An Investigation on the Ecology of *Triatoma vitticeps* (Stal, 1859) and its Possible Role in the Transmission of *Trypanosoma cruzi*, in the Locality of Triunfo, Santa Maria Madalena Municipal District, State of Rio de Janeiro, Brazil

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From January 1989 to April 1995, 465 specimens of Triatoma vitticeps *were collected in the locality of Triunfo, 2nd District of Santa Maria Madalena municipal district, State of Rio de Janeiro. The bugs were found indoors by local residents with predominance of adults. The flight activity was high in hot months when the incidence in the domicile also increased. Two hundred and two bugs (111 alive and 91 dead) were examined for* Trypanosoma cruzi *infection. This was detected in 31 of the dead bugs (34%) and 88 (79%) of the live bugs examined. With a view to investigate the possible vertebrate hosts of the* T. cruzi *isolates, the blood of 122 mammals was examined through Giemsa-stained smears, hemocultures and xenodiagnosis.* T. cruzi *was detected in three specimens of* Didelphis marsupialis *and* T. (M.) theileri *was detected in one specimen of* Bos taurus*. The parasites were isolated from triatomine feces, xenoculture and hemoculture. No evidence of human infection was detected in 58 inhabitants examined, as evaluated by indirect imunofluorescence technique using* T. cruzi *epimastigotes as antigens. These results show that* T. vitticeps *is still a sylvatic species although nymphs have been found inside the domicile. Thus, an epidemiological vigilance is necessary to know the behaviour of this species following the continuous modifications promoted by the presence of man.*

Key words: *Triatoma vitticeps* - ecology - *Trypanosoma cruzi* - State of Rio de Janeiro - Brazil

Triatoma vitticeps has been found in the states of Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro (Silveira et al. 1984, Carneiro et al. 1985, Corrêa 1986).

This species was found colonizing the domicile for the first time in Espírito Santo (Santos et al. 1969b). Lent and Jurberg (1978) confirmed its colonization in Espírito Santo, Minas Gerais and Rio de Janeiro. In Espírito Santo, Silveira et al. (1983) and Sessa and Carias (1986) registered the colonization in seven and in one municipal districts, respectively. Dias et al. (1989) emphasized the high dispersion in this state, reporting signs of colonization in some districts. Ferreira et al. (1986) described the occurrence of *T. vitticeps* in the State of Rio de Janeiro, from the city of Rio de Janeiro to the north of the state.

The present study was carried out in the locality of Triunfo, 2nd District of Santa Maria Madalena municipal district, which has the higher incidence of the triatomine infected with *T. cruzi*-like (Ferreira et al. 1986). The aim of the ecology study of *T. vitticeps* in natural habitat and the investigation of the infection in domestic, synanthropic and wild triatomines and mammals, is to know the actual situation of this area and to alert to the possible risk of colonization contribuing to programs of vigilance and control.

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MATERIALS AND METHODS

Santa Maria Madalena municipal district is situated at an altitude of 620 m, latitude 21°37'S and longitude 42°05'W, in the State of Rio de Janeiro, Brazil. Triunfo, the 2nd District of Santa Maria Madalena is situated in the south, latitude 22°02'52"S and longitude 41°56'32"W (Fig. 1), at the bottom of a mountain, cut by water courses flowing into the Macabu River. The vegetation of the region is typical of an open perennial hygrophyle forest, partly modified by the formation of fields for pasture and banana cultivation.

The triatomines were captured in five areas: area A - 250 m altitude, 3.5 km from the village, profoundly modified by deforestation for banana cultivation; area B - 130 m altitude, 4 km from the village, in a valley with a preserved vegetation (secondary forest). These areas are 2 km far from each other, separated by a mainly rocky geographical barrier (mountain) with altitude 400 m to 900 m. The area C - 40 m altitude, is the village itself, greatly modified by the formation of fields for pasture. The areas D and E - greatly preserved, 10 km and 12 km, from the area C, respectively (Fig. 1).

Monthly, from January 1989 to April 1995, 44 trips of one day were made just to collect the triatomines captured by the population, and 22 trips of one week were made to investigate: (1) the natural ecotopes of the bugs (birds nest, hollow trees and cracks and barks, holes on the ground, railing, bromelia, etc.), and the peridomicile, using in all situations a pyrethrum solution to dislodge the bugs; (2) previously demarcated areas, using the methodology of transect strip; (3) the attraction of the bugs to light-traps; (4) the climatic gauging. The trips were also made to capture the mammals using iron traps (22x22x45 cm and 10x14x31 cm), which were distributed in the peridomestic and sylvatic environments of areas A, B and C.

From January 1989 to April 1990, two light-traps with electric and gas light were set eight times from 8 p.m. to 10 p.m. with climate gauging at the end of each hour. From June 1993 to April 1995 only electric light-traps were set 14 times from dusk to dawn with climate gauging at the end. The traps were placed always close to the house, and preferably, in the new moon period.

The triatomines collected were identified according to the place of capture, sex and if they were alive or dead.

Data on temperature during 1989/1995 were supplied by National Meteorology Institute.

In the laboratory, the triatomines were immersed in 80% ethyl alcohol solution and later dissected under sterile conditions. Their guts were ground in saline with fluorocytosine and then distributed into test tubes containing blood-agar

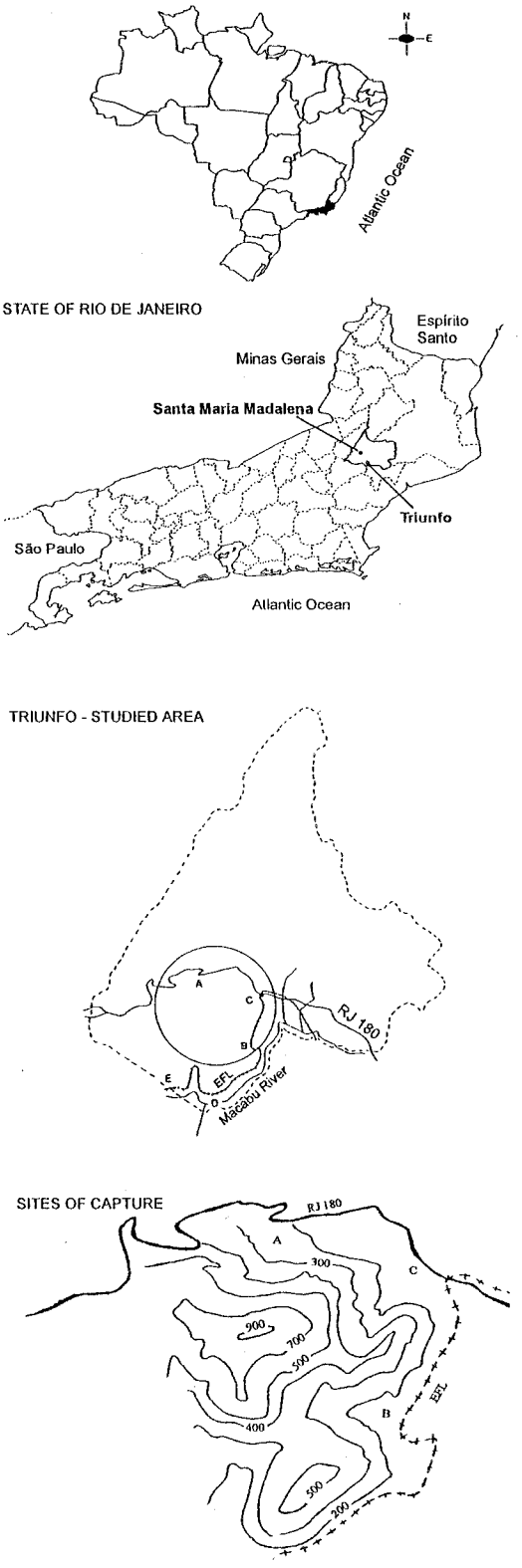

Fig. 1: studied area and sites of capture in Triunfo, Santa Maria Madalena municipal district, State of Rio de Janeiro, Brazil.

medium (NNN) with a LIT overlay. The positive cultures were cryopreserved in liquid nitrogen for subsequent molecular and biochemical characterization (Gonçalves et al. 1996).

To verify the possibility of *T. cruzi* infection, 18-20 g swiss mice were intraperitoneally (IP) inoculated with 0.1 ml of gut content of the bugs examined. The deteccion of the flagellates was verified by microscopic exam.

To identify the possible reservoir hosts of *T. cruzi*, marsupials, rodents, edentates, equines, bovines and canines, trapped in peridomestic and sylvatic environments, were examined through Giemsa-stained blood smears, by hemoculture and xenodiagnosis. Afterwards, the animals were cool marked with a special paste with 20% of sodium hydroxide (NaOH) (Paste R-5) in order to identified an animal captured twice. The hemocultures were examined on day 11, 30, 45 and 60, according to Fernandes et al. (1990).

The total of 40 specimens of *Panstrongylus megistus, Rhodnius prolixus, T. infestans* and *T. vitticeps* (4th instar nymphs), unfed during a two weeks period, were used for xenodiagnosis. These insects were obtained from the insectary of the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz. Unfed or little engorged bugs were discarded. Results of xenodiagnosis, on the 45th day after feeding, were confirmed by xenoculture of the intestinal contents of the bugs, following the same methodology used for hemoculture.

The occurrence of *T. cruzi* human infection was assayed by the sorologic test (indirect imunofluorescence reaction) in inhabitants of Triunfo and Amorosas, Conceição de Macabu municipal district boundary with the area under investigation. The persons examined were those presenting evidences of bite by bugs or living in houses where bugs were found.

RESULTS

From 465 specimens, 455 (98%) were found in indoors by local residents, 9 (2%) were caught in light-traps placed near houses and 1 (0.2%) was found in the domiciliary area. Of the bugs found indoors, 294 (65%) were females, 156 (34%) were males and 15 (3%) were nymphs including all instars. All specimens were identified as *T. vitticeps* and no other species was found. In spite of the difference between the number of triatomines captured mainly in areas A and B, in both areas and all months of the year the number of females was markedly higher than the males. In area C, where the number of houses was higher, the triatomines were captured only in those at the boundary close to the forest. In areas D and E, the distance from the village hampered the contact with the inhabitants. The search in the bug's natural habitats were always negative.

The number of bugs captured per month showed a pronounced annual cycle correlated with average temperature, with a maximum in January and a minimum in June. During this time the number of females was always higher than the males, although in the months of January, May, June, July, August, September, October and December the incidence of females was ³ 50% than the males. The nymphs were found in January, March, April, May, November and December (Fig. 2).

Among the 22 houses included in the survey, only 2, one in area A and the other in area B, were invaded with high frequency. The highest annual incidence of triatomines occurred in 1990 (63 and 45 bugs) in both houses and the lowest in 1994 (5 bugs) and 1995 (13 bugs), in houses of areas A and B, respectively. The highest nymph incidence occurred in 1991 and 1992 (Fig. 3).

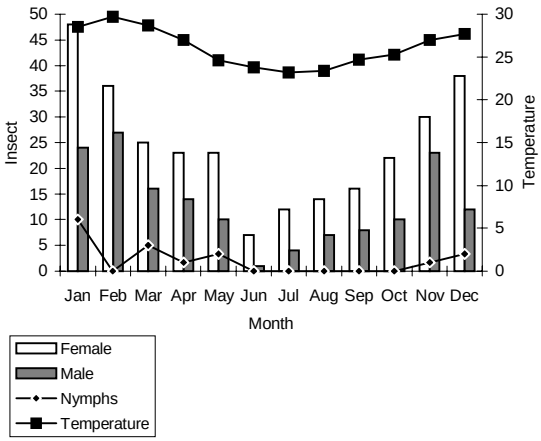

Fig. 2: mean monthly temperature and distribution of *Triatoma vitticeps*, captured in Triunfo, Santa Maria Madalena municipal district, State of Rio de Janeiro, during 1989/1995.

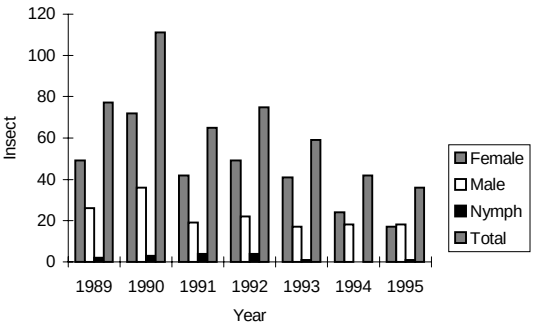

Fig. 3: annual distribution of *Triatoma vitticeps*, captured in Triunfo, Santa Maria Madalena municipal district, State of Rio de Janeiro, during 1989/1995.

The natural ecotopes examined included aproximately 50 birds' nests, 20 marsupial nests, 200 armadillo holes, 50 hollow trees and cracks and barks, 5 railings and 15 bomelias, all of them were negative for triatomines.

The nine bugs captured in light-traps were taken on nights with mean temperature between 24.5°C and 30°C with the occurrence of gust of wind. The highest incidences (five insects) occurred in February 1994 and the others in January 1989, March 1989, August 1993 and November 1994.

From the total specimens captured, 202 (111 alive and 91 dead) were examined for *T. cruzi* infection. *T. cruzi* was detected in 88 (79%) of the live bugs and 31 (34%) of the dead bugs examined. Rates of infection in adults were similar for males (59%) and females (62%) and did not differ significantly between areas. One out of nine nymphs (11%) examined was positive for *T. cruzi* (Tables I, II). Some females were fertile but, unfortunately, this number was not quantified.

In 68 isolates from the bugs, the flagellates were identified as *T. cruzi* either regarding the morphology of the trypomastigotes (metacyclics) seen in their intestinal content or of the bloodstream trypomastigotes seen in mice experimentally inoculated with these forms. Fifty axenic cultures of these flagellates were obtained and were cryopreserved in liquid nitrogen.

The investigation of the natural reservoir was performed in 122 mammals: 22 domestic animals (3 cows, 8 horses, 1 donkey, 1 mule and 9 dogs); 16 black rats (*Rattus rattus*) 41 native rodents including 5 *Holochilus brasiliensis*, 1 *Oxymycterus quaestor*, 2 *Oxymycterus* sp., 1 *Akodon* sp. and 32 unindentified; 32 marsupials including 12 *Didelphis marsupialis*, 4 *Philander opossum* and 1 *Metachirus nudicaudatus* and 11 armadillos including 6 *Dasypus novemcinctus* and 4 *D. septemcinctus*. *T. cruzi* was detected only in 3 *D. marsupialis* (25%) and *T. (M.) theileri* was detected in 1 cow.

TABLE II

Incidence and percentual of dead *Triatoma vitticeps*, infected by *Trypanosoma cruzi* flagellates

Area	Female	%	Male	%
A	8	9	7	8
B	9	10	4	4
C	0	0	3	3
D	0	0	0	0
E	0	0	0	0

All mammals were examined through Giemsa-stained blood smear, with negative results. Fifty two were submitted to xenodiagnosis, 20 to hemoculture and 35 to both xenodiagnosis and hemoculture. One marsupial male from area A had the xenodiagnosis positive to *T. cruzi*. Among the triatomines used for xenodiagnosis in the captured mammals, the specimens of *T. vitticeps*, *P. megistus* and *R. prolixus* which had fed in the marsupial were positive to *T. cruzi* on the 45th day for the first one and 53th day for the last two. Two marsupial males from area B showed hemoculture positive on the 9th and 14th day, but, unfortunately, the parasite isolation was unsuccessful. One cow had the hemoculture positive to *T. (M.) theileri.*

Sorologic tests (indirect imunofluorescence), were negative for all 58 residents tested (35 from Triunfo and 23 from Amorosas) in whose house bugs had been found or who reported having been bitten by triatomines.

DISCUSSION

T. vitticeps may be considered a sylvatic species by the low prevalence of human infection (Santos et al. 1969a,c, Pinto et al. 1969, 1986, Barros et al. 1975, Peçanha et al. 1983, Silveira et al. 1983, Dias et al. 1989) and the high rates of infection by *T. cruzi* observed only in adults bugs (Santos et al. 1969b, Silveira et al. 1983, Ferreira et al. 1986, Sessa & Carias 1986, Dias et al. 1989),

TABLE I

Triatoma vitticeps captured according to the area, developmental stage and infection by *Trypanosoma cruzi* flagellates

Area	Female			Male			Nymphs		
	+	-	N/R	+	-	N/R	+	-	N/R
A	49	29	78	21	16	42	1	7	4
B	26	17	77	15	11	41	0	0	1
C	2	2	10	3	0	7	0	1	1
D	1	0	2	0	0	0	0	0	0
E	1	0	0	0	0	0	0	0	0
Total	79	48	167	39	27	90	1	8	6

N/R: no results

evidencing that the parasite has been acquired outside the houses. However, it is begining to colonize in some localities (Sessa & Carias 1986, Dias et al. 1989, Santos et al. 1969b, Silveira et al. 1983), suggesting a wide ecological valence which was previously low (Forattini et al. 1979).

The results of the present work show a great difference in the number of insects trappeded in areas A and B of the locality of Triunfo, which seems to be related with the preservation of the second area, since both were isolated from each other and from area C.

From the specimens trapped in the houses, 65% presented no intestinal content and some were without internal structures, showing their difficulty to find hosts to feed on and consequently a bad nutricional conditions which may be influencing dispersive flight (Schofield 1985).

The high incidence of *T. vitticeps* in domicile during hot months is in agreement with the previous observation of the high temperature correlation and the increased avidity of the insect to seek blood meal (Zeledón & Rabinovich 1981), which is responsible for the starting point and the duration of the flight (Schofield et al. 1992). However, it is worth mentioning that no correlation has been found between high temperature and incidence of triatomines, based on data obtained by the National Meteorology Institute, in the 1989/1995 period.

According to declarations of inhabitants, the incidence of triatomines inside houses was associated with gust of warm wind, suggesting that the flight might have enabled the triatomines to reach long distances.

In this study the number of females trapped (63%) was higher than the males (34%), both in areas A and B, in aggreement with the findings of Sessa and Carias (1986) and Schofield et al. (1991) in this case related to *T. infestans*, showing that females migrate more than males. This can be due to a higher necessity of blood meal by females (Dias et al. 1989, Gonçalves et al. 1991).

The discovery of some nymphs of *T. vitticeps* in the domicile in Triunfo, cannot be an evidence of its domiciliation, because first of all not all the developmental stages (including eggs) were found together in the same house.

The light-trap set at dusk and down were not efficient in trapping triatomines, contradicting the observations of Bertram (1971) and Ekkens (1981) apud Zeledón (1983), which displayed a higher attraction of these insects to light-traps used in certain hours of the night. Tonn et al. (1978) had results related to kerosene, white and black electric lights, with *R. prolixus*, in opposition to Zárate et al. (1980) who did not observe the attraction to *T. barberi*, by black light set close to the house. In the present work, although gas and white electric lights trapped *T. vitticeps*, they did so in low numbers.

In Triunfo, despite the low socio-economic status of the population the majority of the houses were built with brick and have skylight rather than a ceiling light, which makes them similar to a big light-trap, faciliting the incidence of *T. vitticeps*, but not the establishment of colonies.

The infection rate (59.31%) of adult triatomines from Triunfo by *T. cruzi* confirms the results obtained by Silveira et al. (1983) and Sessa and Carias (1986) in Espírito Santo. However, in the present study it was registered for the first time one 5th instar nymph infected with *T. cruzi* in the bedroom, possibly acquired outside the domicile.

In the present paper, one marsupial has the xenodiagnosis positive to *T. cruzi* using *T. vitticeps*, *P. megistus and R. prolixus*, but not to *T. infestans* evidencing that sylvatic species and others, showing current tendency to domiciliation, are more susceptible to the *T. cruzi* infections than the domiciliate ones. The mutations suffered by the domiciliated species due to the gradual adaptation to the domicile, also make the adaptation of the parasite difficult (Perlowagora-Szumlewicz & Müller 1982, Moreira & Perlowagora-Szumlewicz 1997).

The finding of three marsupials positive between the 122 mammals examined and the failure to detect infection in *R. rattus* and armadillos, importants reservoirs, may be justified by the inefficiency of xenodiagnosis and hemoculture (Fernandes et al. 1990, Perlowagora-Szumlewicz et al. 1990).

The number of infected sylvatic bugs found in houses and the absence of human trypanosomiasis may be explained by its low agressivity and slow defecation (Diotaiuti et al. 1987, Gonçalves et al. 1988). This fact should alert medical personnel do acute Chagas' disease as possible diagnosis in febrile cases, and householder should be warned to protect foodstuff from contamination by these insects.

The epidemiologic vigilance and the working conciousness of the population will be necessary to render difficult the establishment of domestic colonies of the insect.

Complementary studies about food sources of *T. vitticeps* (Gonçalves et al. 1991), and the biochemistry characterization of the parasite isolates (Gonçalves et al. 1996) will allow researchers to identify the possible natural reservoirs and to evaluate the degree of parasite circulation in the area, contributing for a better knowledge of the local epidemiological situation.

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