# Prevalence and intensity of infection, metacyclogenesis and nuclear phenotypes in *Panstrongylus megistus* (Burmeister, 1835) after ingestion of *Trypanosoma cruzi* (Chagas, 1909) II and subjection to heat shock

*Garcia, SL.*[a,b]*, Rodrigues, VLCC.*[c]*, Garcia, NL.*[d] *and Mello, MLS.*[a]*

[a]Departamento de Anatomia, Biologia Celular e Fisiologia e Biofísica, Universidade Estadual de Campinas – UNICAMP, Rua Charles Darwin, s/n, Cidade Universitária Zeferino Vaz, CEP 13083-863, Campinas, SP, Brazil

[b]Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Rua Charles Darwin, s/n, Cidade Universitária Zeferino Vaz, CEP 13083-863, Campinas, SP, Brazil

[c]Superintendência de Controle de Endemias – SUCEN, Rua Afonso Pessini, 86, CEP 13845-206, Mogi Guaçu, SP, Brazil

[d]Departamento de Estatística, Instituto de Matemática, Estatística e Computação Científica, Universidade Estadual de Campinas – UNICAMP, Rua Sérgio Buarque de Holanda, 651, CEP 13083-859, Cidade Universitária Zeferino Vaz, Campinas, SP, Brazil

*e-mail: mlsmello@unicamp.br

Received November 23, 2009 – Accepted May 14, 2010 – Distributed May 31, 2011

## Abstract

This study aimed to contribute to our knowledge of the parasite-vector interaction associated with *Trypanosoma cruzi* (Chagas, 1909) infection in *Panstrongylus megistus* (Burmeister, 1835), an important vector of Chagas' disease in Brazil. The prevalence and intensity of *T. cruzi* infection, the incidence of metacyclogenesis and the frequency of nuclear phenotypes in Malpighian tubules were investigated in nymphs of *P. megistus*, reared at 28 °C and subjected to heat shock (40 °C, 1 hour) two days after infection with *T. cruzi* II (Y strain). Following the 45-day post-infection period, the frequency of epimastigotes was much higher than that of trypomastigotes in both heat-shocked and non-shocked insects, and the prevalence of infection was not altered by heat shock. Fewer epimastigotes and trypomastigotes were found in the infected insects subjected to the heat shock, indicating that the multiplication and metacyclogenesis of the parasites were affected by the stress. In infected specimens heat shock promoted an increased frequency of cell nuclei with heterochromatin decondensation, a cell survival response to stress, and did not affect insect survival. The effects of infection and heat shock, especially on the multiplication and metacyclogenesis of *T. cruzi*, and the observed resistance to heat shock developed by *P. megistus* nymphs are suggestive that they should be considered when adequate conditions for rearing these infected insects in the laboratory are pursued.

*Keywords: Panstrongylus megistus, Trypanosoma cruzi,* heat shock, metacyclogenesis, nuclear phenotypes.

# Prevalência e intensidade de infecção, metaciclogênese e fenótipos nucleares em *Panstrongylus megistus* (Burmeister, 1835) após ingestão de *Trypanosoma cruzi* (Chagas, 1909) II e choque de temperatura

## Resumo

O presente estudo teve como objetivo contribuir para o conhecimento sobre a interação parasita-vetor associada à infecção por *Trypanosoma cruzi* (Chagas, 1909) em *Panstrongylus megistus* (Burmeister, 1835), importante vetor da doença de Chagas no Brasil. A prevalência e a intensidade da infecção por *T. cruzi*, a incidência de metaciclogênese e a frequência de fenótipos nucleares presentes em túbulos de Malpighi foram investigadas em ninfas de *P. megistus* criadas à temperatura de 28 °C e submetidas a choque térmico por 1 hora a 40 °C, dois dias após alimentação em camundongos infectados com *T. cruzi* II (linhagem Y). A análise realizada 45 dias pós-infecção revelou que tanto nos insetos submetidos ao choque térmico como nos respectivos controles a frequência de epimastigotos se apresentava muito maior do que a dos tripomastigotos; a prevalência da infecção não foi alterada pelo choque térmico. Menos epimastigotos e tripomastigotos foram encontrados nos insetos submetidos ao choque térmico, indicando que a multiplicação e a metaciclogênese dos parasitas possam ser afetadas com o estresse. Nos espécimes infectados, o choque térmico induziu aumento na frequência de núcleos com descompactação da heterocromatina, uma resposta de sobrevivência celular ao estresse, e não afetou a sobrevivência propriamente dita do inseto. Os efeitos da infecção e do choque térmico, especialmente sobre a multiplicação e a metaciclogênese de *T. cruzi*, e a resistência ao choque térmico desenvolvidos pelas ninfas de *P. megistus* são indicativos de que devam ser considerados quando se buscam condições adequadas de criação em laboratório de insetos infectados.

*Palavras-chave: Panstrongylus megistus, Trypanosoma cruzi,* choque térmico, metaciclogênese, fenótipos nucleares.

## 1. Introduction

*Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas' disease, is a flagellate protozoan with variable patterns of virulence, pathogenicity and infectivity in animals (Contreras et al., 1998). Blood-sucking hemipterans are vectors of Chagas' disease. Although several factors are important in the parasite-vector interaction, including the parasite strain and features related to the vector species, such as its genetic characteristics and developmental stage (Garcia and Azambuja, 1991; Lana et al., 1998), our understanding of the vector-parasite relationship remains limited. Quantitative studies are difficult to compare because the population density of the parasites is influenced by several factors and because small amounts of metacyclic forms are eliminated in the feces of the vector (Piesman and Sherlock, 1985; Contreras et al., 1998).

Although *T. cruzi* is pathogenic in humans and other mammals there is no evidence that it is harmful to its insect vector besides competing with the vector for nutrients (Schaub, 1989a), reducing the value of some insect reproduction parameters (Lima et al., 1992), and interfering with development of the symbionts required by triatomines to complete nymphal development (Vallejo et al., 2009). The main criterion for identifying an insect as an experimental vector model is its ability to maintain a high parasite load after feeding on infected hosts (Perlowagora-Szumlewicz et al., 1990). In the case of *T. cruzi,* some vector species can potentially limit human and animal infection because they do not favour metacyclogenesis (Perlowagora-Szumlewicz and Moreira, 1994). The use of xenodiagnosis in the chronic phase of Chagas' disease, either as a diagnostic aid or in conjunction with the administration of drugs against trypanosomes, has led to a search for hemipteran species that are highly susceptible to *T. cruzi* and thus could serve as experimental models (Alvarenga and Bronfen, 1984; Silva et al., 1994).

*Panstrongylus megistus* (Burmeister, 1835) is a reduviid triatomine currently considered as the principal vector of *T. cruzi* in the eastern, southern, and some northeastern states of Brazil (Barbosa et al., 2001). In addition to its wide geographic distribution in this country, *P*. *megistus* demonstrates high rates of *T. cruzi* infection and the ability to colonise artificial ecotopes (Forattini, 1980); it is a native species whose adaptation to the human domiciliary ecotope is directly related to the reduction of its usual sources of nutrition (Silveira, 2000). The efficiency of *P. megistus*, especially its extraordinary capacity for allowing the rapid multiplication of a few parasites ingested during the chronic phase of disease, is well documented (Perlowagora-Szumlewicz et al., 1988; Lima et al., 1992).

Knowledge of the best conditions for rearing triatomines in the laboratory could help to explain many of the protozoan infection responses observed in the wild (Rodrigues et al., 1991; Silva and Silva, 1993; Garcia et al., 1999, 2001a,b, 2003; Schmuñis, 2000). Although a short heat shock lasting 1 hour at 40 °C does not affect the survival rate of fasted or fully-nourished non-infected *P. megistus* (Garcia et al., 1999), the effect of this experimental condition on the survival of the infected specimens needed to be studied. Forms of cell death and cell survival responses which have been previously demonstrated in Malpighian tubules of *P. megistus* after a heat shock at 40 °C (Garcia et al., 2000b) have not yet been documented for *T. cruzi*-infected specimens. In addition, development of tolerance to sequential insults by stressors separated by a short time period and followed for 30 days has been reported for *P. megistus* in terms of increased survival rates, decrease in apoptosis and increase in cell survival responses (Garcia et al., 2002). The stress potentially inflicted by protozoan infection (Schaub, 1989a; Lima et al., 1992; Vallejo et al., 2009) accompanied by a heat shock in *P. megistus* has not yet been studied either.

In this study, we examined the prevalence and intensity of infection by *T. cruzi* and the level of metacyclogenesis 45 days post-infection in *P. megistus* reared at 28 °C and also subjected to heat shock. The frequency of Malpighian tubule nuclear phenotypes in specimens subjected to infection by *T. cruzi* and to heat shock was also investigated.

## 2. Material and Methods

### *2.1. Mammalian hosts*

*T. cruzi* II (Y strain) specimens obtained from the Institute of Tropical Medicine at the University of São Paulo (USP) were maintained in the laboratory at Sucen by successive passages in 21-day-old male Swiss mice. The latter were inoculated intraperitoneally with 0.1 mL of infected mouse blood containing ~$10^6$ parasites. All mice developed parasitaemia. Because the number of parasites inoculated was high, the mice were used four days after infection. The number of flagellates in each animal was determined by a modification of Pizzi and Prager's procedure (Andrade, 1974). Blood (5 µL) was collected from mice using a micropipette dipped in liquemine heparin (5.000 IU/mL), placed on a glass slide and covered with a coverslip. The preparations were examined in a Zeiss microscope; the number of parasites was counted in 50 microscopic fields using a 40 x objective.

### *2.2. Insects*

One hundred fourth-instar nymphs of *P. megistus* reared in the laboratory at Sucen were fasted for 30 days and then allowed to feed on infected mice, then placed in glass cylinders covered with cheesecloth and fastened with elastic bands. Each cylinder containing eight to nine insects was weighed before and after insect feeding. From this group, 50 nymphs were subjected to heat shock (40 °C for 1 hour) after two days (group 1). The remaining nymphs were maintained at 28 °C, the temperature used to rear *P. megistus* in the laboratory (group 2). Other two groups of 50 fourth instar nymphs each, also fasted for 30 days and subsequently fed chicken blood, were subjected (group 4) or not (group 3) to heat shock at 40 °C for 1 hour two days after feeding.

Forty-five days after infection, the proportion of insects positive for *T. cruzi* and the intensity of infection were determined. The 45-day period used for assessing infection is considered optimal for the examination of insects fed on infected mice (Perlowagora-Szumlewicz and Muller, 1987). The intensity or magnitude of the parasite density was determined using the classification established by Perlowagora-Szumblewicz and Muller (1987), in which the parasite counts obtained from 50 microscopic fields are scored in four groups: low (1-5), moderate (6-10), dense (11-100) and very dense (≥ 101).

*2.3. Insect preparations*

Examination of the infected insects involved sectioning the terminal portion of their abdomens and pulling out their rectum, which is supposed to contain two to five times more parasites than the small intestine (Schaub, 1989b). This tissue was placed in 100 µL of PBS on a glass slide and macerated, and in 5 µL homogenate the number of parasites was determined as described for the parasite counts in the blood (dilution of homogenate in PBS: 5:100) (Andrade, 1974). Epimastigotes were distinguished from metacyclic trypomastigotes based on their morphology and motility in a fresh faecal sample viewed with a 40 x objective (Piesman and Sherlock, 1985). When the material appeared to be free of parasites, another smear obtained from the same insect was prepared and examined.

Malpighian tubules were fixed in an absolute ethanol: acetic acid solution (3:1, v/v) for 1 minute, rinsed in 70% ethanol for 5 minutes and subjected to the Feulgen reaction with acid hydrolysis in 4 M HCl at 25 °C for 65 minutes (Mello, 1997; Garcia et al., 2000a,b, 2002). The Feulgen-stained material was rinsed in sulphurous and distilled water, air-dried, cleared in xylene and mounted in Canada balsam. The total number of Malpighian tubule epithelial cell nuclei counted per specimen and the identification of their phenotypes and frequencies were performed using a Nikon light microscope. The nuclear phenotype considered to be "normal" shows a small heterochromatic body only in males, as it is formed by several copies of the Y chromosome (Mello et al., 1986). Some phenotypes have been considered as indicative of cell survival (heterochromatin decondensation and nuclear fusion) or cell death (apoptosis and necrosis) in response to stress conditions (Garcia et al., 2000a,b). Some nuclei were assumed as suspicious of undergoing apoptosis on the basis of their deep staining capability paired with a lack of extreme condensation (Garcia et al., 2000a,b; Mello et al., 2001).

*2.4. Statistics*

The parametric Weibull test was used to assess the influence of the stress conditions on the survival of the infected insects. Some insects were expected to remain alive after the 45-day experimental period; these subjects represented censored observations. The Weibull model has been used to analyse survival data for parasitoids (Tingle and Copland, 1989) and *Rhodnius prolixus*, another Chagas' disease vector (Chaves et al., 2004). A more detailed explanation of survival analysis can be found elsewhere (Kalbfleisch and Prentice, 1980).

Homogeneity tests for subpopulations were used to assess the influence of stress on the parasite ingestion, parasite multiplication and metacyclogenesis. The idea was to compare the number of parasites in insects which were subjected to heat shock after parasite ingestion with those not subjected to heat shock after parasite ingestion, using Chi-square tests. Fisher's exact test was used to examine the influence of stress on the prevalence and intensity of infection. Use of this test was advisable because some cell counts in the present study were low. The computation for the p-value made by the software R was based on a C version of the FORTRAN subroutine FEXACT, which implemented the network developed by Mehta and Patel (1986) and improved by Clarkson et al. (1993). The FORTRAN code can be obtained at http://www.netlib.org/toms/643. More details on the statistical methods suitable for the analysis of categorical data can be found in Agresti's paper (1990).

In order to assess the influence of the heat stress on the nuclear phenotypes displayed by non-infected and infected insects, the relative frequency of the various nuclear phenotypes were compared in both cases using a *t*-test with stabilisation and normalizations of variables through the arcsine transformation. This method is commonly used for the analysis of binary data summarised as proportions (Kalbfleisch and Prentice, 1980).

All statistical analyses were completed using one of the most reliable statistical software programs (R), which can be obtained for free at www.r-project.org.

## 3. Results

*3.1. Survival of T. cruzi-infected P. megistus*

All infected specimens and 98% of those subjected to heat shock survived until 45 days following infection. Survival is thus not significantly affected by heat shock in both infected and non-infected *P. megistus* nymphs (Weibull distribution - coefficient, 0.087; Z, 0.425; P, 0.671).

*3.2. Parasite ingestion and evaluation of stress on the ingested parasites*

The number of the parasites ingested by insects in each glass cylinder (Table 1) when compared with the Chi-square and homogeneity tests demonstrated no significant difference (p = 0.559). The frequency of *T. cruzi* - 77.7% in non-shocked insects (n = 36) and 71.8% in shocked insects (n = 39) found 45 days after parasite ingestion did not significantly differ (p = 0.410), indicating that heat shock did not alter the prevalence of infection. No relationship between the amount of ingested parasites and the number of parasites eliminated in the insect's faeces was observed (Table 1).

The intensity of the infection, as assessed by counting the parasites in 50 microscopic fields, showed no significant difference in parasite density between heat-shocked and

**Table 1.** Number of parasites ingested by the insects per glass cylinder and of epimastigotes and trypomastigotes counted in 50 microscopic fields per specimen subjected or not to heat shock obtained in a snigle experiment.

| Heat shock | Glass cylinder code | Number of parasites ingested in each glass cylinder | Number of parasites eliminated in the insect faeces | | |
|---|---|---|---|---|---|
| | | | Epimastigotes | Trypomastigotes | Total |
| Yes | 1 | 6,972,240 | 57 | 0 | 57 |
| | 2 | 2,958,471 | 111 | 0 | 111 |
| | 3 | 6,901,275 | 76 | 0 | 76 |
| | 4 | 7,645,296 | 148 | 1 | 149 |
| | 5 | 17,943,258 | 38 | 1 | 39 |
| | 6 | 7,603,002 | 68 | 2 | 70 |
| No | 7 | 9,428,712 | 83 | 8 | 91 |
| | 8 | 8,339,442 | 216 | 31 | 247 |
| | 9 | 11,134,513 | 116 | 71 | 187 |
| | 10 | 22,311,225 | 111 | 4 | 115 |
| | 11 | 4,862,936 | 131 | 10 | 141 |
| | 12 | 10,008,250 | 111 | 3 | 114 |

**Table 2.** Influence of heat shock on the intensity of *T. cruzi* infection. Parasite density determined in 50 microscopic fields 45 days after infection.

| Heat shock | Parasite density | | | |
|---|---|---|---|---|
| | + | ++ | +++ | ++++ |
| Yes | 13 | 2 | 13 | 0 |
| No | 6 | 3 | 17 | 2 |

Number of insects, 28; Fisher test: p = 0.157. +, low (1-5 parasites); ++, moderate (6-10 parasites); +++, dense (11-100 parasites); ++++, very dense (≥101 parasites).

non-shocked insects (Table 2). When values referred to low (+) and moderate (++) densities were combined and compared to values referred to intense (+++) and very intense (++++) densities (also combined), there was still no difference between shocked and non-shocked insects (p = 0.085).

*3.3. Influence of heat shock on metacyclogenesis*

The frequency of epimastigotes in the post-infection period here studied was much higher than that of trypomastigotes, in both shocked and non-shocked insects (Table 1) (Chi-square test, p = 0.00001). Fewer epimastigotes and trypomastigotes were observed following heat shock (Table 1) (p = 0.048). Whereas the relative frequency of epimastigotes in non-shocked specimens varied from 62.0 to 97.4%, in heat-shocked specimens it varied from 97 to 100%. When considering the trypomastigotes, their relative frequency varied from 2.6 to 38.0% in non-shocked specimens, and from 0 to 2.9% in heat-shocked specimens.

*3.4. Nuclear phenotypes*

Not only nuclei with the normal phenotype, but also nuclei with heterochromatin decondensation or showing necrotic and apoptotic characteristics were observed in the Malpighian tubules of *P. megistus* specimens infected with *T. cruzi* and subjected or not to heat shock as well as in the non-infected specimens subjected or not to heat shock (Tables 3-6). Giant nuclei arisen by nuclear/cellular fusion were rare in all the situations (Tables 3-6).

Heat shock did not significantly affect the frequency of the different nuclear phenotypes present in the infected insects except for heterochromatin decondensation which increased after heat shock in non-infected and infected specimens. The relative frequency of the heterochromatin decondensation in non-infected and non-shocked specimens varied from 0.04 to 0.69%, whereas in non-infected and shocked specimens it varied from 0.06 to 2.69% (Tables 3 and 4). In non-shocked infected specimens this phenotype varied from 0.01 to 0.38%, whereas in heat-shocked infected specimens it varied from 0.14 to 2.29% (Tables 5 and 6). In both non-infected and infected insects the differences regarding effect of the heat shock on heterochromatin decondensation were statistically significant (Table 7).

## 4. Discussion

The results of the present study, using fourth-instar nymphs of *P. megistus* infected with *T. cruzi* II, demonstrated that insect survival is not affected by the parasite ingestion followed or not by a heat shock at 40 °C for 1 hour.

The high percentage of dense infestation by *T. cruzi* in *P. megistus* as observed in this study (68.9% in non-shocked specimens) is consistent with previous findings by Perlowagora-Szumlewicz and Muller (1987), who reported that over 60% of the insect specimens used harbored dense or very dense parasite populations after feeding on animals infected with *T. cruzi* II (Y strain). Heat shock did not significantly change the intensity of infection.

**Table 3.** Frequencies of nuclear phenotypes in Malpighian tubule epithelial cells of fourth-instar *P. megistus* nymphs.

| Insect code | Nuclear phenotypes (%) | | | | | | No. of nuclei counted |
|---|---|---|---|---|---|---|---|
| | A | $A_s$ | NE | G | HD* | N | |
| $I_1$ | 0 | 0.53 | 6.45 | 0 | 0.23 | 92.79 | 15,126 |
| $I_2$ | 0 | 1.27 | 12.13 | 0 | 0.20 | 86.40 | 11,315 |
| $I_3$ | 0 | 2.19 | 9.77 | 0 | 0.04 | 88.00 | 10,407 |
| $I_4$ | 0 | 0.86 | 9.12 | 0 | 0.23 | 89.78 | 10,324 |
| $I_5$ | 0 | 3.38 | 7.82 | 0 | 0.33 | 88.47 | 15,377 |
| $I_6$ | 0 | 1.60 | 5.29 | 0 | 0.06 | 93.05 | 8,558 |
| $I_7$ | 0 | 1.09 | 2.38 | 0 | - | 96.53 | 11,793 |
| $I_8$ | 0 | 1.18 | 4.85 | 0 | - | 93.97 | 14,365 |
| $I_9$ | 0 | 0.17 | 4.38 | 0 | 0.14 | 95.31 | 12,585 |
| $I_{10}$ | 0 | 0.84 | 4.44 | 0 | 0.23 | 94.48 | 14,202 |
| $I_{11}$ | 0 | 2.10 | 3.15 | 0 | - | 94.75 | 14,358 |
| $I_{12}$ | 0 | 0.70 | 3.08 | 0 | - | 96.22 | 15,504 |
| $I_{13}$ | 0 | 0.27 | 14.56 | 0 | - | 85.17 | 11,028 |
| $I_{14}$ | 0 | 1.05 | 3.39 | 0 | 0.17 | 95.39 | 9,048 |
| $I_{15}$ | 0 | 2.48 | 2.73 | 0 | 0.30 | 94.49 | 11,935 |
| $I_{16}$ | 0 | 0.44 | 12.41 | 0 | 0.35 | 86.8 | 13,036 |
| $I_{17}$ | 0 | 0.94 | 7.10 | 0.01 | - | 91.95 | 11,110 |
| $I_{18}$ | 0.05 | 0.31 | 7.84 | 0.04 | - | 91.76 | 10,260 |
| $I_{19}$ | 0 | 0.52 | 4.39 | 0 | - | 95.09 | 10,006 |
| $I_{20}$ | 0 | 0.56 | 5.32 | 0.02 | - | 94.10 | 13,301 |
| $I_{21}$ | 0.01 | 0.52 | 2.47 | 0 | 0.58 | 96.42 | 11,414 |
| $I_{22}$ | 0.11 | 0.12 | 8.60 | 0.01 | 0.69 | 90.47 | 8,017 |

A: apoptosis; $A_s$: suspected apoptosis; NE: necrosis; G: giant nuclei; HD: heterochromatin decondensation; $I_7$, $I_8$, $I_{11}$-$I_{13}$, $I_{17}$-$I_{20}$: female nymphs; N: normal nuclei; *: phenotype detected only in male nymphs.

The individual variations observed in the insect groups exposed simultaneously to the same infection conditions could have been caused by factors such as different parasite nutrient assimilation capacities. Alternatively, the accumulation of a toxic metabolic end product may have caused the premature death of some parasites (Perlowagora-Szumlewicz and Muller, 1987). These factors could result in the spontaneous elimination of some parasites, with a consequent reduction in their numbers below the levels of detection (Perlowagora-Szumlewicz and Muller, 1987).

Prolonged starvation of blood-sucking insects has deleterious effects on the multiplication and persistence of their parasites (Perlowagora-Szumlewicz and Muller, 1987; Garcia et al., 2007). Furthermore, provision of additional blood meals to the insect accelerates parasite multiplication and stimulates epimastigote division and subsequent differentiation into metacyclic forms (Piesman and Sherlock, 1985; Perlowagora-Szumlewicz and Muller, 1987). Similar results have been observed by Garcia and Azambuja (2000) for specimens fasted up to four weeks. In the present investigation, the period over which insects were starved (45 days) is considered to be moderate, as the starved insects were seen to still contain undigested food.

In this study, the *T. cruzi* present in the infected insects were mostly represented by epimastigotes, which, in the case of the non-shocked insects made up 62 to 97.4% and in the case of the heat-shocked insects, made up 97 to 100% of the total parasites. The lower frequencies of trypomastigotes in comparison to the frequency reported by Schaub (1989b) was probably a consequence of the lack of an additional blood meal offered to the insects (Perlowagora-Szumlewicz and Muller, 1987; Schaub and Lösch, 1988), as discussed above. According to Perlowagora-Szumlewicz and Moreira (1994), on the 45th day post-infection, the proportion of epimastigotes in *T. cruzi*-infected *P. megistus* was 86.2% and that of the metacyclics was 2.0%. However, 90 days after infection, the relative frequency of the metacyclic forms began to increase whereas that of the epimastigotes decreases. There is a report of trypomastigotes representing 3-11 and 15-25% of the parasites present in the rectum of *Triatoma infestans* four to nine weeks after infection depending on the *T. cruzi* strain used; after this period, trypomastigotes increase to 30-50%, with a concomitant decrease in the percentage of epimastigotes (Schaub, 1989b). Although the infected insects were followed up only 45 days long in a single experiment, predominance of epimastigotes

**Table 4.** Frequencies of nuclear phenotypes in Malpighian tubule epithelial cells of fourth-instar *P. megistus* nymphs subjected to heat shock at 40 °C for 1 hour.

| Insect code | Nuclear phenotypes (%) | | | | | | No. of nuclei counted |
|---|---|---|---|---|---|---|---|
| | A | $A_s$ | NE | G | HD* | N | |
| $I_1$ | 0.30 | 0.19 | 11.57 | 0.01 | 0.23 | 87.70 | 11,992 |
| $I_2$ | 0.01 | 0.72 | 6.15 | 0 | 0.13 | 92.99 | 14,292 |
| $I_3$ | 0 | 1.00 | 3.27 | 0 | 0.08 | 95.65 | 15,387 |
| $I_4$ | 0 | 2.51 | 3.17 | 0 | 0.36 | 93.96 | 14,452 |
| $I_5$ | 0 | 0.28 | 27.60 | 0.02 | - | 72.10 | 19,078 |
| $I_6$ | 0 | 1.35 | 4.91 | 0 | - | 93.74 | 10,867 |
| $I_7$ | 0 | 1.61 | 7.25 | 0 | - | 91.14 | 11,661 |
| $I_8$ | 0 | 0.50 | 7.07 | 0.01 | 0.06 | 92.36 | 12,673 |
| $I_9$ | 0 | 0.60 | 3.97 | 0.01 | - | 95.41 | 7,477 |
| $I_{10}$ | 0 | 2.95 | 6.19 | 0 | - | 90.86 | 8,903 |
| $I_{11}$ | 0 | 1.75 | 5.01 | 0 | 0.18 | 93.06 | 8,900 |
| $I_{12}$ | 0 | 1.36 | 2.22 | 0 | - | 96.42 | 11,295 |
| $I_{13}$ | 0 | 0.53 | 2.84 | 0 | 0.36 | 96.27 | 11,966 |
| $I_{14}$ | 0 | 0.22 | 8.22 | 0 | 0.98 | 90.58 | 9,257 |
| $I_{15}$ | 0 | 0.70 | 2.55 | 0.01 | 2.69 | 94.05 | 8,155 |
| $I_{16}$ | 0 | 1.12 | 1.86 | 0 | 2.61 | 94.41 | 9,344 |
| $I_{17}$ | 0 | 0.67 | 4.30 | 0 | - | 95.03 | 12,325 |
| $I_{18}$ | 0 | 0.61 | 1.95 | 0 | - | 97.44 | 8,659 |
| $I_{19}$ | 0 | 1.58 | 0.88 | 0.1 | 0.57 | 96.96 | 9,831 |
| $I_{20}$ | 0.02 | 1.01 | 2.88 | 0 | 0.49 | 95.60 | 12,453 |

A: apoptosis; $A_s$: suspected apoptosis; NE: necrosis; G: giant nuclei; HD: heterochromatin decondensation; $I_5$-$I_7$, $I_9$-$I_{10}$, $I_{12}$, $I_{17}$-$I_{18}$: female nymphs; N: normal nuclei; *: phenotype detected only in male nymphs.

**Table 5.** Frequencies of nuclear phenotypes in Malpighian tubule epithelial cells of fourth-instar *P. megistus* nymphs infected with *T. cruzi*.

| Insect code | Nuclear phenotypes (%) | | | | | | No. of nuclei counted |
|---|---|---|---|---|---|---|---|
| | A | $A_s$ | NE | G | HD* | N | |
| $I_1$ | 0 | 3.82 | 7.53 | 0 | 0.20 | 88.45 | 9,530 |
| $I_2$ | 0 | 1.34 | 6.58 | 0.08 | 0.12 | 91.88 | 15,374 |
| $I_3$ | 0.01 | 3.07 | 3.73 | 0.01 | 0.01 | 93.17 | 16,590 |
| $I_4$ | 0.02 | 1.01 | 4.95 | 0 | 0.06 | 93.96 | 13,425 |
| $I_5$ | 0.04 | 0.86 | 10.64 | 0.02 | 0.10 | 88.34 | 9,937 |
| $I_6$ | 0 | 1.60 | 4.22 | 0 | 0.06 | 94.12 | 10,625 |
| $I_7$ | 0 | 0.64 | 7.84 | 0 | - | 91.52 | 12,850 |
| $I_8$ | 0 | 0.49 | 4.29 | 0 | - | 95.22 | 14,758 |
| $I_9$ | 0 | 2.49 | 6.33 | 0 | 0.21 | 90.97 | 12,956 |
| $I_{10}$ | 0 | 2.52 | 7.65 | 0 | 0.38 | 89.45 | 10,805 |
| $I_{11}$ | 0 | 0.43 | 3.23 | 0 | - | 96.34 | 13,351 |
| $I_{12}$ | 0 | 0.21 | 2.62 | 0 | - | 97.17 | 10,921 |
| $I_{13}$ | 0 | 0.78 | 4.12 | 0.03 | 0.19 | 94.88 | 11,942 |
| $I_{14}$ | 0 | 1.13 | 2.63 | 0 | 0.05 | 96.19 | 12,666 |
| $I_{15}$ | 0.03 | 1.92 | 4.48 | 0 | 0.23 | 93.34 | 13,994 |
| $I_{16}$ | 0 | 0.91 | 5.00 | 0 | 0.07 | 94.02 | 11,168 |

A: apoptosis; $A_s$: suspected apoptosis; NE: necrosis; G: giant nuclei; HD: heterochromatin decondensation; $I_7$, $I_8$, $I_{11}$, $I_{12}$: female nymphs; N: normal nuclei; *: phenotype detected only in male nymphs.

**Table 6.** Frequencies of nuclear phenotypes in Malpighian tubule epithelial cells of fourth- instar *P. megistus* nymphs infected with *T. cruzi* and subjected to heat shock at 40 °C for1 hour.

| Insect code | Nuclear phenotypes (%) | | | | | | No. of nuclei counted |
|---|---|---|---|---|---|---|---|
| | A | $A_s$ | NE | G | HD* | N | |
| $I_1$ | 0 | 3.58 | 2.57 | 0.06 | - | 93.79 | 7,794 |
| $I_2$ | 0 | 7.63 | 7.36 | 0.01 | - | 85.00 | 12,235 |
| $I_3$ | 0 | 1.03 | 5.56 | 0 | - | 93.41 | 10,307 |
| $I_4$ | 0 | 2.36 | 8.09 | 0 | - | 89.55 | 11,502 |
| $I_5$ | 0 | 2.41 | 4.01 | 0.02 | 0.37 | 93.19 | 9,292 |
| $I_6$ | 0 | 1.97 | 4.81 | 0 | 0.41 | 92.81 | 11,125 |
| $I_7$ | 0 | 1.97 | 3.03 | 0.02 | 0.30 | 94.68 | 8,121 |
| $I_8$ | 0.01 | 1.00 | 8.92 | 0.01 | 0.63 | 89.43 | 7,827 |
| $I_9$ | 0 | 4.96 | 2.31 | 0 | - | 92.73 | 7,245 |
| $I_{10}$ | 0 | 1.18 | 3.90 | 0 | - | 94.92 | 9,253 |
| $I_{11}$ | 0 | 1.56 | 3.70 | 0 | 0.55 | 94.19 | 9,048 |
| $I_{12}$ | 0.02 | 0.58 | 1.72 | 0.02 | - | 97.66 | 10,762 |
| $I_{13}$ | 0 | 0.09 | 3.70 | 0.02 | 2.29 | 93.90 | 6,114 |
| $I_{14}$ | 0 | 0.95 | 2.91 | 0 | - | 96.14 | 7,054 |
| $I_{15}$ | 0 | 3.30 | 2.80 | 0 | 0.14 | 93.76 | 9,503 |
| $I_{16}$ | 0 | 1.88 | 6.63 | 0 | - | 91.49 | 11,157 |

A: apoptosis; $A_s$: suspected apoptosis; NE: necrosis; G: giant nuclei; HD: heterochromatin decondensation; $I_1$-$I_4$, $I_9$, $I_{10}$, $I_{12}$, $I_{14}$, $I_{16}$: female nymphs; N: normal nuclei; *: phenotype detected only in male nymphs.

**Table 7.** Analysis of variance (ANOVA) with two factors for the various nuclear phenotypes.

| Nuclear phenotype | Heat shock vs. control conditions | Infection vs infection plus heat shock |
|---|---|---|
| A | 0.82 | 0.87 |
| As | 0.75 | 0.15 |
| NE | 0.44 | 0.24 |
| G | 0.19 | 0.32 |
| HD (*) | <u>0.01</u> | <u>0.01</u> |
| N | 0.64 | 0.87 |

A: apoptosis; $A_s$: suspected apoptosis; NE: necrosis; G: giant; HD: heterochromatin decondensation; N: normal nuclei. *: phenotype detected only in male nymphs. Phenotype frequencies that are statistically significant with $p < 0.05$ are underlined.

on trypomastigotes at this period was in agreement with data by Perlowagora-Szumlewicz and Moreira (1994).

Confirmation of the hypothesis of *T. cruzi* parasitism followed by heat shock inducing thermotolerance in *P. megistus* was partly prejudiced as survival of both shocked and non-shocked infected nymphs was high and not affected during the period in which they were accompanied. However, the increase in frequency of heterochromatin decondensation in Malpighian tubules of the infected specimens subjected to heat shock may be in agreement with this hypothesis.

The influence of temperature on *T. cruzi* in cell cultures, in experimentally infected mammals and in vectors infected naturally or in the laboratory has been previously reported (Wood, 1954; Neves, 1971). In insects infected in the laboratory, cold temperatures (0 and 5 °C) inhibit the development of *T. cruzi*, which, in *Triatoma infestans*, is maintained in blood forms for 35 days. Exposure to higher temperatures (36-37 °C) for 40 days also inhibits the development of *T. cruzi*, especially its multiplication, giving rise to a small number of parasites (Neves, 1971). Thermal changes accompanied by induction of heat shock protein translation have been reported for *T. cruzi* (Gambiagi-deMarval et al., 1993, 1996).

With regard to the analysis of the nuclear phenotypes in the Malpighian tubules of infected *P. megistus*, the apparently high frequency of nuclei suspected of apoptosis was not sufficient to affect all the insects survival until 45 days after infection, a situation which was not significantly affected by heat shock. The significant increase in heterochromatin decondensation in infected as well as in non-infected fourth-instar nymphs with the heat shock, suggestive of cell survival processes, probably contributed to Malpighian tubule cell survival (Garcia et al., 2000a,b; Mello et al., 2001).

Of the parameters examined herein, the alteration of *T. cruzi* multiplication in *P. megistus* is of great importance. Considering that this event is affected by even a short heat shock, greater care should be taken in the manipulation of infected insects during experimentation in order to avoid jeopardising results; insect exposure to stressors such as

temperature changes and handling should be avoided. Heat shock did not decrease the survival rate of *P. megistus*, but it decreased *T. cruzi* multiplication and metacyclogenesis. The changes associated with the multiplication and metacyclogenesis of *T. cruzi* and the mechanisms of resistance developed by the vector should be considered when one intends to promote adequate conditions for rearing infected *P. megistus* in the laboratory. Such knowledge is important because this species is a very convenient model for xenodiagnosis (Perlowagora-Szumlewicz et al., 1988; Lima et al., 1992; Pereira et al., 1996).

The results presented here are restricted to infection of *P. megistus* by *T. cruzi* II, formerly designated as Y strain (Zingales et al., 2009). It is not to be overlooked that differences in *P. megistus* responses to *T. cruzi* infection followed by heat shock may occur, when considering different strains of this parasite. In *T. cruzi* strains CL Brener (VI group) and PBOL, hsp60 genes, important agents of the heat shock response, are differently organized (Giambiagi-deMarval et al., 1993, 1996). In addition, different *T. cruzi* populations have been reported to cause different effects on rat organs (Camargos et al., 2000) and to present chromosomal polymorphism, genome heterogeneity both structurally and in overall size, and variation in abundance of repetitive DNA sequences (Dvorak et al., 1982; Vargas et al., 2004; Lewis et al., 2009).

*Acknowledgements* – LSG thanks Liléia Diotaiuti and Evandro M. M. Machado for their hospitality at Fiocruz (Rio de Janeiro), and Raquel M. Pacheco and Leilaine R. Oliveira (IMECC-UNICAMP, Campinas) for helpful discussions on the statistical analyses. This study was part of a thesis presented by SLG to the Institute of Biology, UNICAMP, Campinas, in partial fulfilment of the requirements for the PhD degree, and received financial support from FAPESP (grants no. 98/0826-4 and 99/2547-8) and CNPq.

## References

AGRESTI, A., 1990. *Categorical Data Analysis.* New York: Wiley.

ALVARENGA, NJ. and BRONFEN, E., 1984. Interação do *Trypanosoma cruzi* com diferentes vetores: uso para o xenodiagnóstico. *Revista da Sociedade Brasileira de Medicina Tropical,* vol. 17, p. 145-149.

ANDRADE, SG., 1974. Caracterização de cepas do *Trypanosoma cruzi* isoladas no Recôncavo Baiano. (Contribuição ao estudo da patologia geral da doença de Chagas em nosso meio). *Revista de Patologia Tropical,* vol. 3, p. 65-121.

BARBOSA, SE., SOARES, RPP., PIRES, HHR. and DIOTAIULI, L., 2001. Experimental evidence for a demographic cline in *Panstrongylus megistus* populations. *Memórias do Instituto Oswaldo Cruz,* vol. 96, p. 773-775.

CAMARGOS, ERS., FRANCO, DJ., GARCIA, CMMG., DUTRA, AP., TEIXEIRA, AL., CHIARI, E. and MACHADO, CRS., 2000. Infection with different *Trypanosoma cruzi* populations in rats: myocarditis, cardiac sympathetic denervation, and involvement of digestive organs. *American Journal of Tropical Medicine and Hygiene*, vol. 62, p. 604-612.

CHAVES, LF., HERNANDEZ, MJ., REVILA, TA., RODRIGUEZ, DJ. and RABINOVICH, JE., 2004. Mortality profiles of *Rhodnius prolixus* (Heteroptera: Reduviidae), vector of Chagas disease. *Acta Tropica*, vol. 92, p. 119-125. PMid:15350863. doi:10.1016/j.actatropica.2004.06.005

CLARKSON, DB., FAN, Y. and JOE, H., 1993. A remark on algorithm 643: FEXACT: an algorithm for performing Fisher's Exact Test in r x c contingency tables. *ACM Transactions on Mathematical Software,* vol. 19, p. 484-488. doi:10.1145/168173.168412

CONTRERAS, VT., De LIMA, AR. and ZORRILLA, G., 1998. *Trypanosoma cruzi*: Maintenance in culture modifies gene and antigenic expression of metacyclic trypomastigotes. *Memórias do Instituto Oswaldo Cruz,* vol. 93, p. 753-760.

DVORAK, JA., HALL, TE., CRANE, MSJ., ENGEL, JC., MCDANIEL, JP. and URIEGAS, R., 1982. *Trypanosoma cruzi*: flow cytometric analysis. I. Analysis of total DNA/organism by means of mithramycin-induced fluorescence. *Journal of Protozoology*, vol. 29, p. 430-437.

FORATTINI, OP., 1980. Biogeografia, origem e distribuição da domiciliação de triatomíneos no Brasil. *Revista de Saúde Pública,* vol. 14, p. 256-299.

GAMBIAGI-deMARVAL, M., SOUTO-PADRON, T. and RONDINELLI, E., 1996. Characterization and cellular distribution of heat-shock proteins HSP70 and HSP60 in *Trypanosoma cruzi. Experimental Parasitology*, vol. 83, p. 335-345. PMid:8823250. doi:10.1006/expr.1996.0081

GARCIA, ES. and AZAMBUJA, P., 1991. Development and interaction of *Trypanosoma cruzi* within the insect vector. *Parasitology Today*, vol. 7, p. 240-244. doi:10.1016/0169-4758(91)90237-I

-, 2000. Fisiologia dos triatomíneos: desenvolvimento, reprodução e interação com *Trypanosoma cruzi*. In BRENER, Z., ANDRADE, ZA. and BARRAL-NETTO, M. (Eds.) *Trypanosoma cruzi e Doença de Chagas*. Rio de Janeiro: Guanabara Koogan Ed. p. 41-47.

GARCIA, ES., RATCLIFFE, NA., WHITTEN, MM., GONZALEZ, MS. and AZAMBUJA, P., 2007. Exploring the role of insect host factors in the dynamics of *Trypanosoma cruzi-Rhodnius prolixus* interactions. *Journal of Insect Physiology*, vol. 53, p. 11-21. PMid:17141801. doi:10.1016/j.jinsphys.2006.10.006

GARCIA, SL., GARCIA, NL., OLIVEIRA, LR., RODRIGUES, VLCC. and MELLO, MLS., 2003. Experimentally induced heat- and cold-shock tolerance in adult *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae). *Brazilian Journal of Biology*, vol. 63, p. 449-455.

GARCIA, SL., GARCIA, NL., RODRIGUES, VLCC. and MELLO, MLS., 2001a. Effect of sequential cold shocks on survival and molting incidence in *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae). *Cryobiology,* vol. 41, p. 74-77.

GARCIA, SL., MELLO, MLS., GARCIA, NL. and RODRIGUES, VLCC., 2000a. Changes in nuclear phenotypes following cold shock in *Panstrongylus megistus* (Burmeister). *Memórias do Instituto Oswaldo Cruz,* vol. 95, p. 893-898.

-, 2001b. Experimentally induced heat-shock tolerance in *Panstrongylus megistus* (Hemiptera: Reduviidae). *Journal of Medical Entomology*, vol. 38, p. 510-513. PMid:11476330. doi:10.1603/0022-2585-38.4.510

GARCIA, SL., MELLO, MLS., RODRIGUES, VLCC. and GARCIA, NL., 2000b. Nuclear phenotype changes after heat

shock in *Panstrongylus megistus* (Burmeister). *Memórias do Instituto Oswaldo Cruz*, vol. 95, p. 271-277.

GARCIA, SL., PACHECO, RM., RORIGUES, VLCC. and MELLO, MLSM., 2002. Effect of sequential heat and cold shocks on nuclear phenotypes of the blood-sucking insect, *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae). *Memórias do Instituto Oswaldo Cruz*, vol. 97, p. 1111-1116.

GARCIA, SL., RODRIGUES, VLCC., GARCIA, NL., FERRAZ-FILHO, NA. and MELLO, MLS., 1999. Survival and molting incidence after heat and cold shocks in *Panstrongylus megistus* Burmeister. *Memórias do Instuto Oswaldo Cruz,* vol. 94, p. 131-137.

GIAMBIAGI-deMARVAL, M., GOTTESDIENER, K., RONDINELLI, E. and VAN DER PLOEG, LHT., 1993. Predicted amino acid sequence and genomic organization of *Trypanosoma cruzi hsp*60 genes. Molecular and Biochemical Parasitology, vol. 58, p. 25-32. doi:10.1016/0166-6851(93)90087-E

GIAMBIAGI-deMARVAL, M., SOUTO-PADRÓN, T. and RONDINELLI, E., 1996. Characterization and cellular distribution of heat-shock proteins HSP70 and HSP60 in *Trypanosoma cruzi*. *Experimental Parasitology*, vol 83, p. 335-345. PMid:8823250. doi:10.1006/expr.1996.0081

KALBFLEISCH, JD. and PRENTICE, RL., 1980. *The Statistical Analysis of Failure Time Data*. New York: John Wiley & Sons.

LANA, M., PINTO, AS., BARNABÉ, C., QUESNEY, V. and TIBAYRENC, M., 1998. *Trypanosoma cruzi*: compared vectorial transmissibility of the major clonal genotypes by *Triatoma infestans*. *Experimental Parasitology,* vol. 90, p. 20-25. doi:10.1006/expr.1998.4304

LEWIS, MD., LLEWELLYN, MS., GAUNT, MW., YEO, M. and CARRASCO, HJ., 2009. Flow cytometric analysis and microsatellite genotyping reveal extensive DNA content variation in *Trypanosoma cruzi* populations and expose contrasts between natural and experimental hybrids. *International Journal for Parasitology*, vol. 39, p. 1305-1317. PMid:19393242. PMCid:2731025. doi:10.1016/j.ijpara.2009.04.001

LIMA, MM., PEREIRA, JB., DOS SANTOS, JAA., PINTO, ZT. and BRAGA, MV. 1992.. Development and reproduction of *Panstrongylus megistus* (Hemiptera, Reduviidae) infected with *Trypanosoma cruzi*, under laboratory conditions. *Annals of the Entomological Society of America*, vol. 85, p. 458-461.

MEHTA, CR. and PATEL, NR , 1986. Algorithm 643. FEXACT: A FORTRAN subroutine for Fisher's exact test on unordered r x c contingency tables. *ACM Transactions on Mathematical Software,* vol. 12, p. 154-161. doi:10.1145/6497.214326

MELLO, MLS., 1997. Cytochemistry of DNA, RNA, and nuclear proteins. *Brazilian Journal of Genetics,* vol. 20, p. 257-264.

MELLO, MLS., RANDI, MA., GIORGIO, S., FERRAZ-FILHO, AN., RODRIGUES, VLCC., ROCHA-E-SILVA, EO. and CORDEIRO, JA., 1986. Number of chromosomes, Feulgen-DNA content and nuclear phenotypes in domestic and wild specimens of *Panstrongylus megistus*. *Annals of Tropical Medicine and Parasitology*, vol. 80, p. 641-648.

MELLO, MLS., TAVARES, MCH., DANTAS, MM., RODRIGUES, VLCC., MARIA-ENGLER, SS., CAMPOS, SGP. and GARCIA, NL., 2001. Cell death and survival alterations in Malpighian tubules of *Triatoma infestans* following heat shock. *Biochemistry and Cell Biology,* vol. 79, p. 709-717. PMid:11800011. doi:10.1139/bcb-79-6-709

NEVES, DP., 1971. Influência da temperatura na evolução do *Trypanosoma cruzi* em triatomíneos. *Revista do Instituto de Medicina Tropical, São Paulo*, vol. 13, p. 155-161.

PEREIRA, JB., JUNQUEIRA, ACV., SANTOS, LC., DE CASTRO, JAF., DE ARAÚJO, IB.and COURA, JR., 1996. Xenodiagnóstico na doença de Chagas crônica I – sensibilidade de *Panstrongylus megistus* e *Triatoma infestans*. *Revista da Sociedade Brasileira de Medicina Tropical,* vol. 29, p. 341-347.

PERLOWAGORA-SZUMLEWICZ, A. and MOREIRA, CJC., 1994. In vivo differentiation of *Trypanosoma cruzi*. 1. Experimental evidence of the influence of vector species on metacyclogenesis. *Memórias do Instituto Oswaldo Cruz,* vol. 89, p. 603-618.

PERLOWAGORA-SZUMLEWICZ, A. and MULLER, CA., 1987. Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas' disease. 2- Attempts to upgrade the reliability and the efficacy of xenodiagnosis in chronic Chagas'disease. *Memórias do Instituto Oswaldo Cruz,* vol. 82, p. 259-272. doi:10.1590/S0034-89101988000500004

PERLOWAGORA-SZUMLEWICZ, A., MULLER, CA. and MOREIRA, CJC., 1988. Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas' disease: 3- On the interaction of the vector species and parasite strain in the reaction of bugs to infection by *Trypanosoma cruzi*. *Revista de Saúde Pública*, vol. 22, p. 390-400.

-, 1990. Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas' disease. 4- The reflection of parasite stock in the responsiveness of different vector species to chronic infection with different *Trypanosoma cruzi* stocks. *Revista de Saúde Pública,* vol. 24, p.165-177.

PIESMAN, J. and SHERLOCK, IA., 1985. *Trypanosoma cruzi*: kinetics of metacyclogenesis in adult and nymphal *Panstrongylus megistus*. *Experimental Parasitology,* vol. 59, p. 231-238. doi:10.1016/0014-4894(85)90077-3

RODRIGUES, VLCC., MELLO, MLS., FERRAZ-FILHO, NA. and DANTAS, MM., 1991. Sobrevivência e ocorrência de muda em *Triatoma infestans* Klug (Hemiptera, Reduviidae) após choque de temperatura. *Revista de Saúde Pública*, vol. 25, p. 461-467.

SCHAUB, GA., 1989a. Does *Trypanosoma cruzi* stress its vectors? *Parasitology Today*, vol. 5, p. 185-188. doi:10.1016/0169-4758(89)90142-7

-, 1989b. *Trypanosoma cruzi*: quantitative studies of development of two strains in the small intestine and rectum of the vector *Triatoma infestans*. *Experimental Parasitology,* vol. 68, p. 260-273. doi:10.1016/0014-4894(89)90108-2

SCHAUB, GA. and LÖSCH, P., 1988. *Trypanosoma cruzi*: origin of metacyclic trypomastigotes in the urine of the vector *Triatoma infestans*. *Experimental Parasitology,* vol. 65, p. 174-186. doi:10.1016/0014-4894(88)90121-X

SCHMUÑIS, GA., 2000. A tripanosomíase americana e seu impacto na saúde pública das Américas. In BRENER, Z., ANDRADE, ZA. and BARRAL-NETTO, M. (Eds.). *Trypanosoma cruzi e Doença de Chagas* Rio de Janeiro: Guanabara Koogan Ed. p. 1-12.

SILVA, IG. and SILVA, HHG., 1993. Influência da temperatura na biologia de triatomíneos. III *Panstrongylus megistus* (Burmeister, 1835) (Hemiptera, Reduviidae). *Revista Brasileira de Entomologia,* vol. 37, p. 489-496.

SILVA, IG., SANTOS, LGP., NAKANO, R. and BADAUY, RC., 1994. Capacidade de replicação da cepa Y *Trypanosoma*

*cruzi* em diferentes espécies de triatomíneos. *Revista de Patologia Tropical,* vol. 23, p. 197-204.

SILVEIRA, A., 2000. Profilaxia. In BRENER, Z., ANDRADE, ZA. and BARRAL-NETTO, M. (Eds.). *Trypanosoma cruzi e Doença de Chagas* Rio de Janeiro: Guanabara Koogan Ed. p. 75-86

TINGLE, CCD. and COPLAND, MJW., 1989. Progeny production and adult longevity of the mealybug parasitoids *Anagyrus pseudococci, Leptomastix dactylopi* and *Leptomastidea abnormis* (Hym.: Encyrtidae) in relation to temperature. *Entomophaga*, vol. 34, p. 111-120. doi:10.1007/BF02372594

VALLEJO, GA., GUHL, F. and SCHAUB, GA., 2009. Triatominae-*Trypanosoma cruzi/T. rangel*i: vector-parasite interactions. *Acta Tropica*, vol.110, p. 137-147. PMid:18992212. doi:10.1016/j.actatropica.2008.10.001

VARGAS, N., PEDROSO, A. and ZINGALES, B., 2004. Chromosomal polymorphism, gene synteny and genome size in *T. cruzi* I and *T. cruzi* II groups. *Molecular and Biochemical Parasitology*, vol 138, p. 131-141. PMid:15500924. doi:10.1016/j.molbiopara.2004.08.005

WOOD, SF., 1954. Environmental temperature as a factor in development of *T. cruzi* in *Triatoma protracta*. *Experimental Parasitology,* vol. 3, p. 227-233. doi:10.1016/0014-4894(54)90021-1

ZINGALES, B., ANDRADE, SG., BRIONES, MRS., CAMBELL, DA., CHIARI, E., FERNANDES, O., GUHL, F., LAGES-SILVA, E., MACEDO, AM., MACHADO, CR., MILES, MA., ROMANHA, AJ., STURM, NR., TIBAYRENC, M. and SCHIJMAN, AG., 2009. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. *Memórias do Instituto Oswaldo Cruz*, vol. 104, p. 1051-1054.