

Comparison of feeding and defecation patterns between fifth-instar nymphs of *Triatoma patagonica* (Del Ponte, 1929) and *Triatoma infestans* (Klug, 1934) under laboratory conditions

Comparação dos padrões alimentar e de defecação em ninfas de quinto estágio de *Triatoma patagonica* (Del Ponte, 1929) e *Triatoma infestans* (Klug, 1934), em condições de laboratório

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ABSTRACT

Among the vectors of Chagas disease, *Triatoma patagonica* is a species in the process of adaptation to the human environment. However, its vector competence is not well known. This study had the aim of evaluating and comparing feeding and defecation patterns among fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans* that were fed *ad libitum*. The results showed that nymphs of *Triatoma patagonica* had a feeding pattern similar to that of *Triatoma infestans*. Sixty nine percent and 58% of nymphs of *Triatoma patagonica* and *Triatoma infestans*, respectively, produced their first defecation within five minutes after being fed. *Triatoma patagonica* defecated during feeding, with an average time until first defecation that was shorter than that of *Triatoma infestans* (3.4 and 6.2 min, respectively). The nymphs of *Triatoma patagonica* were capable of defecating during or immediately after feeding.

Key-words: Peridomestic vector. Vector capacity. Chagas disease vector. *Triatoma patagonica*. *Triatoma infestans*.

RESUMO

Entre os vetores da doença de Chagas, *Triatoma patagonica* é uma espécie que se encontra em processo de adaptação ao ambiente humano; embora sua competência vetorial não seja bem conhecida. O estudo teve como objetivo avaliar e comparar padrões de alimentação e defecação em ninfas de quinto estágio de *Triatoma patagonica* e *Triatoma infestans* *ad libitum*. Os resultados mostraram que as ninfas de *Triatoma patagonica* apresentou padrão de alimentação semelhante a *Triatoma infestans*. Sessenta e nove por cento e 58% das ninfas de *Triatoma patagonica* e *Triatoma infestans* respectivamente, realizaram a primeira defecação cinco minutos após de serem alimentadas; sendo que a primeira defecou durante a alimentação, com um tempo médio de defeção menor do que para *Triatoma infestans* (3,4 vs 6,2 respectivamente). As ninfas de *Triatoma patagonica* foram capazes de defecar durante ou imediatamente após de se alimentarem.

Palavras-chaves: Vetor peridoméstico. Capacidade vetorial. Vetor da doença de Chagas. *Triatoma patagonica*. *Triatoma infestans*.

In the transmission cycle from *Trypanosoma cruzi* Chagas, 1909, to humans, the peridomestic area is showing increasing importance because progressive control over the main domestic vector species of Triatominae may provide opportunities for other species of Triatominae to become epidemiologically relevant^{2 4 16}.

Among the peridomestic vectors of Chagas disease, *Triatoma patagonica* has the most southerly distribution. This vector is only found in Argentina, at latitudes of 20° to 50° south^{3 8}, and it has been reported in domestic and peridomestic areas in the provinces of Rio Negro⁷ and Chubut¹⁶, Argentina. Susceptibility of this species

to infection with *Trypanosoma cruzi* has been demonstrated experimentally⁹ and also naturally¹. Regarding its efficiency as a vector, Nattero et al¹⁰ suggested that females of *Triatoma patagonica* would be capable of transmitting *Trypanosoma cruzi*, because they defecate while feeding on the host.

The biological characteristics of greatest impact on the epidemiology of Chagas disease involve the feeding and defecation processes of the vector¹⁵. Fifth-instar nymphs and females constitute the most important fractions of the population, from an epidemiological point of view, since nymphs take more blood

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than adults, ingesting between eight and nine times their body weight^{11,14}.

In order to better understand the efficiency of *Triatoma patagonica* in *Trypanosoma cruzi* transmission, the aim of this study was to evaluate the feeding and defecation patterns of fifth-instar nymphs of this species and to compare them with those of nymphs of *Triatoma infestans*, which is the principal domestic vector in Argentina.

MATERIAL AND METHODS

This study was carried out with 62 and 55 fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans*, respectively. The insects used in this study came from laboratory colonies of the insectary of the National Chagas Service (Argentina). Nymphs of *Triatoma patagonica* were the seventh generation obtained from insects collected in chicken coops at the locality of Monte Fiori, Santa Fe, Argentina, and nymphs of *Triatoma infestans* were the second generation obtained from insects collected in peridomestic areas at the locality of Loma Blanca, La Rioja, Argentina.

The experiment was carried out between September and December 2005, in a laboratory under constant temperature conditions ($26^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and relative humidity ($60 \pm 10\%$). The bugs were kept in glass jars, covered with nylon mesh and provided with vertically placed strips of paper. The nymphs were starved for 15 days after molting. Then, they were allowed to feed ad libitum on restrained pigeons (*Columba livia* Gemelin, 1789). The pigeons were not given anesthesia during the insects' meal. The nymphs were observed continuously during feeding and for 30 min afterwards. For each feeding, the following variables were observed:

Blood meal size. This was determined as the difference in weight between before and after feeding.

Duration of feeding. This was obtained by recording the time elapsed (in minutes) from the time when the insect's rostrum came into contact with the host's skin until the time when the rostrum was detached.

Number and frequency of defecations by the insect during feeding and within 30 min after feeding. The number of defecations was registered by direct observation of the insect while it was on the host and for 30 min after feeding.

All weights were measured using a Mettler balance with a precision of 0.001g. The duration of feeding and frequency of defecations by the insects were timed using a stopwatch. To compare the meal sizes between nymphs of different species of different body sizes, a relative meal size index (RMS) was obtained: $\text{RMS} = \text{meal size (mg)} / \text{insect weight before feeding (mg)}$.

The t-test for samples with independent variables was used as the statistical treatment for comparing the variables of different species. Correlations between variables were investigated using standard liner regression. To verify the homogeneity of variance, the Levene test was used⁶.

RESULTS

Feeding patterns. The variables analyzed with regard to the feeding behavior of fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans* are shown in Table 1. The nymphs of *Triatoma patagonica* needed significantly more time than did the nymphs of *Triatoma infestans* to feed to repletion. The two species ingested similarly sized blood meals, but *Triatoma patagonica* had greater ingestion capacity than presented by *Triatoma infestans*, ingesting up 3.52 times its body weight.

Table 1 - Feeding behavior of fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans*.

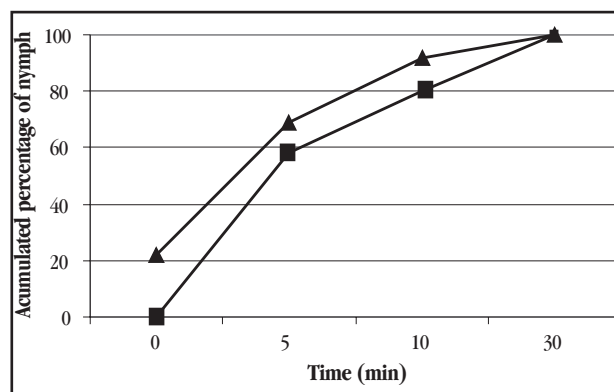
Species	n ^o	Duration of feeding (min)		Blood meal size (mg)		RMS
		x	SD	x	SD	
<i>Triatoma patagonica</i>	62	21.40*	9.82	128.76	79.33	3.52
<i>Triatoma infestans</i>	55	12.42*	6.69	143.30	68.00	2.67

*nymphs of *Triatoma patagonica* differed significantly from nymphs of *Triatoma infestans* ($p < 0.05$), RMS: relative meal size, x: mean, SD: standard deviation

As expected, blood meal size (mg) presented a correlation with duration of feeding (min) in both species (*Triatoma patagonica* $r = 0.54$; $p < 0.05$; $n^{\circ} = 62$; and *Triatoma infestans* $r = 0.53$; $p < 0.05$; $n^{\circ} = 55$). The regression analysis between the initial weight (mg) and the blood meal size (mg) showed a significant positive linear correlation to nymphs of *Triatoma infestans* ($r = 0.34$; $p < 0.05$; $n^{\circ} = 55$), such that higher weight nymphs ingested more blood. No significant relationship was found between these variables for the nymphs of *Triatoma patagonica*.

Defecation patterns. From the total number of fifth-instar nymphs feeding of both species, 84% ($n^{\circ} = 52$) of *Triatoma patagonica* and 75% ($n^{\circ} = 41$) of *Triatoma infestans* defecated while feeding and within the first 30 min thereafter.

Figure 1 shows that 69% and 58% of the nymphs of *Triatoma patagonica* and *Triatoma infestans* respectively, had produced their first defecation within five minutes after feeding. Ten minutes after feeding, the values recorded were 92% and 80% for *Triatoma patagonica* and *Triatoma infestans*, respectively.



(▲) *Triatoma patagonica* (■) *Triatoma infestans*.

Figure 1 - Accumulated percentages of fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans* which defecated during feeding (time 0) and at different times afterwards.

The average numbers of defecations produced per insect within 5, 10 and 30 min after feeding were significantly higher for nymphs of *Triatoma patagonica* than for nymphs of *Triatoma infestans*: 20% of specimens of *Triatoma patagonica* defecated during feeding (time 0), with an average time to first defecation that was shorter than that of *Triatoma infestans* (Table 2).

Table 2 - First defecation time and number of defecations by fifth-instar nymphs of *Triatoma patagonica* and *Triatoma infestans* during the feeding and within 5, 10 and 30 min after feeding.

Species	First time defecation (min)	Number of defecations per insect			
		0 min	5 min	10 min	30 min
<i>Triatoma patagonica</i>	3.39 ± 5.41 ^a	0.29 ± 0.66 ^b (0-3)	0.92 ± 0.89 ^c (0-4)	1.39 ± 1.25 ^d (0-6)	2.24 ± 1.87 ^e (0-9)
<i>Triatoma infestans</i>	6.22 ± 7.08 ^a	0.00 ^b (0-2)	0.56 ± 0.71 ^c (0-3)	0.94 ± 0.95 ^d (0-5)	1.73 ± 1.39 ^e

a, b, c, d, e: nymphs of *Triatoma patagonica* differed significantly from nymphs of *Triatoma infestans* (p < 0.05).

Relationship between blood meal size and defecation pattern. In both species, blood meal size was significantly greater among nymphs that defecated (149.7 ± 73.4 and 164.9 ± 62.1 mg for nymphs of *Triatoma patagonica* and *Triatoma infestans*, respectively) than among nymphs that did not defecate (67.3 ± 81.8 and 80.0 ± 39.3mg for nymphs of *Triatoma patagonica* and *Triatoma infestans*, respectively) (p < 0.05).

Regression analysis on blood meal size at the time of first defecation did not show any significant correlation in either species.

For the nymphs of *Triatoma patagonica* that defecated, the blood meal size was correlated with the number of defecations per insect within five minutes after feeding (r = 0.2796; p < 0.05; n^o = 52). In *Triatoma infestans*, no significant relationship was found.

DISCUSSION

Comparison between the RMS observed for *Triatoma patagonica* in the present study and for other peridomestic species by other authors shows that the capacity of nymphs of *Triatoma patagonica* (3.52) is lower than that of nymphs of *Triatoma sordida* (4.10)⁵ and higher than that of nymphs of *Triatoma guasayana* (2.23)¹³. As seen with other Triatominae^{5,10,13}, in fifth-instar nymphs of *Triatoma patagonica* the blood meal size controls the excretion behavior, i.e. the frequency of defecations within the first five minutes after feeding is directly related to the blood meal size.

On the other hand, transmission of *Trypanosoma cruzi* is crucially dependent on the defecation time. The most efficient vectors are those that defecate during or soon after feeding, while the bug is still in contact with its host¹⁸. Fifth-instar nymphs of *Triatoma patagonica* not only defecated quickly after feeding, but some of them (20%) also defecated during the feeding. However, in our experiment, nymphs of *Triatoma infestans* did not defecate

at these times. Nevertheless, Zeledón et al¹⁸ observed that about 5% of the nymphs of *Triatoma infestans* defecated during feeding.

Comparison of the defecation patterns observed in this study among nymphs of *Triatoma patagonica* and *Triatoma infestans* showed that the nymphs of *Triatoma patagonica* were more efficient in terms of average time to first defecation and number of defecations per insect at different times during the first 30min after feeding. Zeledón¹⁷, considered that defecation within 10 min after feeding appeared to present the highest risk for transmission of *Trypanosoma cruzi*. The percentage of nymphs of *Triatoma patagonica* that defecated within this time was approximately 90% (to do this required a minimum ingestion of 86.5mg), while for *Triatoma infestans* it was 80%. Comparing this percentage with those for other peridomestic species, it can be seen that fifth-instar nymphs of *Triatoma patagonica* present a pattern that is similar to those shown for *Triatoma sordida* (88%)⁵ and for *Triatoma guasayana* (89.3%)¹³.

The results obtained here suggest that, like the peridomestic species *Triatoma guasayana* and *Triatoma sordida*, the fifth-instar nymphs of *Triatoma patagonica* have a feeding pattern that is similar to the pattern shown by *Triatoma infestans*, and they are capable of defecating during or immediately after feeding. These conditions would allow nymphs of *Triatoma patagonica* to transmit *Trypanosoma cruzi*.

In Triatominae, factors like the dependence of defecation time on the quantity of blood ingested have important implications for disease transmission¹⁵. However, other factors like the density inside the home, the affinity with the host and the degree of adaptation to the human dwelling are also epidemiologically important with regard to determining this potential¹². It would be important to continue with studies on the relationships of biological and morphological characteristics with the capacity of *Triatoma patagonica* to colonize domestic habitats.

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