Triatoma rubrovaria (Blanchard, 1843) (Hemiptera-Reduviidae-Triatominae) III: Patterns of Feeding, Defecation and Resistance to Starvation

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Data from the Chagas Disease Control Program indicate a growing domiciliary and peridomiciliary invasion of Triatoma rubrovaria *in the State of Rio Grande do Sul, where it has become the most frequent triatomine species captured there since the control of* T. infestans. *Bionomic characteristics that could influence the vectorial capacity of* T. rubrovaria *as vector of* Trypanosoma cruzi *were evaluated: patterns of (i) feeding, (ii) defecation, and (iii) resistance to starvation, using insects fed on mice. Fifty three percent of the females showed a defecation pattern conducive to chagasic transmission, defecating either on or near the bite site. The averages of the resistance to starvation varied from 48.1 to 179 days, for the first and fifth nymphal stages, respectively. Our study shows that with respect to the patterns of feeding, defecation and resistance to fasting,* T. rubrovaria *presented similar rates to the ones observed for other effective vector species, such as* T. infestans. *Thus, based on our studies we conclude that* T. rubrovaria *has biological characteristics that can positively influence its capacity to become infected and transmit* T. cruzi, *and also to keep residual populations after chemical control interventions.*

Key words: *Triatoma rubrovaria* - feeding patterns - resistance to starvation

Triatoma rubrovaria (Blanchard, 1843) (Hemiptera, Reduviidae, Triatominae) is widespread in Uruguay, and some neighboring parts of Northeastern Argentina. In Brazil, its presence has been confirmed only in the southern states of Paraná (PR) and Rio Grande do Sul (RS). This species of triatomine is found mainly among exfoliate rocks known as 'pedregales' (Salvatella et al. 1995). Lent (1942) has earlier reported *T. rubrovaria* (*Eutriatoma rubrovaria*) as a sylvatic species inhabiting rock piles and may rarely be found in human dwellings. Further, Silva and Silva (1993) showed that it may be a highly competent vector of *Trypanosoma cruzi,* the causative agent of Chagas disease. Vinhaes and Dias (2000) reported 1.02% of natural infection for a total of 1,746 *T. rubrovaria* specimens examined.

In RS, data from the Chagas Disease Control Program indicate an increasing of domiciliary and peridomiciliary invasion of *T. rubrovaria*, where it has become the most frequently triatomine species captured in that State since the control of *Triatoma infestans* (Klug, 1834) (Almeida et al. 2000). Isoenzymatic and chromatic studies developed by Almeida et al. (2002b) showed two distinct phenotypic

patterns for this species. Salvatella et al. (1994), utilizing the precipitin test, demonstrated that it feeds on humans in Uruguay. Later, studies performed by Almeida et al. (2002a) in several localities of the RS, indicated its food eclecticism in this State. A total of 85 samples were analyzed, and the highest reactivity was observed for the anti-rodent serum in most of the localities. Just 1.3% of positivity for human blood was recorded.

As originally demonstrated by Dias (1956), the success of vectorial transmission of human Chagasic infection depends among other factors, on the capacity of the infected domiciliated triatomines to defecate during or shortly after the blood meal.

Pellegrino (1952) emphasized the epidemiological importance of the starvation resistance for the triatomines. Afterwards, Perlowagora-Szumlewicz (1954, 1969) noted that insecticide applications must be carried out in pre-established regular periods of time for species that present a high resistance to fast, stating that only then the domiciliary reinfestations can be minimized. Several other authors have conducted extensive studies about various species of triatomines analyzing these biologic aspects, such as Zeledon et al. (1970), Costa and Perondini (1973), Jurberg and Costa (1989), and Costa and Jurberg (1989).

The assessment of the vectorial capacity of the triatomines through biological studies is essential to define monitoring actions and serves as a basis for control measures (Perlowagora-Szumlewics 1969, Costa 1999, Soares et al. 2000). In order to clarify bionomic aspects that could influence the vectorial capacity of *T. rubrovaria*, the patterns of feeding, defecation and resistance to starvation were evaluated.

MATERIALS AND METHODS

Insects - Specimens used in this investigation were originated from a colony maintained for one year under laboratory conditions: temperature (min. 21.5ºC, max. 32ºC, $\overline{X} = 27.63$ºC), relative humidity (min. 52%, max. 96%, $\overline{X} = 77.5\%$). This colony was established with 58 specimens collected in Santana do Livramento municipality (30º53'27"S; 55º31'58"W), RS, Brazil. The insects were fed weekly on blood of Swiss mice on a free access basis.

Feeding and defecation patterns - A group of 30 nymphs, and eggs (F1) were selected according to their different stages. These nymphs were fed weekly until their ecdysis. After the ecdysis or egg hatching, all specimens were submitted to a starvation period (SP). The SP was standardized, for nymphs (n = 15 per instar) and adults (n = 15 per sex), in a pilot experiment, carried out in order to assess the elapsed time for the majority (> 50%) of the specimens to start feeding not longer than 20 min after the blood source has been offered. Thus, for the first, and second nymphal stages (N1, N2) the SP was standardized respectively in 7, 15, and in 20, days for the third, forth and fifth nymphal stages (N3, N4, N5). For males and females the SP was standardized in 20 and 15 days, respectively. Then, at least 30 specimens of each instar were randomly picked up from the stock of insects mentioned above. Each individual was kept in a circular plastic container, with a radius of 5 cm, and 15 cm high. Filter paper was used to cover the inner surface of the container. The mouse anesthetized intramuscularly with ketamin in the dosage of 0.35 mg/kg of body weight and immobilized in a nylon web was placed into the container. The elapsed time for the insect to start feeding, the duration of feeding, and the elapsed time between the end of feeding and defecation were registered. Observations of insect movements after feeding and the distance between the feeding source and defecation places were recorded.

Resistance to starvation - For these studies, we essentially followed the protocol as established by Costa and Jurberg (1989), Jurberg and Costa (1989) and Costa and Marchon-Silva (1998). Thirty eggs and 30 nymphs (F1) were grouped according to their stages. These insects were observed daily until ecdysis or hatching. Afterwards, the insects were kept in plastic containers of 15 cm high with a quadrangular base of 8 × 8 cm with perforated lid and containing folded filter paper inside (10 insects per container). In this experiment, the period of starvation resistance of the insects, after the ecdysis or hatching, was noted until their death.

RESULTS

Feeding and defecation patterns - For the nymphs, N4 took the longest time to begin feeding, 01'43" in average. Males and females spent an average of 02'49" and 03'23", respectively, to initiate feeding and the N1 required the shortest time ($\overline{X} = 21$"). The N5 seems to present the greatest nutritional avidity, since they allied a short time to begin feeding ($\overline{X} = 28$") and the longest average in time ($\overline{X} = 35'15$") of duration of feeding. In spite of the females having delayed to accept the feeding ($\overline{X} = 3'23$") they spent the second longest average of duration of feeding ($\overline{X} = 23'29$") (Fig. 1).

As depicted in Fig. 2, and Table I, females showed among all analyzed developmental stages the reduced elapsed time average (01'19") for defecation after the blood meal. Out of 30 females analyzed, 25 defecated in less than 10 min. A higher percentage (53.3%) of the females defecated in less than 1 min after the end of feeding, without leaving the bit site. Nine of them defecated during feeding and eight of them defecated twice during the feeding. One female defecated three times during feeding and once more within 40 sec after the end of feeding. After standardized post-ecdysis starvation, the number of insects that defecated during the feeding time and at different intervals after the first feeding was determined (Fig. 2).

Both Fig. 2 and Table I concur that the N5 presented a high rate of defecation shortly after feeding. The males
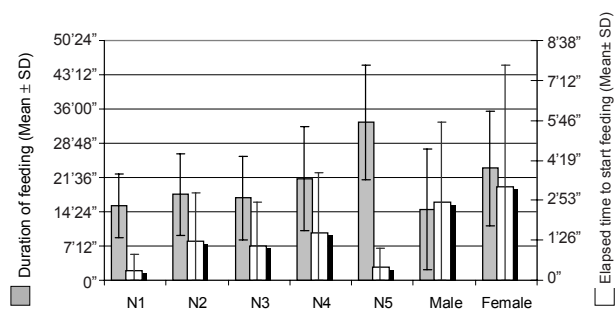

Fig. 1: elapsed time to begin feeding and duration of feeding of *Triatoma rubrovaria*.

TABLE I

Elapsed time between the end of feeding and defecation for *Triatoma rubrovaria* specimens, which defecated in less than 10 min

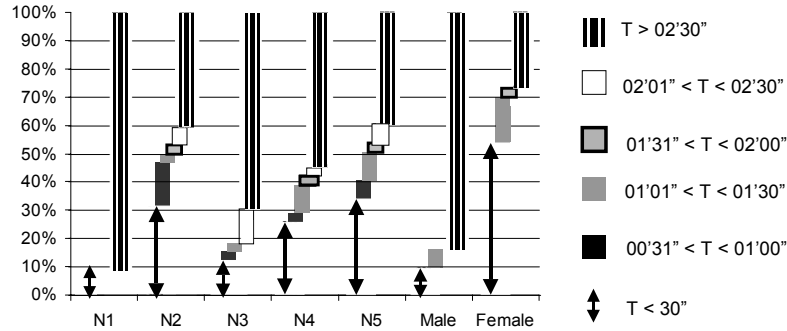|	N1	N2	N3	N4	N5	Male	Female
Pre-established starvation period after the blood meal	7	15	20	20	20	20	20
Number of specimens which defecate in less of 10 min	3/30	29/32	20/33	26/30	25/30	18/31	25/30
$\overline{X}$	02'48"	02'07"	03'12"	02'45"	02'03"	04'17"	01'19"
S	04'33"	02'16"	02'20"	02'31"	02'34"	03'22"	02'17"
S^2	39'34"	05'07'18"	05'28'00"	06'20'17"	06'36'24"	11:21'29"	05'13'25"
Max.	08'03"	06'37"	07'09"	10'00"	08'56"	09'43"	09'08"
Min.	00"	00"	00"	03"	00"	00"	00"

Fig. 2: elapsed time between the end of feeding and defecation (T) of *Triatoma rubrovaria.*

presented a low rate of defecation in less than 1 min, as it is shown in the Fig. 2. Most of defecation delivered by the males occurred mainly between 2'20" and 10'.

From 58 defecations recorded for all stages and in less than 1 min, in 40 cases (69%) the feces were deposited less than 3 cm from the bite site. On the other hand, of the 38 defecations recorded between 1 min and 2.5 min, only nine (23.7%) were deposited less than 3 cm from the bite site.

Resistance to starvation - It is shown in Fig. 3, that the average resistance to fasting during the nymphal stage yielded an ascending curve from the N1 to N5. The averages of resistance to starvation of the N1 and N5 were respectively 48.1 and 179.1 days, and for males and females were 70.5 and 68 days, respectively.
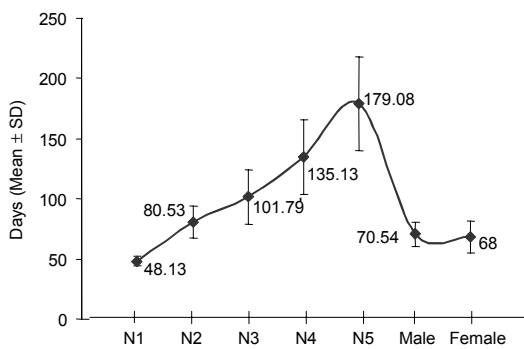

Fig. 3: resistance to starvation (in days) of *Triatoma rubrovaria.*

DISCUSSION

Feeding and defecation patterns - In our present investigation, the results showed that the adults of *T. rubrovaria* took more time to initiate feeding when compared to the nymphs. This correlation was not observed for other triatomine species, such as *Triatoma dimidiata* (Latreille, 1811), *T. infestans,* and *R. prolixus* (Zeledon et al. 1977).

Soares et al. (2000) carried out studies on biological parameters of *T. brasiliensis* Neiva, 1911 and *T. pseudomaculata* Corrêa & Espínola, 1964 associated to their epidemiological importance as vectors of *T. cruzi*. Both species spent a significantly longer time to feed than *T.*

rubrovaria, in all stages. For example, for *T. rubrovaria,* the longest average was recorded to N5 taking 33'15" to feed. In this same stage, *T. brasiliensis* and *T. pseudomaculata* presented a mean of 42'00" and 50'33", respectively.

Wood (1951) demonstrated that, in general, North American species such as *Triatoma protracta* (Ulher 1894)*, Triatoma rubida* (Ulher, 1894) and *Paratriatoma hirsuta* Barber, 1938, did not defecate after biting in an elapsed time effective to the chagasic transmission to man. On the other hand, Dias (1956) showed that some of the most important vectors in South America: *Rhodnius prolixus* Stal, 1859, *T. infestans*, *Panstrongylus megistus* (Burmeister, 1835) and *T. sordida* (Stal, 1859) defecated shortly after feeding. This showed a defecation pattern conductive to Chagas transmission to man. An exception among South American triatomines was *T. vitticeps* (Stal, 1859), which required more time to defecate. Later, Gonçalves et al. (1988) have confirmed this trait for *T. vitticeps*.

Based on the results obtained in this investigation, in the most cases we found that elapsed more than 1 min following feeding, the insects had already moved more than 3 cm from the bite site. Thus, we conclude that *T. cruzi* transmission may occur when the insect defecates either during or less than 1 min after feeding. Under these circumstances, it appears that 53.3% of the observed females (Fig. 2) would present vectorial transmission risks at the time they bite. Our results are in accordance with the observations noted by Zeledon et al. (1977) for *T. dimidiata*, where they showed that females and the N5 of this species defecate quickly and emitted more feces after feeding than did the males and the other nymphal stages.

Table II shows the defecation patterns for males and females reported by several authors for various species during the blood meal or in less than 1 min after the feeding. Accordingly, it was found that the females of *T. rubrovaria* demonstrate defecation rates similar to those obtained for *T. infestans* (Dias 1956, Zeledon et al. 1977) and for *T. brasiliensis,* and *T. pseudomaculata* (Soares et al. 2000). It is important to emphasize that according to the Brazilian Health Foundation (Funasa), in RS, the records of domiciliary positivity for *T. rubrovaria* refer to capture and notification of adult specimens (Almeida et al. 2000).

TABLE II

Comparative defecation rates, reported by several authors, for various triatomine species, which defecated during the blood meal or in less than 1 min after the feeding

Species	Blood source	Male (%)	Female (%)	Author
T. rubrovaria	Mice	9.7	53.3	This paper
T. brasiliensis	Mice	56 [a]		Soares et al. (2000)
T. pseudomaculata	Mice	50 [a]		Soares et. al. (2000)
T. sordida	Bird	40 <X< 60	80 < X < 100	Crocco & Catalá (1996)
R. prolixus	Mice	40 <X< 50	70 < X < 80	Zeledon et al. (1977)
T. infestans	Mice	20 < X < 30	30 < X < 40	Zeledon et al. (1977)
T. dimidiata	Mice	0 < X< 10	20 < X < 30	Zeledon et al. (1977)
R. prolixus	Bird	50x	62 x	Dias (1956)
T. infestans	Bird	44.4	54.4	Dias (1956)
R. neglectus	Bird	50 x	12 x	Dias (1956)
P. megistus	Bird	0 x	40 x	Dias (1956)
T. sordida	Bird	23.1	[b] x	Dias (1956)

a: no specification about the sex; *b*: without information; *T*: *Triatoma*; *R*: *Rhodnius*; *P*: *Panstrongylus*

TABLE III

Comparative starvation resistance averages (in days) for different triatomine species, reported by several authors

Species	Nymphs					Adults		Temp. (°C)	Blood source	Author
	N1	N2	N3	N4	N5	Male	Female			
T. rubrovaria	48.1	80.5	101.7	135.1	179	70.5	68.0	Environmental, RJ	Mice	This paper
T. rubrofasciata	21.6	24.2	46.8	77.9	101.5	51.4 [a]		Environmental, RJ	Rat	Cortez & Gonçalves (1998)
T. brasiliensis [c]	35.5	40	50.5	67.9	121.1	37.1 [a]		Environmental, RJ	Mice	Costa & Marchon-Silva (1998)
T. vitticeps	37	91	136	177	180	63	58	Environmental, RJ	Mice	Gonçalves et al. (1989)
T. rubrovaria	40.9	81.1	91.1	117	133	78.8	74.8	25	Bird	Silva (1985)
T. rubrovaria	20.9	52.8	67.2	83.1	88.9	54.4	57.8	30	Bird	Silva (1985)
T. sordida	46.7	72.2	118	176.7	217.8	54.9	63.9	25	Mice	Juarez & Silva (1982)
T. infestans	60.2	49.4	- [b]	86.3	76.4	48.8 [a]		24-28	Bird	Perlowagora-Szumlewicz (1969)

a: no specification about the sex; *b*: without information; *T. Triatoma*; *c*: "brasiliensis" population

Resistance to starvation - In this investigation, the results obtained for resistance to starvation were similar to the results obtained by Silva (1985) for the same species, but fed on bird (*G. gallus*) and kept at 25°C. In both studies, the nymphal stages showed an increasing curve from the N1 to N5 at about the starvation resistance. Also the results suggest that males are slightly more resistant to starvation than the females. The comparative results of starvation resistance for several species of triatomines reported by other authors are shown in Table III.

Concerning the features studied, *T. rubrovaria* presented biological characteristics comparable to some important Chagas disease vectors, that may favor the transmission of *T. cruzi* to man. Thus, it must be kept under constant entomological surveillance. It should be emphasized that the results of the investigation presented here were carried out utilizing insects collected in one municipality from RS. Almeida et al. (2002b) showed significant phenotypic differences among *T. rubrovaria* populations from different RS municipalities. The comparative study of the bionomic characteristics among different populations could help us to better clarify the vectorial potentiality of *T. rubrovaria* in RS.

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