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Vectorial Capacity of *Triatoma guasayana* (Wygodzinsky & Abalos) (Hemiptera: Reduviidae) Compared with Two other Species of Epidemic Importance

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ABSTRACT - *Triatoma guasayana* (Wygodzinsky & Abalos) is a peridomestic triatomine with epidemiological importance in Bolivia, that may play an important role in the transmission of *Trypanosoma cruzi* (Chagas). In this study, two parameters of vectorial capacity were evaluated: the interval of feeding-defecation time and metacyclogenesis, in adult males and females and nymphal instars II to V of *T. guasayana* with comparisons with *Triatoma infestans* (Klug) and *T. sordida* (Stal). The results showed a close relationship between ingestion of blood and beginning of defecation. Values were negative in *T. infestans*, and *T. sordida* for instars II, III, IV, and V and also males and females but were positive in female *T. sordida*. *Triatoma guasayana* showed only negative values for instar II. Adults and nymphs began defecation as soon as they had finished feeding and required an average of 29.8 min. The analysis of metacyclogenesis showed that *T. guasayana* was superior to *T. infestans* and *T. sordida*. However, the vectorial effectiveness of *T. guasayana* was significantly affected regarding the percentage of metacyclic trypomastigotes during instars III to V and showed a progressive increase. Females had higher proportions despite their ingestion being half that of *T. infestans*. The different instars of *T. guasayana* had a higher parasitic load than those of *T. sordida* and, although ingestion was 1/3 of that of female *T. infestans*, there was a progressive increase in metacyclic trypomastigotes in the different nymphal stages of *T. guasayana* that decreased in adults.

KEY WORDS: Metacyclogenesis, feeding time, attacking time, feeding-defecation interval, Chrono M

In 1991, Southern Cone countries initiated a project whose main objective was to stop vectorial transmission of Chagas disease by *Triatoma infestans* (Klug) and also by blood transfusion in Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay. (Schofield *et al* 2006, OMS 2007). Results showed that the Health Ministries of Latin-American countries had efficient and economically viable methods for establishing vectorial control and blood transfusion programs which would permit the elimination of some vector species (OMS 2007). However, this was at odds with the recent increase of cases of Chagas disease in the Gran Chaco of Argentina (Gürtler *et al* 2005).

At the moment, 141 species of Triatominae are described in the New World (Galvão *et al* 2003, Galvão & Angulo 2006, Costa *et al* 2006, Bérenger & Blanchet 2007, Costa & Félix 2007, Martinez *et al* 2007, Sandoval *et al* 2007). The reduction in *T. infestans* (Klug) populations in residences has created an available niche for other vectors and the tendency for peridomestic or wild species to occupy these available niches in Paraguay and Brazil has been demonstrated (Dias 1988, Ruas Neto & Krug 1995, Rojas-de-Arias 1996). In Uruguay, Rosa & Salvatella (1995) warned of the presence of *Triatoma rubrovaria* ((Blanchard) in residences, and Bar *et al* (1993) and Wisnivesky-Colli *et al* (1993) demonstrated the potential risk that *Triatoma sordida* (Stal) and *Triatoma guasayana* (Wygodzinsky & Abalos) represented in Argentina. *Triatoma sordida* and *T. guasayana* are closely related species of Triatominae since they are morphologically similar and occupy the same ecotopes with an overlapping distribution in northern Argentina and part of the Bolivian Chaco and Paraguay (Schofield 1994,Usinger *et al* 1966). Although originally they were wild species, they were frequently found in peridomestic and domestic habitats (Forattini 1980, Wisnivesky-Colli *et al* 1993, Gajate *et al* 1996, Noireau *et al* 1998) due to their domestic tendency, and may be considered as possible domestic vectors of Chagas disease.

Triatoma sordida and *T. guasayana* both occur in Bolivia (Noireau *et al* 1999), and have adapted to the human environment but they are also found in forest habitats. Both these species might be potential vectors since they frequently substitute *T. infestans* after control operations. In the forest environment, *T. sordida* and *T. guasayana* were found in a variety of ecotopes: bird nests, tree holes, under bark, trees roots, palm trees, in groups of bromeliads and under rocks (Barretto 1971, Forattini *et al* 1971, Carcavallo & Martinez 1985, Diotaiuti *et al* 1993). In the Bolivian Chaco, these species were mainly collected from trees holes and bromeliads (Noireau *et al* 2000) and the adults have a high dispersal potential by flying at night during the hot, dry months (August-November) (Wisnivesky-Colli *et al* 1993, Noireau *et al* 1998). Studies on triatomine species have demonstrated that hunger is the main trigger for starting flight (Sjogren & Ryckman 1996, Ekkens 1981, Lehane & Schofield, 1982, McEwen & Lehane 1993) and, therefore, nutritional condition might indicate whether adults will fly or not. (Lehane & Schofield 1982).

Triatoma guasayana has high populations in the wild, mainly in biotopes that include Cactacea (*Opuntia quimilo*), chaguares (*Bromelia* sp.) and trunks and this species usually feeds on rodents, marsupials and birds (Carcavallo *et al* 1988, Vezzani *et al* 2001). Its epidemiological importance is considered but in 1985 its presence in housing, where *T. infestans* had been controlled by insecticides (Carcavallo *et al*, 1985), was mentioned. Studies in Argentina showed that both this species and *T. sordida* were secondary vectors of *Trypanosoma cruzi* (Chagas) and would be a potential danger for human beings during entomological surveillance, since *T. guasayana,* is not only able to invade homes and attack human beings and dogs but has been found naturally infected with the parasite (Carcavallo *et al* 1985, Wisnivesky-Colli *et al* 1993, Gajate *et al* 1996, Cecere 1999, Canale 2000).

In order to be an efficient vector of *T. cruzi*, a Triatominae species must have the following characteristics (Lent & Wygodzinsky 1979): i) inhabit dwellings and have a high reproductive rate; ii) be susceptible to infection by *T. cruzi* (infection rate by *T. cruzi*: high and efficient metacyclogenesis); iii) be anthropophylic and have a short feeding-defecation interval.

With the objective of evaluating the potential epidemiological importance of *T. guasayana* and *T. sordida*, two important parameters which control the vectorial capacity of both species were studied: the feeding-defecation process and their metacyclogenesis, with comparisons made with the main vector, *T. infestans*. These data may predict differences between the abilities of these species as vectors (Mello 1980, Kirk & Schofield 1987, Trumper & Gorla 1991, Crocco & Catalá 1996).

Material and Methods

The nymphal instars and adult males and females were supplied by the Insectary of the Instituto Boliviano de Biología de la Altura (I.B.B.A.) from fifth generation triatomine colonies. The insects were kept in the laboratory at 28 ± 2ºC and 60-70% RH. The *Tulahuén* strain of *T. cruzi* was provided by the Phytochemistry Department of I.B.B.A and the female *Swiss* mice were supplied by the Laboratory of the Animals Center of the Instituto de Laboratorios de Salud (INLASA). All the research was carried out at the Tropical Diseases Laboratory of I.B.B.A.

Feeding/defecation study. An apparatus called Chrono M, designed by Noireau (unpublished data), was used to determine the feeding-defecation interval of the triatomines (Fig 1). Chrono M is a plastic container of 250 cm^3, which has a millimeter mesh in the upper part that puts the mouse in contact with the food source. Both the upper and lower parts have electronic detectors which are activated when the insect is in contact with the food source and are deactivated when the insect deposits its feces. Chrono M has a digital chronometer which measures time in seconds. This apparatus was used for 5th instar nymphs and adults but the 2nd, 3rd and 4th nymphal instars were observed directly using an ordinary chronometer.

Insects. *Triatoma sordida* of Cotoca from Santa Cruz (216 insects), *T. guasayana* of Izozog from Santa Cruz (180) and *T. infestans* of Izozog from Santa Cruz (173) as the control. After two successive meals, the insects were starved for 30 days and the following parameters were evaluated: (i) time of attack or time interval between placing the insect in the container and its contact with the mouse, with the penetration of the rostrum into the host tegument; **(ii)** feeding time or time interval between the penetration of the insect rostrum into the mouse tegument and its withdrawal after finishing blood ingestion; (iii) defecation time or time interval between the penetration of the insect rostrum into the mouse tegument and excretion of the first drop of urine and/or feces. (iv) the determination of the time interval for feeding-defecation, which is the difference between

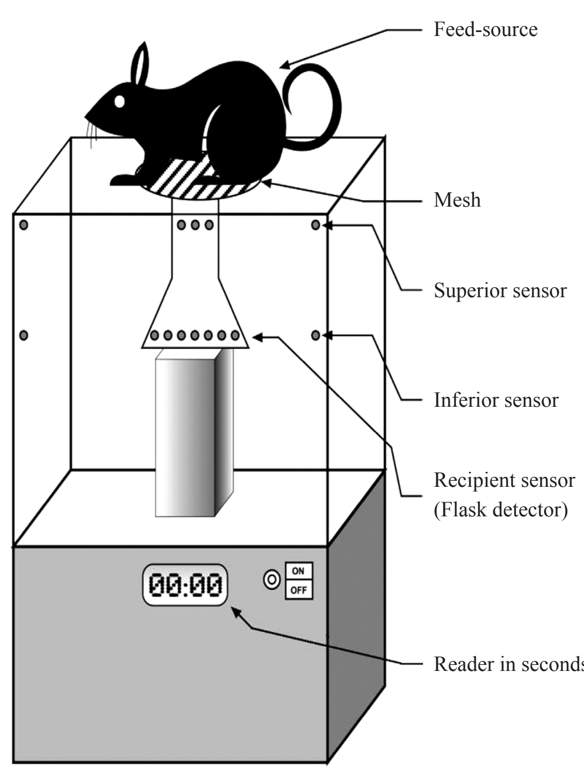

Fig 1 Chrono M.

the feeding and defecation times. If this interval was negative, the insect defecated before finishing blood feeding.

Metacyclogenesis. This is the process of transformation of the epimastigote forms of *T. cruzi* into metacyclic trypomastigote forms which takes place in the triatomine alimentary canal. Six female *Swiss* mice were infected intraperitoneally with an inoculation of 100 µl of the *Tulahuén* strain of *T. cruzi* from a culture that contained 2.4 10^6 parasites/cm^3. Nymphs of the 3rd, 4th and 5th instars, as well as adults of *T. sordida* (39 insects), *T. guasayana* (40 insects) and *T. infestans* (33 insects), were used for evaluating metacyclogenesis. The insects were starved for thirty days before feeding them for 30 min on infected mice. After 30 days, their abdomens were squeezed to obtain feces and if preliminary observations showed the presence of flagellates, 50 µl of their faeces in two or three drops of physiologic solution were centrifuged at 1500 rpm for 3 min in saline solution. The sediment was treated with a mixture of absolute ethanol and giemsa and observations were made using an Olympus CH30 microscope with an immersion objective (100x) and the counts made over 200 fields.

Statistical methods. Means were compared using the Student t test (when one of the samples < 30) and reduced deviation or R.D. (when both samples > 30)

Results

Feeding - defecation study

Attacking time. *Triatoma infestans* had the shortest attacking times; *T. sordida* needed less time for starting the feeding process than *T. guasayana,* for males and for the 3rd and 4th nymphal instars. The differences between both species were not significant for the other instars (Table 1).

Feeding time. *Triatoma infestans* fed faster than *T. guasayana* and *T. sordida*, which fed for longer during all the nymphal instars, except for the 5th instar where there were no significant differences (Table 2).

Defecation time. *Triatoma infestans* defecated faster for all the instars, followed by *T. sordida* and *T. guasayana* and all the differences were significant (Table 3).

Time interval of feeding-defecation. Except for the 2nd instar, *T. guasayana* defecated after having finished feeding whereas the other two species defecated before withdrawing their rostrum from the host tissue. *Triatoma sordida* showed a significant advantage in the 1st, 2nd and 3rd nymphal instars over *T. infestans*. A tendency to defecate rapidly was observed in these instars for both species, and especially in *T. sordida* (Table 4).

Metacyclogenesis. In the 6th mice infected with *T. cruzi*, the highest peak of parasitemia was observed on the 7th day and animals died on the 10th day. The triatomines were infected on the 7th day when the parasitemia had the highest peak (Table 5). No differences in parasite populations were observed among the different stages of the same species. *Triatoma infestans* had the biggest population of trypomastigotes (20.5 trypomastigotes in 200 fields vs. 15.4 for *T. guasayana* and 13.6 for *T. sordida*, R.D. = ($P < 0.001$). The percentages of metacyclic trypomastigotes were not significantly different between the three species (11.5% in *T. infestans* vs. 9.2% in the other two species; R.D. = ($P < 0.05$) (Table 6).

Discussion

According to Noireau (1999), the vector candidates are triatomines found in the wild but that may occasionally invade artificial ecotopes where they establish small colonies. Various reports of *T. sordida* and *T. guasayana* in human environments in Southern Cone countries (Forattini *et al* 1982, Carcavallo *et al* 1988, Noireau *et al* 1997) make them vector candidates and this should encourage more studies on these species. With regard to the vectorial control of Chagas disease, the peridomicile vectors have a special importance due to their role in these processes and the re-infestation of housing during entomological surveillance. Among these vectors, *T. guasayana* and *T. sordida*, are species which might play a significant role in the transmission of *T. cruzi* to human beings, specially after the eradication of *T. infestans* from houses (Carcavallo *et al* 1985, Wisnivesky-Colli *et al* 1993, Gajate *et al* 1996, Cecere *et al* 1999, Noireau *et al* 1999, Canale 2000).

According to Wisnivesky-Colli *et al* (1993), *T. guasayana* would develop bigger populations in a domiciliary environment. It is clear that domiciliary transmission of *T. cruzi* depends on factors, such as the population size of triatomines, which has to reach a critical size (Noireau *et al* 1997). For this reason, from just considering the population dynamics, it is improbable that any of these species might be as efficient a vector as *T. infestans* (Schofield 1994). However, it is known that this biological criterion is not the only one and others, such as the process of feeding-defecation and metacyclogenesis of *T. cruzi*, are also very important (OMS 1991).

The dependence of defecation time on the quantity of ingested blood was observed in *T. infestans* (Trumper & Gorla 1991) and *Rhodnius prolixus* (Kirk & Schofield 1987); these two variables show a similarity in the feeding-defecation interval. A minimum blood feeding of 80 mg was enough for defecations to occur during feeding by 5th instar nymphs, while nymphs of the 5th instar of *T. infestans,* which fed on the same quantity of blood, had a feeding-defecation interval of 15 min after feeding (Trumper & Gorla 1991). Comparison of these two parameters between *T. infestans* and *T. sordida* show that adult females of *T. sordida* are more efficient when defecating compared to the other instars (Perlowagora 1973, Zeledon *et al* 1977). The 5th nymphal instar of *T. sordida* feeds on more blood than *T. infestans*, which allows this species to survive longer with just one meal (Perlowagora 1973, Juarez & Castro 1982). Nymphs and adults of *T. sordida* defecated immediately after feeding or frequently defecated during feeding (Crocco & Catalá 1996); the quantity ingested depended on the nutritional state of insect at the moment of feeding, which is related to dispersal activity. Different studies have shown that a low nutritional state is a determinant for adult dispersal by flying

Table 1 Mean time (min) for beginning an attack at the blood source, comparision of the different vectors and different instars (*Triatoma infestans, T. sordida, T. guasayana*).

Vector	Instar	No	Min.	Max.	Mean ± SD	DM	P <	t	P <
T. sordida	II	31	0.60	2.20	1.40 ± 0.80	0.77	N.S.		
T. guasayana	II	32	0.80	2.40	1.60 ± 0.80[a]				
T. sordida	II	31	0.60	2.20	1.40 ± 0.80	3.19	0.01		
T. infestans	II	36	0.40	1.40	0.90 ± 0.50				
T. guasayana	II	32	0.80	2.40	1.60 ± 0.80[a]	4.27	0.001		
T. infestans	II	36	0.40	1.40	0.90 ± 0.50				
T. sordida	III	40	0.10	1.50	0.80 ± 0.70	5.58	0.001		
T. guasayana	III	42	0.80	2.80	1.80 ± 1.00[a]				
T. sordida	III	40	0.10	1.50	0.80 ± 0.70	2.12	0.05		
T. infestans	III	39	0.10	0.90	0.50 ± 0.40				
T. guasayana	III	42	0.80	2.80	1.80 ± 1.00[a]	8.34	0.001		
T. infestans	III	39	0.10	0.90	0.50 ± 0.40				
T. sordida	IV	44	0.10	1.50	0.70 ± 0.80	5.59	0.001		
T. guasayana	IV	44	0.80	2.40	1.60 ± 0.80[a]				
T. sordida	IV	44	0.10	1.50	0.70 ± 0.80	1.54	N.S.		
T. infestans	IV	43	0.20	0.80	0.50 ± 0.30				
T. guasayana	IV	44	0.80	2.40	1.60 ± 0.80[a]	8.61	0.001		
T. infestans	IV	43	0.20	0.80	0.50 ± 0.30				
T. sordida	V	61	0.30	0.11	0.60 ± 0.30	1.17	N.S.		
T. guasayana	V	64	0.30	1.60	0.70 ± 0.60[a]				
T. sordida	V	61	0.30	0.11	0.60 ± 0.30	1.55	N.S.		
T. infestans	V	64	0.20	0.99	0.50 ± 0.30				
T. guasayana	V	64	0.30	1.60	0.70 ± 0.60[a]	2.76	0.05		
T. infestans	V	64	0.20	0.99	0.50 ± 0.30				
T. sordida	Males	17	0.10	0.39	0.20 ± 0.10			5.12	0.001
T. guasayana	Males	26	0.60	1.90	1.10 ± 0.70[a]				
T. sordida	Males	17	0.10	0.39	0.20 ± 0.10			1.69	N.S.
T. infestans	Males	25	0.10	0.60	0.30 ± 0.20				
T. guasayana	Males	26	0.60	1.90	1.10 ± 0.70[a]			5.20	0.001
T. infestans	Males	25	0.10	0.60	0.30 ± 0.20				
T. sordida	Females	23	0.10	0.40	0.30 ± 0.20			0.38	N.S.
T. guasayana	Females	26	0.20	0.44	0.30 ± 0.10[a]				
T. sordida	Females	23	0.10	0.40	0.30 ± 0.20			0.21	N.S.
T. infestans	Females	27	0.20	0.60	0.30 ± 0.30				
T. guasayana	Females	26	0.20	0.44	0.30 ± 0.10[a]			0.06	N.S.
T. infestans	Females	27	0.20	0.60	0.30 ± 0.30				

No: number of insects; SD: standard deviation; a: test comparision of the mean: DM = reduced deviation > 30; t = student test < 30

Table 2 Mean feeding time (min) of instar at blood source for the three vectors and different instars (*Triatoma infestans, T. sordida, T. guasayana*).

Vector	Instar	No	Min.	Max.	Mean ± SD	DM	P <	t	P <
T. sordida	II	30	24.5	39.0	32.0 ± 6.9	2.30	0.05		
T. guasayana	II	30	24.5	32.0	28.7 ± 3.7[a]				
T. sordida	II	30	24.5	39.0	32.0 ± 6.9			4.00	0.001
T. infestans	II	28	19.3	32.0	25.2 ± 5.8				
T. guasayana	II	30	24.5	32.0	28.7 ± 3.7[a]			2.72	0.01
T. infestans	II	28	19.3	32.0	25.2 ± 5.8				
T. sordida	III	40	23.0	46.0	35.1 ± 10.4	5.05	0.001		
T. guasayana	III	37	20.0	30.0	25.9 ± 4.7[a]				
T. sordida	III	40	23.0	46.0	35.1 ± 10.4	7.99	0.001		
T. infestans	III	30	15.0	25.0	20.4 ± 4.6				
T. guasayana	III	37	20.0	30.0	25.9 ± 4.7[a]	4.89	0.001		
T. infestans	III	30	15.0	25.0	20.4 ± 4.6				
T. sordida	IV	40	20.5	45.0	33.3 ± 11.5	4.68	0.001		
T. guasayana	IV	32	21.0	28.5	24.3 ± 3.5[a]				
T. sordida	IV	40	20.5	45.0	33.3 ± 11.5	4.60	0.001		
T. infestans	IV	30	18.5	28.5	24.1 ± 4.6				
T. guasayana	IV	32	21.0	28.5	24.3 ± 3.5[a]	0.21	N.S.		
T. infestans	IV	30	18.5	28.5	24.1 ± 4.6				
T. sordida	V	28	21.9	42.5	31.8 ± 9.3			0.08	N.S.
T. guasayana	V	29	29.0	34.0	31.6 ± 2.1[a]				
T. sordida	V	28	21.9	42.5	31.8 ± 9.3			2.97	0.01
T. infestans	V	30	19.5	31.5	25.8 ± 5.7				
T. guasayana	V	29	29.0	34.0	31.6 ± 2.1[a]			5.15	0.001
T. infestans	V	30	19.5	31.5	25.8 ± 5.7				
T. sordida	Males	28	25.5	40.0	33.2 ± 6.5			0.90	N.S.
T. guasayana	Males	26	28.5	35.0	31.9 ± 3.0[a]				
T. sordida	Males	28	25.5	40.0	33.2 ± 6.5			2.62	0.01
T. infestans	Males	34	21.5	34.6	28.9 ± 6.2				
T. guasayana	Males	26	28.5	35.0	31.9 ± 3.0[a]			1.47	N.S.
T. infestans	Males	34	21.5	34.6	28.9 ± 6.2				
T. sordida	Females	29	26.0	35.0	31.4 ± 4.2			0.12	N.S.
T. guasayana	Females	26	27.5	34.0	31.2 ± 2.7[a]				
T. sordida	Females	29	26.0	35.0	31.4 ± 4.2			5.93	0.001
T. infestans	Females	21	17.5	28.0	23.5 ± 5.1				
T. guasayana	Females	26	27.5	34.0	31.2 ± 2.7[a]			6.64	0.001
T. infestans	Females	21	17.5	28.0	23.5 ± 5.1				

No: number of insects; SD: standard deviation; a: test comparison of the mean: DM: reduced deviation > 30; t = student test < 30

Table 3 Mean defecation time (min) of different instars of *Triatoma infestans, T. sordida, T. guasayana*.

Vector	Instar	No	Min.	Max.	Mean ± DS	DM	P <	t	P <
T. sordida	II	31	18.9	25.0	21.8 ± 2.4	11.36	0.001		
T. guasayana	II	32	25.5	31.0	28.3 ± 2.2[a]				
T. sordida	II	31	18.9	25.0	21.8 ± 2.4	10.29	0.001		
T. infestans	II	36	12.5	19.0	15.6 ± 2.5				
T. guasayana	II	32	25.5	31.0	28.3 ± 2.2[a]	22.20	0.001		
T. infestans	II	36	12.5	19.0	15.6 ± 2.5				
T. sordida	III	39	18.8	25.7	22.9 ± 3.3	3.86	0.001		
T. guasayana	III	44	20.7	31.8	26.7 ± 5.5[a]				
T. sordida	III	39	18.8	25.7	22.9 ± 3.3	11.98	0.001		
T. infestans	III	39	11.6	16.9	15.2 ± 2.3				
T. guasayana	III	44	20.7	31.8	26.7 ± 5.5[a]	12.58	0.001		
T. infestans	III	39	11.6	16.9	15.2 ± 2.3				
T. sordida	IV	44	18.4	25.3	23.1 ± 3.2	6.17	0.001		
T. guasayana	IV	44	22.8	35.0	28.8 ± 5.2[a]				
T. sordida	IV	44	18.4	25.3	23.1 ± 3.2	6.13	0.001		
T. infestans	IV	44	15.9	20.8	19.6 ± 2.3				
T. guasayana	IV	44	22.8	35.0	28.8 ± 5.2[a]	10.81	0.001		
T. infestans	IV	44	15.9	20.8	19.6 ± 2.3				
T. sordida	V	61	25.0	35.0	30.3 ± 4.4	6.99	0.001		
T. guasayana	V	64	31.0	39.0	35.3 ± 3.6[a]				
T. sordida	V	61	25.0	35.0	30.3 ± 4.4	14.19	0.001		
T. infestans	V	64	16.9	23.0	20.7 ± 2.9				
T. guasayana	V	64	31.0	39.0	35.3 ± 3.6[a]	25.25	0.001		
T. infestans	V	64	16.9	23.0	20.7 ± 2.9				
T. sordida	Males	17	28.6	34.8	32.2 ± 3.1			2.75	0.01
T. guasayana	Males	26	31.5	36.5	34.5 ± 2.5[a]				
T. sordida	Males	17	28.6	34.8	32.2 ± 3.1			5.29	0.001
T. infestans	Males	25	19.5	28.0	25.0 ± 4.9				
T. guasayana	Males	26	31.5	36.5	34.5 ± 2.5[a]			8.71	0.001
T. infestans	Males	25	19.5	28.0	25.0 ± 4.9				
T. sordida	Females	23	28.8	33.0	31.6 ± 2.1			3.59	0.001
T. guasayana	Females	26	30.7	36.9	34.4 ± 3.1[a]				
T. sordida	Females	23	28.8	33.0	31.6 ± 2.1			7.73	0.001
T. infestans	Females	27	17.0	28.0	22.6 ± 5.3				
T. guasayana	Females	26	30.7	36.9	34.4 ± 3.1[a]			9.87	0.001
T. infestans	Females	27	17.0	28.0	22.6 ± 5.3				

No: number of insects; SD: standard deviation; a: test comparison of the mean: DM: reduced deviation > 30; t = student test < 30

Table 4 Mean feeding-defecation interval (min) for each instar of *Triatoma infestans, T. sordida, T. guasayana*.

Vector	Instar	No	Feeding time Mean ± SD	Feeding-defecation Mean ± SD	Feeding-defecation interval Mean ± SD
T. sordida	II	31	32.0 ± 6.9	21.8 ± 2.4	-10.2 ± 4.50
T. guasayana	II	32	28.7 ± 3.7	28.3 ± 2.2	-0.40 ± 1.50
T. infestans	II	36	25.2 ± 5.8	15.6 ± 2.5	-9.60 ± 3.30
T. sordida	III	40	35.1 ± 10.4	22.9 ± 3.3	-12.2 ± 7.10
T. guasayana	III	42	25.9 ± 4.7	26.7 ± 5.5	+0.70 ± 0.80
T. infestans	III	32	20.4 ± 4.7	15.2 ± 2.3	-5.10 ± 2.40
T. sordida	IV	44	33.3 ± 11.5	23.1 ± 3.2	-10.1 ± 8.30
T. guasayana	IV	44	24.3 ± 3.5	28.8 ± 5.2	+4.60 ± 1.70
T. infestans	IV	44	24.1 ± 4.6	19.6 ± 2.3	-4.50 ± 2.30
T. sordida	V	61	31.8 ± 9.3	30.3 ± 4.4	-1.50 ± 4.90
T. guasayana	V	64	31.6 ± 2.1	35.3 ± 3.6	+3.70 ± 1.50
T. infestans	V	64	25.8 ± 5.7	20.7 ± 2.9	-5.10 ± 2.80
T. sordida	Males	17	33.2 ± 6.5	32.2 ± 3.1	-1.00 ± 3.40
T. guasayana	Males	26	31.9 ± 3.0	34.5 ± 2.5	+2.60 ± 0.50
T. infestans	Males	25	28.9 ± 6.2	25.0 ± 4.9	-3.90 ± 1.30
T. sordida	Females	23	31.4 ± 4.2	31.6 ± 2.1	+0.30 ± 2.10
T. guasayana	Females	26	31.2 ± 2.7	34.4 ± 3.1	+3.10 ± 0.40
T. infestans	Females	27	23.5 ± 5.1	22.6 ± 5.3	-1.00 ± 0.20

No: number of insects; SD: standard deviation

Table 5 Mean quantity of blood ingested (mg) for each instar of *Triatoma infestans*, *T. sordida* and *T. guasayana*.

Vector	Instar	Nr	Min.	Max.	Mean ± DS	F	P <
T. sordida	II	31	3.50	6.0	5 ± 1x	60.90	0.001
T. guasayana	II	32	4.10	24.5	14 ± 11[1]		
T. infestans	II	36	17.6	44.4	32 ± 13		
T. sordida	III	39	9.80	21.9	16 ± 6x	58.40	0.001
T. guasayana	III	44	13.50	25.1	20 ± 6x[1]		
T. infestans	III	39	23.0	72.0	50 ± 25		
T. sordida	IV	44	28.0	55.0	40 ± 13	57.40	0.001
T. guasayana	IV	44	18.0	55.0	40 ± 20[1]		
T. infestans	IV	44	56.0	90.0	74 ± 17		
T. sordida	V	61	53.0	140.0	100 ± 45	12.40	0.001
T. guasayana	V	64	68.0	170.0	115 ± 51[1]		
T. infestans	V	64	107.0	176.0	139 ± 34		
T. sordida	Males	17	51.0	73.0	64 ± 11	127.90	0.001
T. guasayana	Males	26	31.0	71.0	53 ± 20[1]		
T. infestans	Males	25	103.0	135.0	124 ± 16		
T. sordida	Females	23	55.0	88.0	71 ± 14	281.70	0.001
T. guasayana	Females	26	30.0	70.0	48 ± 19[1]		
T. infestans	Females	27	130.0	165.0	146 ± 14		

No: number of insects; SD: standard deviation; [1]test comparison of the mean: DM: reduced deviation >30; F = student test >30

Table 6 Mean number of different forms found in triatomine feces of *Trypanosoma cruzi* (metacyclic-trypomastigotes, epimastigotes and other forms) in feces of triatomine instars (mean for vectors after examination at 100x to quantify the number of parasites in 200 fields).

Instar	No	Metacyclicstrypomastigotes			Epimastigotes			Other forms			Total forms		
		Min.	Max.	Mean ± DS	Min.	Max.	Mean ± DS	Min.	Max.	Mean ± DS	Min.	Max.	Mean ± DS
T. guasayana													
Males	5	7.6	16.0	12.2 ± 2.8	88.9	113.7	100.4 ± 10.2	14.7	20.6	18.4 ± 3.0	111.2	150.3	131.0 ± 13.0
Females	5	9.5	13.5	10.8 ± 1.3	90.6	125.3	108.8 ± 14.3	9.9	14.9	13.4 ± 2.3	110.0	153.7	133.0 ± 16.8
V	10	10.9	18.0	15.2 ± 3.4	102.5	153.7	128.6 ± 27.3	12.9	19.7	18.4 ± 4.2	126.3	191.4	162.2 ± 33.6
IV	10	14.8	25.9	21.0 ± 5.9	125.2	220.6	174.1 ± 43.3	19.5	34.4	28.8 ± 8.3	159.5	280.9	223.9 ± 53.4
III	10	8.7	18.9	13.9 ± 4.5	89.2	147.6	122.1 ± 29.7	12.9	19.9	18.3 ± 3.6	110.8	186.4	154.3 ± 36.0
Total	40	9.4	21.0	15.4 ± 5.4	89.4	165.6	132.4 ± 39.0	12.6	25.8	20.4 ± 7.2	111.4	212.4	168.1 ± 49.6
T. sordida													
Males	4	5.8	15.9	11.5 ± 5.2	70.6	128.7	104.8 ± 31.4	30.6	51.7	45.0 ± 12.5	107.0	196.3	161.3 ± 44.4
Females	4	6.8	15.7	10.8 ± 3.2	69.7	91.4	86.5 ± 8.8	17.3	33.1	28.0 ± 8.1	93.8	140.2	125.3 ± 10.5
V	10	9.8	15.6	13.5 ± 2.9	76.4	135.2	111.2 ± 28.8	13.4	25.2	21.3 ± 5.9	99.6	176.0	146.0 ± 34.5
IV	11	8.5	21.9	16.5 ± 6.6	76.5	148.2	120.5 ± 36.2	8.3	30.3	21.5 ± 11.1	93.3	200.4	158.5 ± 49.9
III	10	5.8	18.9	12.4 ± 7.5	68.4	136.3	107.1 ± 35.9	7.6	26.2	18.7 ± 9.8	81.8	181.4	138.2 ± 50.6
Total	39	8.2	20.3	13.6 ± 5.8	69.3	136.1	109.6 ± 32.1	10.7	33.6	23.8 ± 11.8	88.2	190.0	146.9 ± 42.9
T. infestans													
Males	6	15.7	28.8	23.3 ± 6.5	88.4	210.9	157.8 ± 64.5	15.8	34.2	26.0 ± 9.3	120.2	273.9	207.2 ± 73.0
Females	5	14.9	28.5	21.6 ± 5.9	107.4	159.8	144.2 ± 24.3	18.5	34.7	29.8 ± 8.7	140.8	223.0	195.6 ± 19.4
V	10	12.8	25.5	20.2 ± 6.5	69.3	149.7	119.3 ± 40.7	9.8	28.7	20.3 ± 9.7	91.9	203.9	159.8 ± 50.9
IV	5	18.9	27.3	23.8 ± 4.4	88.4	209.7	158.2 ± 58.3	25.2	39.6	36.0 ± 7.3	132.5	276.6	218.0 ± 59.7
III	7	10.5	19.1	15.6 ± 4.2	78.9	111.6	102.6 ± 14.9	10.6	27.9	21.3 ± 9.0	100.0	158.6	139.4 ± 17.2
Total	33	13.8	25.6	20.5 ± 6.1	74.9	168.3	132.4 ± 46.4	13.6	33.4	25.4 ± 10.2	102.3	227.3	178.3 ± 54.7

No: number of insects; SD: standard deviation

(Schofield *et al* 1991). The low mean weight of *T. sordida* adults allows them to easily disperse and at the same time create new colonies (Crocco & Catalá 1996).

The results obtained in this study support the statements made by some authors. *Triatoma sordida* needs average blood feeding of 5, 16, 40, 100 mg of blood for nymphal stages: N2, N3, N4, N5 respectively, and 64 mg and 71 mg for adult males and females respectively, in order to defecate during feeding or after finishing it. Whereas for *T. guasayana* 14, 20, 40, 115 mg of blood for nymphal stages: N2, N3, N4, N5, and 53 mg and 48 mg for male and females adults are needed respectively.

The results of the attacking time, feeding time and defecating time demonstrated the superiority of *T. infestans* since it started feeding, ingested blood and defecated faster than the other species. Although *T. sordida* is faster than *T. guasayana* in attacking time, its feeding time is prolonged in the first stages. Its blood ingestion is frequently incomplete due to interruptions and the insect will not start to defecate. Crocco & Catalá (1996) observed in *T. infestans* and *T. sordida,* that the first species was the most efficient except for the females. Our data do not show any differences regarding sex, but the authors used another index (Zeledon *et al* 1977) without considering the excretions emitted before the end of feeding. However, we agree that the fastest defecation occurs in the first nymphal instars (2 to 4).

Of all the Triatominae of the genus *Triatoma*, *T. infestans* seems to be the species which produces the highest percentage of trypomastigotes. However, the metacyclogenesis of *T. cruzi* would be more efficient in the genera *Rhodnius* and *Panstrongylus* (Perlowagora-Scumlewicz & Carvalho Moreira 1994), and from a comparative study of the differentiation of *T. cruzi* in various species of triatomines, these authors concluded that there was a direct relationship between Triatominae species and metacyclogenesis. Our data showed a higher production of metacyclic forms in *T. infestans* compared to *T. sordida* and *T. guasayana*, and we did not observe any differences between the different instars of the same species. Various intrinsic factors, such as hemolytic factors and lecithins, may affect the development of strains of *T. cruzi* and explain the differences observed in metacyclogenesis among species (Garcia & Azumbuja 1991).

Of the three species studied, *T. infestans* has, without a doubt, the biggest vectorial capacity in the laboratory, and this is fully confirmed in the field where this species constitutes the main vector of Chagas disease in the Southern Cone countries

(Schofield 1994). *Triatoma sordida* is in a more advanced domiciliary process (Noireau *et al* 1997), and this species is superior to *T. guasayana* regarding the feeding-defecation interval. Both species have very similar characteristics of metacyclogenesis, but this is insufficient for *T. sordida* to be considered an efficient vector in infested housing and its population dynamics may explain its low vectorial capacity (Noireau *et al* 1997). This last parameter, which was not considered in this study, should be studied in *T. guasayana* to see if it can be considered as a possible vector.

In the wild, *T. sordida* and *T. guasayana* are species which occupy different peridomiciliary areas without coexisting, except in the "monte chaqueño" of Santiago del Estero, Argentina (Gajate *et al* 1996, Cecere *et al* 1999). Studies in Argentina on their dispersal capacity show that *T. guasayana* actively invades not only the peridomiciliary area but also the domestic area, where it is numerically superior to *T. sordida*. However, none of these species has been able to colonize houses, probably because during the stage of domiciliary re-infestation, they have to compete with *T. infestans* which has a greater capacity for building important colonies within the same area (Carcavallo *et al* 1988, Wisnivesky-Colli *et al* 1993, Cecere *et al* 1999, Noireau *et al* 1999).

Since *T. guasayana* was found infected with *T. cruzi* in the wild, as well as invading housing, although without colonizing it, various authors support the hypothesis that this species would not only be acting as a link between the three cycles of parasite transmission (wild – peridomiciliary – domiciliary), but is also behaving as a possible secondary vector of the parasite in Argentina and is a potential substitute of *T. infestans* during the surveillance stage (Wisnivesky-Colli *et al* 1993, Gajate *et al* 1996, Cecere *et al* 1999).

Although the need for more studies on the vectorial capacity of *T. sordida* and *T. guasayana* is recognized, the results obtained in this study show that 5th instar nymphs would be capable of transmitting *T. cruzi*, due to characteristics of the vectorial capacity of these species which show that they begin ingesting blood and feed and defecate quickly.

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