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Major Article

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 intermolting 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 autochthonous[1] 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 cycles[2]. Field data have shown that *T. rubrovaria* is frequently found in domiciliary and peridomiciliary areas[3]. Only *T. carcavalloi* has been observed in Canguçu, Dom Feliciano, Pinheiro Machado and São Jerônimo[4] (Source: IPB-LACEN/RS), appearing inside domiciles throughout Rio Grande do Sul[5,6].

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 habitats[8].

The life cycles of triatomines vary according to the species, environmental conditions and the availability of suitable sources of blood[9,10].

Resistance to fasting could be of great importance and might directly affect control campaigns targeting these vectors[11]. Tolerance to long periods of fasting helps these

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insects survive difficult periods when food shortages occur. During these periods, they hide in wall cracks and escape residual insecticides[12], 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 nymphal 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*.

		Duration in days			
Stage	N	minimum	maximum	mean	SD
1st	50	15	24	18.52	2.34
2nd	47	25	95	62.77	18.45
3rd	43	44	138	86.93	19.00
4th	42	55	189	119.05	36.38
5th	42	59	295	193.43	70.33

N: number of nymphs; **SD:** standard deviation.

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

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

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

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).

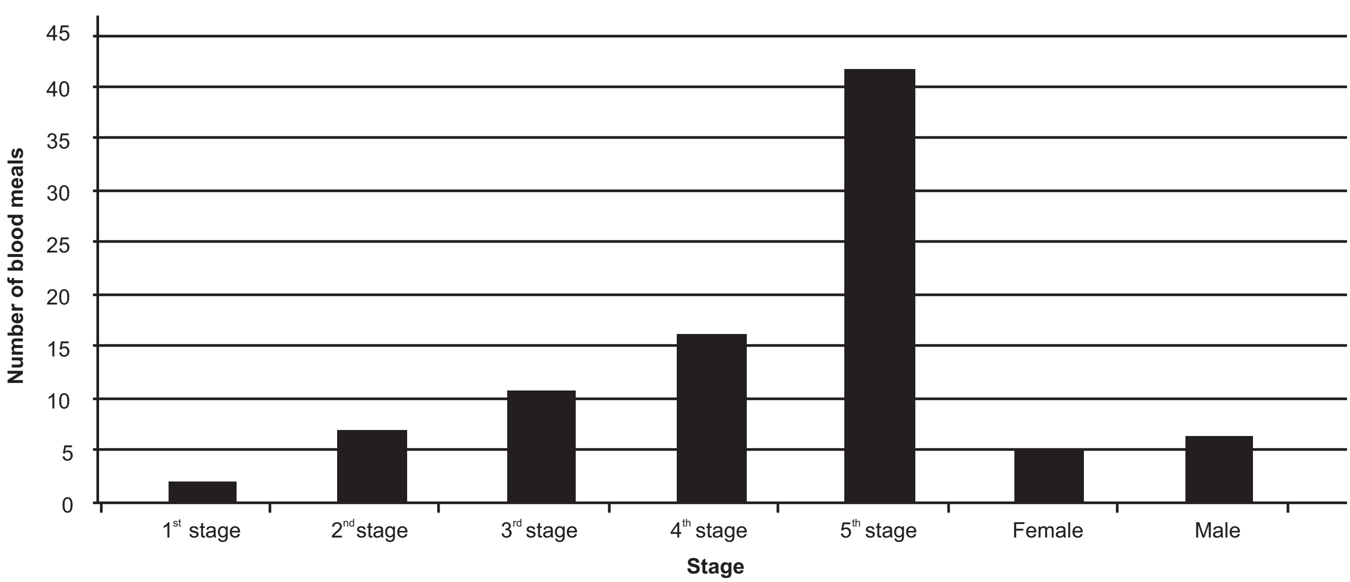

FIGURE 1 - The number of blood meals according to the stage of *Triatoma carcavalloi*.

TABLE 3 - Mortality of *Triatoma carcavalloi*.

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

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

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

Stage	N	Mean (days)	Quantiles 25% - 75%
Egg	60	22.7	21 - 26
1st	50	18.52	15 - 24
2nd	47	62.77	25 - 95
3rd	43	86.93	44 - 138
4th	42	119.05	55 - 189
5th	42	193.43	59 - 295
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[13-16].

Under laboratory conditions, which generally involve more stable abiotic factors, vital cycles are generally shorter[17]. Nevertheless, certain species may present a longer life cycle. For example, the diapause of 5th stage nymphs has been recorded in several wild species[18] and was observed in this study, as fifth-stage nymphs of the examined species require several blood meals to permit molting, which can lengthen this phase up to a year. Similar findings have been reported for *T. rubrovaria*, *T. carcavalloi* and *T. circummaculata*[19].

The first study on the biological cycle of *T. carcavalloi* under laboratory conditions involved allowing the insects to feed on pigeons, blaberids and lizards[20]. Other authors have studied the biological cycles of *T. rubrovaria* and *T. circummaculata* fed the hemolymph of Blattodea (cockroaches) to assess nymphal development[21]. These studies suggested that this type of food may be naturally exploited by these triatomine species and most likely represents a means of surviving under natural conditions. The dependence on hemolymph is obvious in *T. circummaculata*, as this species does not develop into a 1st stage nymph in the laboratory if this food source is not available[1,5]. Certain species of triatomines display rather eclectic eating habits under natural conditions[22].

Triatoma rubrovaria develops well under laboratory conditions at temperatures between 20ºC and 30ºC and a relative humidity maintained at approximately 80%. The various life cycle times were recorded for the two sexes. Males survived for an average of 115.37 days, whereas females survived for an average of 99.6 days[16,23]. The influence of temperature (25ºC and 30ºC) on *T. rubrovaria* was studied, and faster development was observed at 30ºC[17]. In the present study, temperatures equal to or higher than 28ºC influenced the development of *T. carcavalloi*, resulting in a shorter life cycle compared to that of *T. rubrovaria*.

In studies examining the life cycle of *T. infestans*[24] and the resistance to fasting in *Rhodnius prolixus*[25], the authors suggested that the blood meal represents an essential condition for oviposition in general and fertility in particular. In a study of *T. rubrovaria*, it is not known whether the low rate of hatching was due to these factors because the experimental specimens were fed once a week for an hour, without establishing the amount of blood ingested by each individual[26]. In the present study, the feeding was standardized, with *T. carcavalloi* being fed mice once a week until repletion. Therefore, we cannot conclude whether the low rate of hatching recorded was due to food or other factors, such as temperature and humidity, present under the laboratory conditions.

In our experiments, not all of the eggs hatched, indicating that fertility was compromised or that small variations in temperature or humidity (which are known to influence development directly) or some other unknown cause limited hatching.

The hatching 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 *Triatoma flavida*, in which 93% of eggs have been reported to hatch[27]; *T. pseudomaculata*, with a rate was 88.3%[28]; and *T. rubrovaria*[29], *Triatoma dimidiata*[30], *T. infestans*[31], *T. maculata*[32], *Triatoma rubrofasciata*[33] and *Triatoma pallidipennis*[34], 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*[35].

The average incubation period reported for *T. rubrovaria* is 24.84 days[26], 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*[28] but different from that of *Triatoma vitticeps*, which shows a preference for days 3, 6 and 10[36].

It is important to manage newly fed triatomines carefully because improper handling is a major cause of mortality during their development[17,37].

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)[38], *Triatoma vitticeps* (8)[36] and *Triatoma brasiliensis* (11)[39] but lower than the value reported for *T. pseudomaculata* (14.7)[28]. 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 meals[28]. 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 nymphs[38]. *Triatoma brasiliensis*, under standard temperature conditions, tends to show a crescent-shaped scale of intermolt periods from the 1st stage to the 5th stage nymphs[15,17], 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 fasting[40]. Nymphs tolerate longer fasting periods than do adults[18], confirming that our findings indicate a compensation mechanism for the higher dispersion capacity of adults[25].

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 30ºC for *T. rubrovaria*[17] and *T. sordida*[41]. These results contradict results reported for *T. vitticeps*[40] and *T. rubrovaria*[42], 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. rubrovaria*[8] When nymphs of *T. infestans* were fed at longer intervals, their mortality rate increased, indicating the influence of feeding on nymphal mortality[24,43].

In this study, the higher longevity was observed in *T. carcavalloi* males, as was also observed in *T. rubrofasciata*[33] and *T. rubrovaria*[8,17,26,29]. The longevity of *T. carcavalloi* females was found to be higher when they were fed pigeons, suggesting a likely influence of feeding[19].

The developmental cycle of the fifth stage nymphs of *T. carcavalloi* fed with pigeons lasted an average of 259.67 days[19], 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 days[29], 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 individuals[12,14,42,44-47].

The capacity for transmission observed in *T. carcavalloi* is as high as that of *T. rubrovaria*, whereas *T. circummaculata* is infected less often[1], 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*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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