognized as an important emerging anthroponosis. Information from the Brazilian Ministry of Health<sup>4</sup> indicates the occurrence of 756 cases of acute illness in the country during 2005–2010. Most strikingly, 688 (91%) cases occurred in the states belonging to the Amazon region. The Brazilian states with the largest number of these cases were Pará (573 cases; 75.8%) and Amapá and Amazonas (54 cases each; 7.1%), which are all located in the Amazon region. In the State of Amazonas, Chagas disease has a lower morbidity and mortality than in the classic endemic areas, and appears mainly in the chronic latent form<sup>1,5</sup>. Cross-sectional studies in several riverine communities and *piassava* fiber collectors in the Rio Negro micro-region proved the high prevalence of chagasic infection in this area and demonstrated its low morbidity profile in the chronic phase of the disease<sup>6,7</sup>.

The characterization of the biological diversity of *T. cruzi* stocks circulating in the Amazon region is essential to understand the emergence, expansion, and peculiar clinic-epidemiological characteristics

of Chagas disease in this area. Thus, the purpose of this preliminary study was to investigate the behavior in mice of *T. cruzi* stocks from the State of Amazonas, in the Western Brazilian Amazon.

## METHODS

### Isolation of parasites

The 7 sample *T. cruzi* stocks were obtained by the Tropical Medicine Foundation of Amazonas, between April 2006 and January 2010, from 6 municipalities in the State of Amazonas. **Table 1** shows the host, method of isolation, site of origin, and genetic lineage of the 7 *T. cruzi* stocks studied.

For parasite isolation and culturing, heparinized blood samples from chagasic patients in the acute phase were inoculated into tubes containing a biphasic medium consisting of Novy-McNeal-Nicolle medium, covered with an overlay of liver-infusion tryptose medium containing 10% fetal calf serum and 140 mg/mL gentamycin sulfate<sup>8</sup>. Approximately 0.5mL of whole blood was placed in each tube (3-5 tubes for each person). Cultures were kept at 28°C and monitored microscopically for parasite growth twice a week for 2 months. One *T. cruzi* stock was isolated from a cerebrospinal fluid culture of a patient (a resident of the municipality of Coari (AM49)) who died due to acute chagasic meningoencephalitis, by the same method used for blood culture.

Two *T. cruzi* stocks were isolated from marsupials captured in Tomahawk traps with fruit as bait. Parasite isolation from wild mammals was carried out using the same technique as for human blood culture.

To obtain the 2 *T. cruzi* stocks from triatomine bugs, specimens were collected from palm trees in the peridomestic environment. Triatomines were dissected, their intestinal contents were examined by phase microscopy, and positive samples for trypanosomes were inoculated in mice for subsequent isolation by blood culture.

After isolation, the stocks were immediately cryopreserved in liquid nitrogen (-193°C) in the trypanosomatid culture collection of the Department of Entomology, Tropical Medicine Foundation of Amazonas.

To genotype the isolated parasites, total DNA extraction from in vitro isolates of *T. cruzi* was performed using the PureLink Kit (Invitrogen, Life Technologies, USA), according to the manufacturer's protocol. DNA was prepared from 200µL of culture

and eluted with 50µL of milliQ water. DNA from the non-transcribed spacer of the mini-exon was amplified according to the protocol reported by Fernandes et al.<sup>9</sup> The *T. cruzi* isolates were typed as TcI or Z3 lineages (Monteiro et al., in preparation).

### Inoculation of mice

Groups of 7 male Swiss mice (age, 12-15 days) originating from the vivarium of the Department of Venomous Animals, Tropical Medicine Foundation of Amazonas, were inoculated intraperitoneally with  $1.0 \times 10^6$  metacyclic trypomastigotes from a late-stationary-phase culture in liver-infusion tryptose medium, determined in a Neubauer chamber. The mice were maintained in large boxes in a well-ventilated room, with *ad libitum* access to a commercial pellet feed for rodents and drinking water.

### Parasitological parameters

**Fresh blood examination:** we collected 5 µL of blood daily from the tails of the mice and examined it microscopically for living trypomastigotes. The level of parasitemia was measured daily from the 3<sup>rd</sup> day after inoculation, as described by Brener<sup>10</sup>. The results were also expressed as the percentage of mice with positive fresh blood examination results (FBE; %FBE). The pre-patent period (PPP), patent period (PP), maximum parasitemia (MP), and the day of maximum parasitemia (DMP) were determined, according to the protocol reported by Toledo et al.<sup>11</sup>

**Hemoculture:** At 90 days after inoculation (d.a.i.), a blood sample collected by cardiac puncture was cultured as described above, in duplicate. The hemocultures (HCs) were maintained at 28°C and examined at 15, 30, 60, and 90 days later for parasites. The results were expressed as the percentage of mice with a positive HC (%HC)<sup>11</sup>.

### Infectivity and mortality

**Infectivity (%INF)** was determined as the percentage of mice that presented with a positive FBE and/or HC in the first 3 months after inoculation<sup>11</sup>.

**Mortality (%MOR)** was registered daily after inoculation and expressed as the cumulative percentage of death.

### Histopathological parameters

For each *T. cruzi* stock, all surviving mice were necropsied at 90 d.a.i. for histopathological studies. The following organs and tissues were collected: (1) heart, (2) skeletal muscle, (3) lungs, (4) large intestine, (5) brain, (6) liver, and (7) spleen. This material was

**TABLE 1 - Host, municipality of origin, method of isolation, and genetic lineage of the *Trypanosoma cruzi* stocks from the State of Amazonas, Brazil.**

<i>Trypanosoma cruzi</i> stock	Host	Municipality of origin	Method of isolation	Lineage *
AM28	<i>Didelphis marsupialis</i>	Manaus	hemoculture	TcI
AM38	<i>Philander opossum</i>	Coari	hemoculture	TcI
AM41	<i>Rhodnius robustus</i>	Maraã	xenoculture	TcI
AM49	human**	Coari	CSF culture	TcI
AM61	<i>Rhodnius pictipes</i>	Apuí	inoculation in mice	TcI
AM69	human**	Santa Isabel do Rio Negro	hemoculture	Z3
AM70	human**	Coari	hemoculture	Z3

CSF: cerebrospinal fluid. \*molecular characterization performed by PCR of the non-transcribed spacer of the mini-exon gene according to Fernandes et al; \*\*patient in acute phase.

routinely processed and embedded in paraffin. Five-micrometer sections of 3 blocks containing every organ of each mouse were obtained for further evaluation. These preparations were stained with hematoxylin-eosin for microscopic examination. The tissue parasitism (TP) and inflammatory process (IP) displayed by different organs or tissues were classified as absent (-), mild (+), moderate (++), and severe (+++).

#### Statistical analysis

Data were analyzed using SPSS® version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The chi-square test was used to test differences in proportions, and Student's t test was used to test differences in means. Statistical significance was considered if  $p < 0.05$ .

#### Ethical considerations

The use of stocks of *T. cruzi* obtained from patients was approved by the Ethics in Research on Humans Committee of the Tropical Medicine Foundation of the State of Amazonas (approval no. 360/07). The capture and handling of marsupials for blood sample collection were performed according to permits from the Brazilian Institute for Environment (approval no. 1830651/07). The use of mice in this study followed the ethical principles for animal experimentation<sup>12</sup>.

## RESULTS

Four of the stocks evaluated (AM28, AM41, AM69, and AM70) did not lead to patent parasitemia. The parasitological parameters, infectivity, and mortality obtained from the isolates are shown in **Table 2**. Stock AM49 promoted intense lethargy, hind limb paralysis, and ruffled coat in mice. For stocks AM41, AM49, and AM69, death occurred at 68, 49, and 46 d.a.i., respectively. Death occurred at 28 and 35 d.a.i. for stock AM28. One of the 7 stocks (AM61) caused a higher %MOR in mice (71.4%), and all deaths occurred on the 17 d.a.i. This stock led to intense lethargy and ruffled coat in mice.

The stock isolated from the marsupial *Philander opossum* showed a significantly longer mean PPP than the stocks obtained from humans and triatomines ( $p < 0.001$ ); for the stocks from humans, this parameter was significantly longer than that from triatomines ( $p < 0.001$ ). The mean PP was longer for the stocks from triatomines ( $p < 0.005$ ). These stocks led to an MP that was significantly higher than for the stocks derived from the others hosts ( $p = 0.015$ ). The DMP did not differ significantly among stocks from the different hosts (**Table 3**).

Stocks obtained from *Rhodnius robustus* showed a significantly higher %+FBE than human- and marsupial-derived stocks

**TABLE 2 - Parasitological and virulence parameters in Swiss mice inoculated with *Trypanosoma cruzi* stocks from the State of Amazonas, Brazil.**

<i>Trypanosoma cruzi</i> stock	Mean pre-patent period (days)	Mean patent period (days)	Peak of maximum parasitemia*	Day of maximum parasitemia	%+FBE	%+HC	%INF	%MOR
AM28	-	0	0	0	0/7 (0.0)	5/5 (100.0)	100.0	2/7 (28.6)
AM38	78	1	1,400	78	1/7 (14.3)	7/7 (100.0)	100.0	0/7 (0.0)
AM41	-	0	0	0	0/7 (0.0)	0/6 (0.0)	0.0	1/7 (14.3)
AM49	74	1	1,400	74	1/7 (14.3)	5/6 (83.3)	83.3	1/7 (14.3)
AM61	23	15	2,800	35	2/7 (28.6)	2/2 (100.0)	100.0	5/7 (71.4)
AM69	-	0	0	0	0/7 (0.0)	0/6 (0.0)	0.0	1/7 (14.3)
AM70	-	0	0	0	0/7 (0.0)	0/7 (0.0)	0.0	0/7 (0.0)

%+FBE: percentage of mice with a positive fresh blood examination; %+HC: percentage of positive hemocultures for surviving mice; %INF: infectivity rate; %MOR: cumulative mortality rate. \*number of trypomastigotes/0.1 mL blood.

**TABLE 3 - Statistical comparison of 8 parasitological parameters in Swiss mice inoculated with *Trypanosoma cruzi* stocks from different hosts in the State of Amazonas, Brazil.**

Parameter	Humans × triatomines		Humans × marsupials		Triatomines × marsupials	
	$n_H \times n_T$	p	$n_H \times n_M$	p	$n_T \times n_M$	p
Mean pre-patent period (days)	74 × 23	<0.001	74 × 78	<0.001	23 × 78	<0.001
Mean patent period (days)	0.33 × 7.5	<0.001	0.33 × 0.5	NS	7.5 × 0.5	0.002
Peak of maximum parasitemia**	466.7 × 1,400	0.015	466.7 × 700	NS	1,400 × 700	NS
Day of maximum parasitemia	24.7 × 17.5	NS	24.7 × 39	NS	17.5 × 39	NS
%+FBE	4.7 × 14.3	0.012	4.8 × 7.1	NS	14.3 × 7.1	0.012
%+HC	26.3 × 25	NS	26.3 × 100	<0.001	25 × 100	0.002
%INF	26.3 × 25	NS	26.3 × 100	<0.001	25 × 100	0.002
%MOR	9.5 × 42.9	0.021	9.5 × 14.3	NS	42.9 × 14.3	NS
Number of significant differences	5	3	5			

$n_H \times n_T$ : comparison between parameters derived from humans and triatomines;  $n_H \times n_M$ : comparison between parameters derived from humans and marsupials;  $n_T \times n_M$ : comparison between parameters derived from triatomines and marsupials; %+FBE: percentage of mice with a positive fresh blood examination; %+HC: percentage of positive hemocultures for the surviving mice; %INF: infectivity rate; %MOR: cumulative mortality rate.  $p < 0.05$ : significant difference; NS: not significant. \*only for stocks AM38, AM49, and AM61; \*\*number of trypomastigotes/0.1 mL blood.



( $p < 0.05$ ). The parameters %HC and %INF were higher for stocks from marsupials in relation to the others ( $p < 0.05$ ). Stocks isolated from triatomines led to a higher %MOR than stocks from humans ( $p = 0.021$ ) (Table 3).

Heart tissue parasitism was observed in 2 (28.6%) mice infected with stock AM49 (Figure 1) and in 1 (14.3%) mouse infected with stock AM61. Tissue parasitism was not verified in the other organs examined. All mice inoculated with stock AM49 showed focal and

moderate IP in heart tissue (Figure 1). Focal and mild IP was observed in the heart tissue of mice inoculated with stocks AM28, AM38, AM61, and AM69. The IP in skeletal muscle was diffuse and intense in mice inoculated with stock AM49, moderate with stocks AM28 and AM38, and mild with stocks AM61, AM69, and AM70 (Table 4). Examination of brain tissue revealed inflammatory foci and gliosis in 4 (57.1%) mice inoculated with stock AM49 (Figure 1). An IP was not observed in spleen, liver, lungs, or large intestine for any group of mice (Table 4).

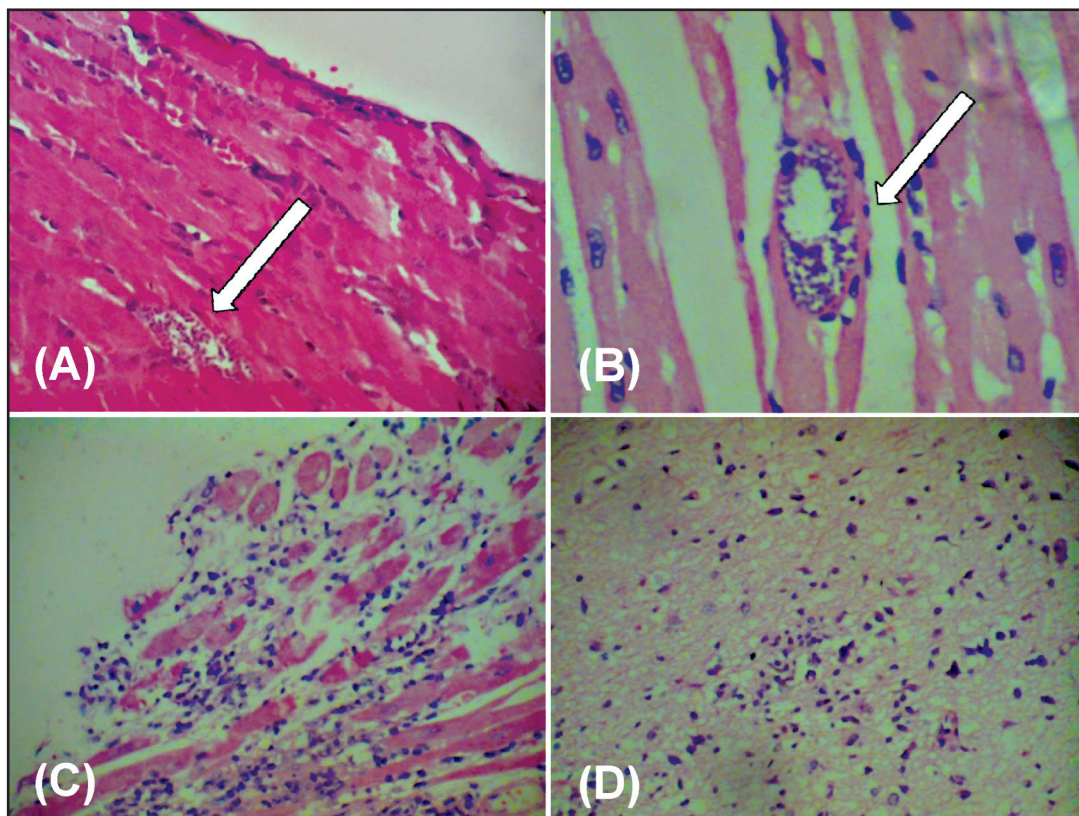


FIGURE 1 - Histopathological alterations in Swiss mice triggered by stock AM49: A and B: nests of amastigotes in cardiac tissue. C: diffuse inflammatory process in skeletal muscle. D: inflammatory process and gliosis in the central nervous system. Magnification  $\times 400$ .

TABLE 4 - Histopathological parameters (tissue parasitism and inflammatory process) in mice inoculated with *Trypanosoma cruzi* stocks from the State of Amazonas, Brazil.

<i>Trypanosoma cruzi</i> stock	Organ						
	heart	skeletal muscle	spleen	liver	lungs	large intestine	brain
AM28	+	++	-	-	-	-	-
AM38	+	++	-	-	-	-	-
AM41	-	-	-	-	-	-	-
AM49	++*	+++	-	-	-	-	+
AM61	+*	+	-	-	-	-	-
AM69	+	+	-	-	-	-	-
AM70	-	+	-	-	-	-	-

+: mild; ++: moderate; +++: severe; -: absent; \*only showing mild tissue parasitism.

## DISCUSSION

In the State of Amazonas, chronic Chagas disease has lower morbidity than in classic endemic areas, appearing mainly in the latent form<sup>1,5</sup>. Chagasic infection was first reported in this state by Ferraroni et al.<sup>13</sup>, who examined 25 individuals who performed

*piassava* fiber extraction in the municipality of Barcelos, and found 6 individuals with positive serological test results. Cross-sectional assessments, including the clinical and radiological evaluations of anti-*T. cruzi* seropositive patients in the Rio Negro micro-region, ratified the high seroprevalence of chagasic infection in this region and showed a clinical profile of low morbidity and mortality in the chronic phase of the disease<sup>5-7</sup>.

*Trypanosoma cruzi* stocks have been characterized on the basis of their biological behavior in mice<sup>14,15</sup>. The present study with stocks from the State of Amazonas, a non-endemic but emerging area for Chagas disease, showed that 4 (AM28, AM41, AM69, and AM70) of the 7 stocks studied produced sub-patent parasitemia. Furthermore, the other 3 stocks that caused patent infections in mice produced low and late parasitemia, with peaks not exceeding 2,800 trypomastigotes/0.1 mL blood. In relation to the parasitemia, we observed that this profile was very different from those observed in endemic areas, where stocks with variable parasitemia circulate, but with frequent rates of patent infections, in particular for stocks isolated from chronic patients<sup>14-18</sup>. In this study, low parasitemia, a very long PPP, and short PP indicate the low virulence of the strains from the western Amazon region. These findings agree with the hypothesis of several authors who suggested that the framework of chronic latent infection in the State of Amazonas is due to the low parasitemia and lower virulence and pathogenicity of the wild parasites in this area.

An important practical aspect that can be explained by the low parasitemia for the *T. cruzi* stocks isolated from the Amazon region is the low performance of the diagnostic methods for Chagas disease in this area. In Bolivia and in the classic Brazilian endemic areas, where the TcII genotype circulates and infections involve high levels of parasitemia, diagnostic tests are very sensitive, in contrast with areas from Amazonia and Mexico, where the TcI genotype occurs<sup>5,19-21</sup>. In the Brazilian Amazon and Venezuela, diagnostic tests employed in the acute phase of Chagas disease showed low sensitivity<sup>22-24</sup>. Brum-Soares et al.<sup>5</sup> also verified that chronic chagasic patients from the Rio Negro micro-region presented with low IgG anti-*T. cruzi* serum levels, probably due to the low parasite load and/or low antigenic power of the circulating parasites. Interestingly, our findings support the first suggestion.

Stocks AM41, AM69, and AM70 were unable to infect mice, both in FBE and HC, confirming the low virulence of these isolates. For stocks AM28, AM38, AM49, and AM61, we found high infectivity rates by HC, despite their low level of parasitemia. If this feature is reproduced in human hosts, an important implication is the lack or delay in diagnosis of acute Chagas disease, since direct methods are being used and recommended in the Amazon region because this is the same approach used for the detection of malaria cases, which would make a parallel program control. These data support the findings of previous studies, which reported that sylvatic stocks from the United States, where the TcI and zymodeme 3 genotypes circulate, as in the Brazilian Amazon, were largely avirulent and did not cause morbidity or mortality in rodent models<sup>25</sup>. In contrast, *T. cruzi* stocks from the classic endemic areas of South America readily infect a wide variety of laboratory mouse strains and many cause significant morbidity and mortality<sup>12,16,26</sup>.

Stocks from marsupials (AM28 and AM38) infected all of the mice inoculated, although with low parasitemia. Previous mouse infection studies using several sylvatic- and domestic-derived isolates from Brazil showed that those from marsupials were generally more infective and generated higher parasitemia than those from vectors or placental mammals<sup>16,26</sup>. Stock AM61, which originated from a specimen of *Rhodnius robustus* collected in the municipality of Apuí, was the other infective isolate and the only one that promoted a high mortality rate, highlighting the southern Amazon region as an area in which the eco-epidemiology of Chagas disease needs to be studied further.

More sensitive methods, e.g., molecular detection by the polymerase chain reaction (PCR), may increase *T. cruzi* infectivity when applied to experimental studies in mice. Thus, we recognize this limitation, although a large number of studies have used the same methods for detecting infection, and stated that caution should be taken when these data are compared with other results. *T. cruzi* infection can be diagnosed by demonstrating the presence of the parasite using direct and indirect parasitological methods, immunodiagnosis to detect specific antibodies against *T. cruzi*, or by molecular methods for parasite DNA. The high rate of positivity using PCR suggested this methodology as a diagnostic tool for the detection of *T. cruzi* DNA in the blood of different host species<sup>27</sup>.

Although the majority of the sylvatic and human stocks isolated from the western Amazon were largely non-virulent and did not cause morbidity or mortality in our model, we noted an interesting result in an experimental infection with stock AM49. This strain was isolated from the cerebrospinal fluid of an infant misdiagnosed primarily as skin abscesses, which evolved to a fatal outcome due to acute Chagas disease meningoencephalitis (unpublished information), and was the only isolate from patients that was able to infect mice. The group of mice infected with this isolate showed relatively low mortality and parasitemia, despite its high infectivity and frequency of observable physical or behavioral changes indicative of Chagas disease, e.g., lethargy, hind limb paralysis, and ruffled coat. Histopathological alterations triggered by this stock included an intense IP of skeletal muscles, moderate inflammation and parasitism in the heart, and, surprisingly, inflammation and gliosis in the brain of the infected mice. These results suggest that this stock could belong to biotopeme III<sup>3</sup>, with a dissimilar neurotropic behavior.

Inflammatory process or tissue parasitism was not observed in the liver or spleen of the infected mice; however, even in animals with no evident parasitemia, IP was observed in skeletal muscle tissue and, less frequently, in cardiac tissue. In addition, 2 stocks also promoted tissue parasitism in rodents, indicating that some stocks of *T. cruzi* from the State of Amazonas can behave like biotopeme III parasites<sup>3</sup>. In fact, infrequent chronic cardiac cases of Chagas disease confirm that parasites circulating in this area cause myocardiopathy, appearing clinically as cardiac insufficiency, arrhythmogenic syndrome, or thromboembolism<sup>28-33</sup>. The absence of histopathological alterations in the large intestine indicates that the parasites studied did not have the capacity to cause digestive Chagas disease, in agreement with the absence of records of *mega* syndromes in Amazonian countries and Central and North America<sup>34</sup>.

In conclusion, our data suggest that the biological characteristics of *T. cruzi* stocks from the western Amazon region may vary considerably. The disproportion between the number of cases of infection and clinical disease in this region may result from the predominance of non-virulent strains. However, our study demonstrated the occurrence of stocks that can cause cardiac and nervous involvement. Therefore, we recommend the biological characterization of a great number of stocks to define the real potential for the emergence of Chagas disease as a public health problem in the Amazonian context.

The belief that Chagas disease may be benign in some geographic areas, as in the Amazon region, derives from misinformation or lack of more thorough investigations. Under no circumstances should it serve to postpone control measures once the risk of transmission to humans has been established.



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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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