Biology of *Triatoma carcavalloi* Jurberg, Rocha & Lent, 1998 under laboratory conditions

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**ABSTRACT**

**Introduction:** *Triatoma carcavalloi* is a wild species that is found in sympatry with *Triatoma rubrovaria* and *Triatoma circummaculata*, which are vectors of *Trypanosoma cruzi* currently found in rural areas of Rio Grande do Sul, Brazil. **Methods:** Fertility was assessed and to determine the incubation period, the eggs were observed until hatching. The first meal was offered to 1st stage nymphs. The intermolt period was also determined. The number of blood meals was quantified at each nymphal stage and the resistance to fasting as the period between ecdysis and death. Mortality was assessed and longevity was determined by recording the time that elapsed from molting to the adult stage and until death. The developmental cycle was assessed by recording the length in days of each stage from molting to adult hood. **Results:** The average incubation period was 22.7 days. The average first meal occurred 3.1 days after hatching. The 5th stage nymph to adult intermolt period was the longest at 193.4 days. The average number of feedings during nymphal development was 13.4. The resistance to fasting assay indicated that the 3rd, 4th and 5th stage nymphs presented higher resistance than did adults. The highest mortality rate was observed in the 3rd stage nymphs (22.2%). The average length of adult survival was 25.6 weeks, and the average total life cycle lasted 503.4 days. **Conclusions:** This study is the first report on the biology of *T. carcavalloi* that fed on mice. The presented findings expand the bionomic knowledge of these species.

**Keywords:** Triatominae. *Triatoma carcavalloi*. Biological behavior.

**INTRODUCTION**

The Brazilian State of Rio Grande do Sul harbors eleven species of triatomines, including introduced species with synanthropic habits. These insects are associated with bird nests, mammals and rocky habitats. They are considered autochthonous1 and are dispersed in a discontinuous pattern throughout the state, with some species restricted to the central-southern region and others in the northwest and northeast regions. Among the wild species of triatomines, *Triatoma rubrovaria* (Blanchard, 1843) is the most important in epidemiological terms due to its wide geographic distribution and capacity to transmit *Trypanosoma cruzi* (Chagas, 1909), followed by *Triatoma circummaculata* (Stål, 1859) and *Triatoma carcavalloi* (Jurberg, Rocha & Lent, 1998). These species are sympatric, inhabiting rocky environments and exhibiting feeding habits that are remarkably eclectic, with irregular intervals observed in their biological cycles2. Field data have shown that *T. rubrovaria* is frequently found in domiciliary and peridomestic areas3. Only *T. carcavalloi* has been observed in Canguçu, Dom Feliciano, Pinheiro Machado and São Jerônimo4 (Source: IPB-LACEN/RS), appearing inside domiciles throughout Rio Grande do Sul3,6.

Morphological observations have led *T. carcavalloi* to be included in the same species complex (*infestans* complex) and subcomplex (*rubrovaria* subcomplex) as *T. rubrovaria*7.

Characterizing the biology of wild species is important while evaluating the efficiency of these insects as vectors of *T. cruzi*, as these species consistently invade environments that are subject to human modification. As a result, knowledge of the biological characteristics of these insects is essential for designing control strategies, mainly in relation to secondary vectors that have the potential to become established in human habitats8.

The life cycles of triatomines vary according to the species, environmental conditions and the availability of suitable sources of blood9,10.

Resistance to fasting could be of great importance and might directly affect control campaigns targeting these vectors11. Tolerance to long periods of fasting helps these...
insects survive difficult periods when food shortages occur. During these periods, they hide in wall cracks and escape residual insecticides, after which they are able to recolonize the household.

The aim of this study was to examine the bionomic features of *Triatoma carcavalloi* under laboratory conditions, including incubation time, fertility, first meal of the 1st stage nymphs, intermolt period, number of blood meals, resistance to starvation, mortality, longevity and developmental cycle to better understand its potential capacity as a vector and to use this knowledge to help monitor this species during control activities.

**METHODS**

Specimens of *T. carcavalloi* were collected in the State of Rio Grande do Sul, Brazil, in Encruzilhada do Sul City (30°32'38"S; 52°31'19"W), from natural ecotopes and peri-household locations. The insects were maintained at 26°C under 70% RH (relative humidity) in the Laboratory of Transmissores de Leishmanioses, Department of Entomologia Médica e Forense, Oswaldo Cruz Institute, Fundação Oswaldo Cruz.

**Fertility**

Sixteen females were individually separated into Borrel tubes closed with nylon mesh and observed daily, and the color of their eggs and whether they hatched was recorded.

**Incubation period**

Sixty eggs from eight females were separated into Borrel tubes closed with nylon mesh (according to the laying date) and then observed daily until they hatched.

**First meal**

Thirty eggs were individually housed in Borrel tubes. After hatching, a blood meal from a Swiss mouse was offered to each nymph within 10 minutes.

**Intermolt period**

Fifty nymphs from each stage, as well as 15 males and 15 females, were separated and placed in individual plastic containers (5.5cm in diameter × 10.5cm in height) containing individuals from the same day of hatching or ecdysis. Filter paper was used to cover the inner surface of the container. The specimens were observed daily from ecdysis until the next stage. The intermolt period was noted as the number of days between two consecutive ecdysis events, and the period of nymphal development was recorded as the number of days between the hatching date and ecdysis into the imago stage.

**Number of blood meals according to the nymphal stage**

The number of blood meals taken during each nymphal stage was quantified. A blood meal was provided once a week until repletion.

**Resistance to fasting**

The period between ecdysis and the death of the insect was recorded. The insects fed on mice (*Mus musculus*) weekly for a period of 60-80 minutes. After ecdysis, the specimens were separated into the nymphs of each phase (n = 50) and adults (n = 30) and stored in Borrel tubes with other individuals from the same day of ecdysis. The specimens were observed daily over the period from ecdysis to death.

**Mortality**

In total, 177 1st stage nymphs were separated, and the mortality was calculated according to the molting of each nymphal stage up to adulthood.

**Longevity**

The time elapsed from the molting of each individual to the adult stage until its death was recorded.

**Developmental cycle**

Sixty eggs laid by females of *T. carcavalloi* obtained from the colony maintained in the laboratory were collected by randomly selection and individually placed in plastic containers that contained filter paper to provide the developing insects with access to a food source. Each container was numbered and examined daily until the egg hatched. The nymphs were observed daily and fed on mice weekly until repletion, and the time required to pass through the various developmental stages and reach adulthood was recorded in days. The development times (total and by stage) were expressed using average values.

**RESULTS**

**Fertility**

The first oviposition was observed on day 6. The mean number of eggs/week was 30. The greatest number of eggs/week was observed during the summer. It is important to note that the eggs that did not hatch were also fertile, as the coloring was the same for all eggs. The eggs were initially light pink and then became red, which intensified as the process of embryogenesis progressed. The eggs exhibited an ellipsoid shape and adhered to the substrate. The oviposition of these females began after the first meal.

**Incubation period**

The mean incubation period of the specimens was 22.7 days, with a minimum of 21 and a maximum of 26 days recorded. Among the 60 eggs observed, only 38.3% hatched.

**First meal**

The 1st stage nymphs obtained their first meal after a mean of 24 days. The first meal occurred during the other nymphal stages after a mean of 3.1 days, with a recorded minimum value of one day and a maximum of five days, although the majority occurred after two or four days.
Intermolting period

Among the 50 nymphs observed, eight died before reaching adulthood (three 1st stage nymphs, four 2nd stage nymphs and one 3rd stage nymph). Table 1 shows that the shortest intermolting period was observed between the 1st/2nd stages (18.5 days) and the longest between the 5th stage/adulthood (193.4 days). The 5th stage nymphs required several blood meals to molt, which occurred after more than one year.

Number of blood meals

The minimum number of blood meals observed in females and males were zero and one meal, respectively, whereas the maximum number (42 meals) was recorded in the 5th stage nymphs. The mean number of feedings throughout all of nymphal development was 13.4 meals (Figure 1).

Resistance to fasting

Observations of all of the nympha and adult (male and female) stages indicated that the 3rd, 4th and 5th stage nymphs presented greater resistance than did the adults, whereas the males displayed similar resistance to the 1st and 2nd stage nymphs and were less resistant overall than were the females (Table 2). The total observation period was 8 months.

Mortality

From the initial 177 Triatoma carcavalloi nymphs, only 95 reached the adult stage (global mortality rate of 57.2%). The mortality rate for each stage varied from 4.0 to 22.2%. The highest mortality rate was recorded in the 3rd (22.2%) stage nymphs, followed by the 2nd (14%) stage nymphs (Table 3).

Longevity

The mean adult survival was 25.6 weeks (n = 42). Males presented a longer life span, with a mean survival of 25.6 weeks, whereas females presented a life span of 23.1 weeks.

### Table 1 - Intermolting period of *Triatoma carcavalloi*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>minimum</th>
<th>maximum</th>
<th>mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>50</td>
<td>15</td>
<td>24</td>
<td>18.52</td>
<td>2.34</td>
</tr>
<tr>
<td>2nd</td>
<td>47</td>
<td>25</td>
<td>95</td>
<td>62.77</td>
<td>18.45</td>
</tr>
<tr>
<td>3rd</td>
<td>43</td>
<td>44</td>
<td>138</td>
<td>86.93</td>
<td>19.00</td>
</tr>
<tr>
<td>4th</td>
<td>42</td>
<td>55</td>
<td>189</td>
<td>119.05</td>
<td>36.38</td>
</tr>
<tr>
<td>5th</td>
<td>42</td>
<td>59</td>
<td>295</td>
<td>193.43</td>
<td>70.33</td>
</tr>
</tbody>
</table>

N: number of nymphs; SD: standard deviation.

### Table 2 - Resistance to fasting in *Triatoma carcavalloi* (days).

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>50</td>
<td>15</td>
<td>59</td>
<td>37</td>
<td>15.52</td>
</tr>
<tr>
<td>2nd</td>
<td>50</td>
<td>10</td>
<td>116</td>
<td>50</td>
<td>29.72</td>
</tr>
<tr>
<td>3rd</td>
<td>50</td>
<td>3</td>
<td>128</td>
<td>65.5</td>
<td>37.54</td>
</tr>
<tr>
<td>4th</td>
<td>50</td>
<td>58</td>
<td>210</td>
<td>134</td>
<td>54.25</td>
</tr>
<tr>
<td>5th</td>
<td>50</td>
<td>7</td>
<td>185</td>
<td>146</td>
<td>43.08</td>
</tr>
<tr>
<td>♂</td>
<td>30</td>
<td>21</td>
<td>81</td>
<td>61</td>
<td>18.30</td>
</tr>
<tr>
<td>♀</td>
<td>30</td>
<td>4</td>
<td>81</td>
<td>42.5</td>
<td>18.24</td>
</tr>
</tbody>
</table>

N: number of nymphs; SD: standard deviation; ♂: male; ♀: female.

Table 4 shows developmental cycle: The total *T. carcavalloi* life cycle lasted 503.4 days (72 weeks). Differences were observed for the 5th stage nymphs, whose development lasted a mean of 193.43 days (27 weeks).

### Figure 1 - The number of blood meals according to the stage of *Triatoma carcavalloi*. 
TABLE 3 - Mortality of Triatoma carcavalloi.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>n</th>
<th>Mortality (%)</th>
<th>Cumulative mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>177</td>
<td>157</td>
<td>11.3</td>
<td>11.3</td>
</tr>
<tr>
<td>2nd</td>
<td>157</td>
<td>135</td>
<td>14.0</td>
<td>25.3</td>
</tr>
<tr>
<td>3rd</td>
<td>135</td>
<td>105</td>
<td>22.2</td>
<td>47.5</td>
</tr>
<tr>
<td>4th</td>
<td>105</td>
<td>99</td>
<td>5.7</td>
<td>53.2</td>
</tr>
<tr>
<td>5th</td>
<td>99</td>
<td>95</td>
<td>4.0</td>
<td>57.2</td>
</tr>
</tbody>
</table>

N: number of nymphs; n: number of nymphs molting into the following stage.

TABLE 4 - Duration of the developmental cycle of Triatoma carcavalloi.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Mean (days)</th>
<th>Quantiles 25% - 75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>60</td>
<td>22.7</td>
<td>21 - 26</td>
</tr>
<tr>
<td>1st</td>
<td>50</td>
<td>18.52</td>
<td>15 - 24</td>
</tr>
<tr>
<td>2nd</td>
<td>47</td>
<td>62.77</td>
<td>25 - 95</td>
</tr>
<tr>
<td>3rd</td>
<td>43</td>
<td>86.93</td>
<td>44 - 138</td>
</tr>
<tr>
<td>4th</td>
<td>42</td>
<td>119.05</td>
<td>55 - 189</td>
</tr>
<tr>
<td>5th</td>
<td>42</td>
<td>193.43</td>
<td>59 - 295</td>
</tr>
</tbody>
</table>

Total: 42, 503.4, 219 - 757

N: number of nymphs.

DISCUSSION

Several authors have highlighted the importance of studying the bionomic features of triatomines under laboratory conditions to increase the biological knowledge of these species, improve breeding conditions for the development of laboratory colonies, and provide recommendations to support control measures.

Under laboratory conditions, which generally involve more stable abiotic factors, vital cycles are generally shorter. Nevertheless, certain species may present a longer life cycle. For example, the diapause of T. carcavalloi showed a higher frequency on days 2 and 4, similar to results reported for T. pseudomaculata, but different from that of T. pallidipennis, which shows a preference for days 3, 6 and 10.

The hatchling incidence varies among species of the Triatoma genus and even within the same species. In T. carcavalloi, the obtained rate of 38.3% is considered low compared to those of T. circunnucula, in which 93% of eggs have been reported to hatch; T. pseudomaculata, with a rate of 88.3%; and T. rubrovaria, T. dimidiata, T. infestans, T. maculata, T. rubrofasciata and T. pallidipennis, in which rates range from 60 to 80%. In the Rhodnius genus, temperature was observed to influence not only the incubation period but also the incidence of hatching, as shown for R. robustus.

The average incubation period recorded for T. rubrovaria is 24.84 days, whereas the incubation period recorded for T. carcavalloi in the present study was 22.7 days.

In this study, the search for the first meals of the 1st stage nymphs of T. carcavalloi showed a higher frequency on days 2 and 4, similar to results reported for T. pseudomaculata, but different from that of T. vitticeps, which shows a preference for days 3, 6 and 10.

It is important to manage newly fed triatomines carefully because improper handling is a major cause of mortality during their development.

The T. carcavalloi life cycle requires an average of 13.4 blood meals, which is higher than the values reported for T. rubrovaria (11.1).
Triatoma vitticeps (8)⁶⁶ and Triatoma brasiliensis (11)⁷⁰ but lower than the value reported for T. pseudomaculata (14.7)⁸⁸. However, differences in the experimental conditions used by the various authors must be considered. We also must consider that these species live in distinct habitats and exhibit different feeding habits. Females of T. pseudomaculata make up for the lower amount of blood ingested by seeking out food sources quickly and consuming a larger number of blood meals⁹⁻¹⁰. The 5th stage nymphs of T. carcavalloi require several feedings to perform ecdysis.

The average intermolt period of the 2nd stage nymphs of T. rubrovaria was longer than that of the 3rd stage nymphs⁹⁻¹⁰. Triatoma brasiliensis, under standard temperature conditions, tends to show a crescent-shaped scale of intermolt periods from the 1st stage to the 5th stage nymphs¹⁵⁻¹⁷, which corroborates our findings.

One of the barriers to successfully combating the vectors of Chagas disease (using insecticides with residual action) is the capacity of these insects to resist fasting⁹⁻¹⁰. Nymphs tolerate longer fasting periods than do adults¹⁸, confirming that our findings indicate a compensation mechanism for the higher dispersion capacity of adults¹⁹.

The ability to resist fasting increased in T. carcavalloi from the 1st to the 5th nymphal stage. During the adult phase, lower resistance was observed in males compared to females, corroborating data obtained at a temperature of 30°C for T. rubrovaria¹⁷ and T. sordida¹⁴. These results contradict results reported for T. vitticeps⁶⁶ and T. rubrovaria⁴², in which males display a higher resistance to fasting.

In the present study the mortality rate was higher in the 3rd stage nymphs of T. carcavalloi (22.2%), corroborating the rate observed in T. rubrovaria⁶⁶. When nymphs of T. infestans were fed at longer intervals, their mortality rate increased, indicating the influence of feeding on nymphal mortality¹⁴⁻⁴³.

In this study, the higher longevity was observed in T. carcavalloi males, as was also observed in T. rubrofasciata³³ and T. rubrovaria⁸,¹⁷,²⁶,²⁹. The longevity of T. carcavalloi females was found to be higher when they were fed pigeons, suggesting a likely influence of feeding¹⁹.

The developmental cycle of the fifth stage nymphs of T. carcavalloi fed with pigeons lasted an average of 259.67 days¹⁹, in contrast to the findings of the present study, in which this species was fed mice and showed an average developmental cycle of 193.43 days. The total life cycle of T. rubrovaria has been reported to be approximately 300 days⁴⁹, whereas a much longer duration was observed in T. carcavalloi in the present study (503.4 days).

Our results suggest that biological traits are important criteria for determining the relationships between the Triatoma carcavalloi, Triatoma circummaculata and Triatoma rubrovaria species in the presence of the same food source and climatization and based on specimens collected in domiciles in State of Rio Grande do Sul, Brazil.

The efficacy of vector control campaigns has been impaired by the resistance to fasting. This resistance allows these animals to remain in their shelters, free from insecticides and sometimes even from their residual effects, thereby increasing the possibility of later recolonization by the remaining individuals¹²,¹⁴⁻²⁴,⁴⁶⁻⁴⁷.

The capacity for transmission observed in T. carcavalloi is as high as that of T. rubrovaria, whereas T. circummaculata is infected less often¹, most likely because it feeds on mammals less frequently. These data together with the results of this study demonstrate the need for constant entomological surveillance of T. carcavalloi.

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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**REFERENCES**


