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Factors controlling the volume of feces produced by triatomine vectors of Chagas' disease

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Summary

Triatomine species influenced volume of feces produced; *Triatoma dimidiata* produced the largest volume of feces followed by *Panstrongylus megistus*, *Rhodnius prolixus*, and *Triatoma infestans*. Moreover, stage and sex affected fecal production; 5th-stage female nymphs excreted the largest volume of feces. The amount of blood ingested was significantly correlated with the volume of feces produced in 10 out of 11 experimental groups. Triatome size and volume of feces produced were less closely correlated. Indeed, a "threshold" minimum amount of blood must be ingested before bugs are stimulated to defecate. The defecation habits of triatomines probably influence the vectorial capacity of a triatomine species to a lesser degree than do the density of domestic infestations, host affinity, and the degree of adaptation to the domestic habitat.

Key words: triatomines; defecation; *Trypanosoma cruzi;* Chagas' disease.

Introduction

Since transmission of *Trypanosoma cruzi* generally involves contact with triatomine feces, the defecation habits of triatomines play a role in regulating transmission. Early observers suggested that certain species were likely to transmit *T. cruzi* because they defecated soon after feeding (Wood, 1951; Dias, 1956). More recent workers have attempted to quantify the defecation habits of important vector species, suggesting that vector "efficiency" may be based on a "defecation index" (Pipkin, 1968; Pippin, 1970; Zeledón et al., 1977a).

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Attempts to quantify the defecation patterns of triatomines have focused on the percentage of bugs defecating within specific time intervals and the number of times bugs defecate. Observations of the actual volume of feces produced have been approximations (Petana, 1967; Neal and Miles, 1977). In this study, we measured the volume of feces produced by triatomines during and immediately after feeding. In addition, we determined whether bug species, stage, weight, and amount of blood ingested affect the volume of feces triatomines produce.

Materials and Methods

Triatomine colonies

Triatomine colonies have been maintained for up to 10 years at the *Centro de pesquisas Gonçalo Muniz* (Salvador, Bahia) as previously described (Szumlewicz, 1969; Szumlewicz et al., 1973). The origins of colonies used in this study were: *Panstrongylus megistus*, states of Bahia and São Paulo, Brazil; *Triatoma infestans*, states of Bahia and São Paulo, Brazil; *Rhodnius prolixus*, Venezuela; *Triatoma dimidiata*, Belize. We used the method described by Szumlewicz and Cruz (1972) to separate male and female 5th-stage nymphs. Colonies were generally maintained at room temperature (25–30° C; 70–90% RH). Bugs were separated from their respective colonies on the day of molting. Subsequently, they were starved for 10–30 days before being used in these experiments.

Collection of feces

Triatomines were allowed to feed on laboratory mice (males and females weighing 15–20 g) as previously described (Zeledón et al., 1977a). Briefly, mice were restrained with masking tape applied to a plastic tray 35×32 cm. Individual triatomines were placed into contact with mice; the time feeding was commenced, of termination of feeding, and of each defecation during the 30 min following feeding were recorded. Every drop of feces deposited onto the plastic tray surface were aspirated into Clay Adams (Parsippany, N. J., USA) micropipettes calibrated at 1–5 μl. The volume of feces contained in each drop was determined to the nearest 0.1 μl.

Amount of blood ingested

In order to calculate the amount of blood ingested, we weighed insects before feeding and at the end of the 30 min observation period following feeding. Triatomines were weighed on an H80 Mettler balance accurate to 0.1 mg. The total amount of blood ingested was computed as follows: [Weight after feeding (mg) – Weight before feeding (mg)] + [Volume feces produced (μl) $\times$ Specific gravity of bug feces (mg/μl)] = Amount of blood ingested (mg).

Specific gravity of feces

We determined the specific gravity of bug feces. Fecal droplets produced by 73 *P. megistus* were aspirated into micropipettes and the volume of feces estimated to the nearest 0.1 μl. Each micropipette was weighed to the nearest 0.1 mg before aspiration of feces and subsequently weighed with the fecal sample present. The specific gravity of each fecal droplet was computed as follows: [Weight of micropipette with feces (mg) – Weight of micropipette (mg)] $\div$ Volume of fecal drop (μl) = Specific gravity of droplet (mg/μl). Triatomines produced 3 types of defecatory material; dark (feces), clear (urine), and mixed. Although the specific gravity of feces was slightly higher than urine, this difference was not significant (Table 1). The overall specific gravity of triatomine defecatory material was 1.04. This value was subsequently used in the formula to determine the amount of blood ingested by triatomines.

Table 1. Specific gravity of feces produced by *Panstrongylus megistus*

Type of defecation	No. of bugs examined	Specific gravity (wt. feces/vol. feces) (mg/μl)
Dark	32	1.05 ± 0.32*
Mixed	18	1.05 ± 0.35
Clear	23	1.01 ± 0.37
Total	73	1.04 ± 0.34

* $\bar{x} \pm$ S.D.

Results

We compared the volume of feces produced by 4 species of triatomines. Fifty 4th-stage nymphs of *T. infestans*, *R. prolixus*, *P. megistus*, and *T. dimidiata* were allowed to feed on mice and the volume of feces these bugs produced was measured. *Triatoma dimidiata* produced the largest volume of feces at all times during and up to 30 min postfeeding, followed by *P. megistus* and *R. prolixus* (Table 2). *Triatoma infestans* produced the least amount of feces. The amount of blood ingested was directly related to the volume of feces produced, being highest in *T. dimidiata* and lowest in *T. infestans*. The size of the insects before feeding was not directly related to the volume of feces produced, since *R. prolixus* weighed less than *T. infestans* but produced more feces. Thus, triatomine species influences the volume of feces produced by bugs.

The influence of stage of development on habits of triatomine defecation was studied by allowing nymphal and adult *P. megistus* to feed on mice. Adults and 5th-stage nymphs were separated as to sex. Fifth-stage nymphs produced the largest volume of feces, followed by adults and 4th-stage nymphs; the first through third stages produced much smaller volumes of feces (Table 3). As in the comparison between species, volume of feces was more closely related to the amount of blood ingested than was the size of the insect. Fifth-stage nymphs were smaller than adults, but these nymphs ingested more blood and produced more feces than did adults. Moreover, female triatomines produced significantly more feces than did males (student "t" = 2.4, df = 48, $p < 0.25$). Thus, insect stage and sex affected the volume of feces produced by triatomines. Female 5th-stage nymphs produced the largest volume of feces.

The amount of blood ingested was significantly correlated with the volume of feces produced in 10 of 11 groups of insects studied (Table 4). Only 1st-stage nymphs did not show such a correlation, since they produced a very small volume of feces ($\bar{x} = 0.2\ \mu$l). In contrast, insect size was correlated with volume of feces in only 7 of the 11 groups studied. The correlation coefficient between amount of blood ingested and volume of feces was higher than the correlation

Table 2. Volume of feces produced by 4th-stage nymphs of 4 triatomine species

Species	No. of bugs examined	Wt. before feeding (mg)	Amt. blood ingested** (mg)	Cumulative volume feces (μl); minutes postfeeding				
				0	1	5	15	30
T. infestans	50	23.9 ± 6.0*	79.5 ± 41.4	0.1 ± 0.6	0.4 ± 1.2	1.9 ± 2.3	3.9 ± 3.3	6.7 ± 4.9
R. prolixus	50	15.3 ± 3.9	124.8 ± 31.1	1.1 ± 2.5	5.5 ± 3.4	7.2 ± 3.9	8.6 ± 4.3	11.8 ± 5.0
P. megistus	50	30.5 ± 7.2	195.2 ± 94.4	8.1 ± 7.7	8.8 ± 7.7	10.4 ± 7.7	13.0 ± 8.6	18.9 ± 10.9
T. dimidiata	50	87.8 ± 67.1	484.8 ± 231.3	14.8 ± 19.5	16.3 ± 19.7	23.9 ± 19.4	33.1 ± 19.8	55.3 ± 25.0

* $\bar{x} \pm$ S.D.

** [Wt. after feeding (mg) − Wt. before feeding (mg)] + [Vol. feces during 30 min (μl) × 1.04 (mg/μl)] = Amount blood ingested (mg)

Table 3. Volume of feces produced by different stages of *Panstrongylus megistus*

Stage	No. of bugs examined	Wt. before feeding (mg)	Amt. blood ingested** (mg)	Cumulative volume feces (μl); minutes postfeeding				
				0	1	5	15	30
1st	25	0.7 ± 0.3*	4.0 ± 1.8	0	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.3	0.2 ± 0.3
2nd	25	2.2 ± 0.4	14.5 ± 8.1	0	0.3 ± 0.6	1.6 ± 2.8	1.8 ± 1.8	2.0 ± 1.9
3rd	25	9.3 ± 2.2	73.3 ± 31.5	2.2 ± 2.4	2.7 ± 2.4	3.2 ± 2.3	4.3 ± 2.8	6.0 ± 3.2
4th	50	30.5 ± 7.2	195.2 ± 94.4	8.1 ± 7.7	8.8 ± 7.7	10.4 ± 7.7	13.0 ± 8.6	18.9 ± 10.9
5th/Female△	25	109.9 ± 22.3	869.2 ± 280.6	79.3 ± 53.8	79.5 ± 53.6	82.2 ± 54.2	91.6 ± 55.4	115.3 ± 56.5
5th/Male△△	25	92.9 ± 17.6	627.1 ± 282.5	63.4 ± 74.5	63.5 ± 74.8	66.3 ± 74.9	75.4 ± 78.0	91.0 ± 81.1
Female□	25	168.6 ± 43.9	357.1 ± 109.3	20.1 ± 30.5	20.3 ± 30.6	20.7 ± 30.9	27.5 ± 37.0	45.3 ± 46.2
Male□□	25	140.7 ± 49.9	194.7 ± 57.7	9.6 ± 19.1	10.0 ± 19.2	11.7 ± 21.2	12.4 ± 22.2	19.7 ± 24.8

* $\bar{x} \pm$ S.D.

** Computed as in Table 2

△ Female 5th-stage nymphs

△△ Male 5th-stage nymphs

□ Adult females

□□ Adult males

Table 4. Correlation between amount of blood ingested and volume of feces produced

Species	Stage	No. bugs examined	Correlation coefficient between insect size (mg) and volume feces (μl) (r)	Correlation coefficient between amount blood ingested (mg) and volume feces (μl) (r)	Proportion blood meal excreted (%)**
T. infestans	4th	50	0.01	0.69*	9.0 ± 5.6
R. prolixus	4th	50	0.44*	0.46*	10.2 ± 4.3
T. dimidiata	4th	50	0.25	0.30*	12.8 ± 7.9
P. megistus	1st	25	–0.01	0.15	6.0 ± 7.6
P. megistus	2nd	25	0.13	0.59*	11.3 ± 11.5
P. megistus	3rd	25	0.59*	0.78*	8.9 ± 3.4
P. megistus	4th	50	0.64*	0.65*	10.4 ± 4.8
P. megistus	5th/Female	25	0.43*	0.63*	13.9 ± 6.5
P. megistus	5th/Male	25	0.45*	0.82*	13.9 ± 8.6
P. megistus	Female	25	0.48*	0.54*	12.2 ± 11.6
P. megistus	Male	25	0.74*	0.59*	9.0 ± 10.4

* $p < 0.05$; ** [Volume feces produced (μl) × 1.04 mg/μl] ÷ Amount blood ingested (mg)

Table 5. Defecation behavior of 4th-stage *P. megistus* allowed to feed during specific time intervals.
A. Duration of feeding

Duration of feeding (min)	No. of bugs examined	Percent of bugs defecating
2.5	15	53.6
5.0	15	46.9
7.5	15	66.7
10.0	15	87.1

coefficient between insect size and volume of feces in 10 out of 11 experiments. Moreover, the proportion of the blood-meal excreted was generally about 10%, varying from a low of 6% (1st-stage *P. megistus*) to a high of 13.9% (5th-stage nymphs). Thus, the amount of blood ingested was significantly correlated with the volume of feces produced, while insect size was often not related.

The volume of feces contained in fecal drops produced by triatomines was highly variable. Of the 934 fecal drops produced by the 200 4th-stage insects of the 4 species studied, the volume of individual fecal deposits ranged from 0.1 μl to 50.4 μl, with a mean = 4.7 μl (± 6.7 μl S.D.). *T. dimidiata* fecal droplets averaged 14.3 μl (± 10.0 μl), *P. megistus* 3.3 μl (± 2.3 μl), *T. infestans* 2.3 μl (± 1.5 μl) and *R. prolixus* 1.8 μl (± 2.3 μl).

Table 6. Defecation behavior of 4th-stage *P. megistus* allowed to feed during specific time intervals. B. Amount of blood ingested

Amount of blood ingested (mg)*	No. of bugs examined	Percent of bugs defecating
0–10	13	0
10.1–30	18	61
30.1–50	14	86
>50	15	100

* Computed as in Table 2

To determine whether triatomines must feed for a minimum time period or ingest a minimum amount of blood before being stimulated to defecate, we allowed 4th-stage *P. megistus* to feed on mice for 2.5, 5, 7.5, or 10 min. Bugs were removed from contact with mice after the specified duration of feeding and observed for 30 min. Defecation was not closely related to the duration of feeding (Table 5). The majority of insects allowed to feed for only 2.5 min defecated. In contrast, the amount of blood ingested directly controlled defecation behavior (Table 6). While none of the bugs which ingested $\leqslant 10$ mg blood defecated, 80% of bugs ingesting >10 mg blood defecated. Apparently, a minimum quantity of blood must be ingested by triatomines before they are stimulated to defecate.

Discussion

Previous attempts to measure the volume of feces produced by triatomines have not yielded definitive information. Petana (1967) suggested that the amount of feces deposited by *T. dimidiata* depended upon the age and "hunger" of the insects; hungry bugs produced more feces. Neal and Miles (1977) attempted to measure the amount of blood and *T. cruzi* parasites ingested by *R. prolixus*. These authors weighed bugs before and after feeding, calculating the difference as the amount of blood ingested. However, they noted that the calculated weight of ingested blood should be increased by the weight of fecal deposits, which they estimated as approximately 10 μl or about 10 mg. During our experiments we found that the volume of feces contained in fecal drops produced by triatomines varied from 0.1 μl to 50.4 μl. Fecal drops of *T. dimidiata* were much larger than the other 3 species studied, while *R. prolixus* produced the smallest fecal deposits. Zeledón et al. (1977b) reported that 1000–1500 μl of excretion could be collected from the largest of triatomines, *Dipetalogaster maximus*. Generally, smaller triatomines such as *R. prolixus* tend to defecate more often than larger species (e.g., *T. dimidiata* and *D. maximus*), while the fecal deposits of larger species are greater in volume.

The pioneering work of Wood (1951) and Dias (1956) suggested that South American triatomines are more efficient vectors of Chagas' disease than are North American triatomines, due to the tendency of North American triatomines to delay defecation. Several subsequent workers have confirmed this tendency (Pipkin, 1968; Pippin 1970; Zeledón et al., 1977a; Silva et al., 1979). Indeed, Zeledón et al. (1977a) suggested that defecation patterns are probably one of the principal factors influencing the epidemiology of Chagas' disease. Moreover, Zeledón et al. (1977a) proposed a "defecation index" in which *R. prolixus* scored much higher than *T. dimidiata.* These workers suggested that defecation habits of these two vectors may explain the greater incidence of Chagas' disease in *R. prolixus* endemic areas. In our study, *T. dimidiata* produced a much greater volume of feces than did *R. prolixus.* In addition, *T. dimidiata* produced more feces during feeding, when in direct contact with the host, than did all other triatomines. These observations suggest that defecation behavior is not the key factor regulating the vectorial capacity (= "vector efficiency") of triatomine vectors of *T. cruzi.* Other factors such as household density of domestic triatomines (Zeledón et al., 1977a; Mott et al., 1978; Schofield, 1980), host affinity (Minter, 1976), and the degree of ecological adaptation to human dwellings (Zeledón, 1974; Mott et al., 1978; Barretto, 1979) appear to play a more important role in establishing vectorial capacity of triatomines.

The amount of blood ingested appears to be closely correlated with the volume of feces produced by triatomines. Indeed, a minimum quantity of blood, in the case of 4th-stage *P. megistus* equal to 10 mg, must be ingested before bugs are stimulated to defecate. Despite the detailed studies of Wigglesworth (1931a, b, c), the stimulus for triatomine defecation is not known. The signal for triatomines to defecate once the "threshold" volume of blood has been ingested may be under hormonal control (Maddrell, 1963), nervous control (i.e., stretch receptors), or the result of direct mechanical pressure.

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Barretto M. P.: Epidemiologia. In: *Trypanosoma cruzi* e Doença de Chagas, ed. by Z. Brener and Z. Andrade, p. 89–151. Guanabara Koogan, Rio de Janeiro 1979.

Dias E.: Observações sobre eliminação de dejeções e tempo de sucção em alguns triatomineos sulamericanos. Mem. Inst. Osw. Cruz *54*, 115–124 (1956).

Maddrell S. H. P.: Excretion in the blood-sucking bug, *Rhodnius prolixus* Stal. I. The control of diuresis. J. exp. Biol. *40*, 247–256 (1963).

Minter D. M.: Feeding patterns of some triatomine vector species. In: New approaches in American trypanosomiasis research, p. 33–46. Pan American Health Organization Sci. Publ. No. 318, Washington, D.C. 1976.

Mott K. E., Muniz T. M., Lehman J. S., Hoff R., Morrow R. H., Oliveira T. S., Sherlock I., Draper C. C.: House construction, triatomine distribution and household distribution of seroreactivity to *Trypanosoma cruzi* in a rural community in northeast Brazil. Amer. J. trop. Med. Hyg. *27*, 1116–1122 (1978).

Neal R. A., Miles R. A.: The number of trypomastigotes of *Trypanosoma cruzi* required to infect *Rhodnius prolixus*. Rev. Inst. Med. trop. S. Paulo *19*, 177–181 (1977).

Petana W. B.: American trypanosomiasis in British Honduras. IV. Laboratory observations on *Triatoma dimidiata* (Hemiptera: Reduviidae) and its efficiency as a vector of Chagas' disease in British Honduras. Ann. trop. Med. Parasit. *61*, 413–416 (1967).

Pipkin A. C.: Domiciliary reduviid bugs and the epidemiology of Chagas' disease in Panama (Hemiptera: Reduviidae: Triatominae). J. med. Ent. *5*, 107–124 (1968).

Pippin W. F.: The biology and vector capability of *Triatoma sanguisuga texana* Usinger and *Triatoma gerstaecker* (Stål) compared with *Rhodnius prolixus* (Stål) (Hemiptera: Triatominae). J. med. Ent. *7*, 30–45 (1970).

Schofield C. J.: Density regulation of domestic populations of *Triatoma infestans* in Brazil. Trans. roy. Soc. trop. Med. Hyg. *74*, 761–769 (1980).

Silva E. O. R., Ferraz A. N., Souza J. M. P.: Indices de dejeção das principais especies de triatomineos, presentes no Estado de São Paulo, Brasil. In: Anais Abstracts, p. 138. Congresso Internacional sobre doenças de Chagas, Rio de Janeiro 1979.

Szumlewicz A. P.: Estudos sobre a biologia do *T. infestans* o principal vector da doença de Chagas no Brasil. Rev. bras. Malar. *21*, 117–159 (1969).

Szumlewicz A. P., Cruz H. N.: Triatominae (Hemiptera: Reduviidae): sex identification in immature forms of vectors of Chagas' disease. Rev. Inst. Med. trop. S. Paulo *14*, 6–11 (1972).

Szumlewicz A. P., Cruz H. N., Araujo J. A. N.: Species and stage interaction in the feeding, behaviour of vectors of Chagas' disease (the importance of determinants in planing for greater efficacy and standardization of xenodiagnostic procedures). Rev. Inst. Med. trop. S. Paulo *15*, 139–150 (1973).

Wigglesworth V. B.: The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera: Reduviidae). I. Composition of the urine. J. exp. Biol. *8*, 411–427 (1931a).

Wigglesworth V. B.: The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera: Reduviidae). II. Anatomy and histology of the excretory system. J. exp. Biol. *8*, 428–442 (1931b).

Wigglesworth V. B.: The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera: Reduviidae). III. The mechanism of uric acid excretion. J. exp. Biol. *8*, 443–451 (1931c).

Wood S. F.: Importance of feeding and defecation times of insect vectors in transmission of Chagas' disease. J. econ. Ent. *44*, 52–54 (1951).

Zeledón R.: Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In: Trypanosomiasis and leishmaniasis with special reference to Chagas' disease, p. 51–85. Associated Scientific Publishers, Amsterdam 1974.

Zeledón R., Alvarado R., Jiron L. F.: Observations on the feeding and defecation patterns of three triatomine species (Hemiptera: Reduviidae). Acta trop. Basel *34*, 65–77 (1977a).

Zeledón R., Alvarenga N. J., Schosinsky K.: Ecology of *Trypanosoma cruzi* in the insect vector. In: Chagas' disease (Proceedings of an international symposium, New York, June 1977), p. 59–70. Pan American Health Organization Sci. Publ. No. 347, Washington, D.C. 1977b.