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STUDIES IN SEARCH OF A SUITABLE EXPERIMENTAL INSECT MODEL FOR XENODIAGNOSIS OF HOSTS WITH CHAGAS' DISEASE. 1 – COMPARATIVE XENODIAGNOSIS WITH NINE TRIATOMINE SPECIES OF ANIMALS WITH ACUTE INFECTIONS BY *TRYPANOSOMA CRUZI*

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In search of a suitable vector species for xenodiagnosis of humans and animals with chronic Chagas' disease we first investigated the reactions of different vector species to acute infection with Trypanosoma cruzi. *Vector species utilized in this study were:* Triatoma infestans, Rhodnius prolixus *and* Triatoma dimidiata, *all well adapted to human habitats;* Triatoma rubrovaria *and* Rhodnius neglectus *both considered totally wild species;* Panstrongylus megistus, Triatoma sordida, Triatoma pseudomaculata *and* Triatoma brasiliensis, *all essentially sylvatic but some with domiciliary tendencies and others restricted to peridomestic biotopes with incipient colonization of human houses after successful eradication of* T. infestans.

Results summarized in Table IV suggest the following order of infectivity among the 9 studied vector species: P. megistus *with 97.8% of infected bugs,* T. rubrovaria *with 95% of positive bugs a close second followed by* T. pseudomaculata *with 94.3% and* R. neglectus *with 93.8% of infected bugs, almost identical thirds.* R. prolixus, T. infestans *and* T. dimidiata *exhibited low infection rates of 53.1%, 51.6% and 38.2% respectively, coupled with sharp decreases occuring with aging of infection (Fig. 1). The situation was intermediate in* T. brasiliensis *and* T. sordida *infection rates being 76.9% and 80% respectively. Results also point to the existence of a close correlation between prevalence and intensity of infection in that, species with high infection rates ranging from 93.8% to 97.8% exhibited relatively large proportions of insects (27.3%–33.5%) harbouring very dense populations of* T. cruzi. *In species with low infection rates ranging from 38.2% to 53.1% the proportion of bugs demonstrating comparable parasite densities was at most* 6%.

No differences attributable to blood-meal size or to greater susceptibility of indigenous vector species to parasites of their own geographical area, as suggested in earlier publications, have been seen. Results described tend to support an alternative hypothesis linking prevalence, intensity and persistence of infection to the type of biotope the vector

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species inhabits. While the parasite produced a scanty and sometimes transient infection in the assembly of vector species widely recognized as domiciliated, it was rich and stable in the essentially sylvatic and totally wild species. The most striking feature is that the biological parameters, on which segregation of vector species in good and poor responders to infection was based were remarkably similar for different species within the groups. Thus, the pattern of vector infectivity does not seem to be a species specifity. On the other hand, the prominent differences in infectivity efficiency between the two ecologically different groups suggest that domiciliation of the vector implies a change in the nature of vector-parasite interaction the magnitude and character of which is manifested by low infection rates and poor parasite yields. The reason for this and the implication of vector biotope involvement in the infective potential of vector species is discussed with particular reference to its use in the search for a suitable insect for xenodiagnosis.

Although accumulated experiments so far have indicated that feeding of bugs of any triatomine species on animals and humans infected with *Trypanosoma cruzi* can be expected to result in infection of the insects, interspecific and intraspecific differences expressed by variations in the rates of infection upon simultaneous feeding on the same hosts have been invariably reported. These have been attributed by Almeida, Miles & Marsden (1973), Minter, Minter-Goedbloed & Ferro Vela (1977), Neal & Miles (1977), Pifano, Morrell & De Ortiz (1973) to interspecific differences of blood-meal size. However, Cerisola et al (1974) and Miles et al (1975) were unable to confirm this direct relationship between blood-meal size and the proportion of insects that became infected. Variations in rates of infection have been also explained by intrinsic differences in susceptibility to infection among bugs of the same species (Brener, 1971; Little, Tay & Biagi, 1966; Marsden et al, 1969; Mello & Chiarini, 1980; Neal & Miles, 1977; Phillips & Bertram, 1967) and between different vector species, as stated recently by Minter, Minter-Goedbloed & Marshall (1978). The possibility of this operating under genetic control has been suggested, as based on experiments described by Maudlin (1976) and Phillips & Bertram (1967).

Apart from the direct factors that might affect the rates of infection among bugs fed on the same donors, the possibility that increased infection rates may be produced more effectively by the use of local vector species than others in xenodiagnosis of local infections has been emphasized. Support for this view was given first by Dias (1940) who, after exposing both local and Venezualan vector species to a Brazilian isolate of *T. cruzi* fround infection rates ranging fron 81.9% to 90.4% in the former but only 54.7% in the latter. Conversely, Zeledon & Vieto (1957), examining infections by a Costa Rican isolate of *T. cruzi* in a local vector species and in four alien species, found the lowest proportion of positive bugs (74%) in the local species of *Triatoma dimidiata*. However, further reports by Cerisola, Rohwedder & Del Prado (1971), Little, Tay & Biagi (1966), Minter, Minter-Goedbloed & Marshall (1978), Ryckman (1965) and Zeledon (1974) confirmed the superiority of the local vector species to alien species in infection rates produced by local *T. cruzi*.

Thus, considerable controversy presently surrounds the hipotheses stressed to explain the diversity of reaction to infection by *T. cruzi* among different vector species. This may be attributed to several factors including the following two: first, our laboratory experience has indicated that, although a particular vector species may readily become infected upon feeding on an infected host, it will support poorly the multiplication of the parasite, thus leading to low parasite yields and sometimes to spontaneous elimination of the parasite. Therefore, it would appear that for the selection of a suitable vector species for xenodiagnosis three biological parameters should be considered: (1), the proportions of positive bugs, (2), the parasite density within the bugs, and (3), the persistence of infection. Second, on the assumption that there exists a close mutual adaptation

between local insect parasite systems, studies on vector species-dependent responsiveness to infection with *T. cruzi* have been focused mainly on *Triatoma infestans* and *Rhodnius prolixus*, two species well adapted to human dwellings and on *Panstrongylus megistus*, so far domiciliated in certain areas in the States of Minas Gerais and Bahia.

Since adaptation of the vector species to artificial biotopes is rather a slow process which could imply a change in the nature of interaction between hosts and parasites, it appeared of interest to extend studies on vector fitness for xenodiagnosis to wild and peridomestic vector species which tend to colonize human houses. This reasoning prompted us to embark on a study of vector species responsiveness to infection with *T. cruzi* Y strain. The aim of the study was to explore and compare the infectivivy of two distinct groups of sylvatic and domiciliated vector species and of those so far restricted to peridomestic niches.

The present paper describes the first series of experiments initiated in 1978 in which species-dependent responsiveness to *T. cruzi* has been focused on the reaction of nine triatomine species fed on guinea-pigs with acute Chagas' disease. The same animals form the hosts for xenodiagnosis of chronic Chagas' disease perfomed throughout 1979, 1980, 1981. Results obtained will be analyzed in a forthcoming paper.

MATERIAL AND METHODS

Triatominae: Vector species used for these studies were essentially those described previously by the senior author (1973, 1976) with the addition of *Triatoma rubrovaria*, briefly:

Triatoma infestans (Klug, 1834) and *Triatoma sordida* (Stal, 1859) – laboratory bred colonies from specimens collected in 1969 in the State of São Paulo.

Triatoma pseudomaculata Corrêa & Spinola, 1964 – Laboratory bred for 9 years, collected in the State of Pernambuco.

Triatoma brasiliensis Neiva, 1911 and *Panstrongylus megistus* (Burmeister, 1835) – laboratory bred for some 9 years, received from the State of Ceará.

Rhodnius prolixus Stal, 1859 and *Rhodnius neglectus* Lent, 1954 – laboratory bred for some 8 years, found as young nymphs in a birds' nest in the State of São Paulo.

Triatoma dimidiata (Latreille, 1811) Belize stock – laboratory bred for some 6 years, received from a laboratory colony maintained at the Dep. Trop. Med. UFRJ, by Dr. Petana.

Triatoma rubrovaria (Blanchard, 1843) – kept as a laboratory colony for 7 years, found in Sete Quedas, State of Paraná.

All of these triatomines are of sylvatic origin and their adaptation to human dwellings varies from species to species. In some areas some species have become so highly domiciliated that are no longer found in the wild, possibly because their sylvatic habitats were destroyed by human activity or because of changes in their microclimate brought about by agricultural practices. Others have adapted to human habitats with lesser degrees of success and are still well represented in natural biotopes. On the basis of surveys concentrated on household infestations described by Barreto (1976) and Zeledon (1976), the bugs utilized in this study can be classified in three categories:

1. Insects well adapted to human houses with few natural ecotopes: *T. infestans*, highly domiciliated, represents probably an association with men and domestic animals of

several centuries and is rarely found in original habitats. *R. prolixus* has been considered strictly domiciliated until Gamboa (1963), Gomez-Nunez (1969) and Pifano, Morrell & De Ortiz (1973) traced them in natural habitats constituted by palm trees. This species, as well as *T. dimidiata* domiciliated in Costa Rica, Equador and Belize has been found, through precipitin tests and marking recapture studies of bug dispersion, to interchange between sylvatic and domestic habitats (Gomez-Nunez, 1969 and Zeledon, 1976).

2. Insects essentially sylvatic but in process of adaptation to human dwellings: *T. brasiliensis* inhabits natural (rodents' nests) and artificial biotopes. *T. pseudomaculata* lives in peridomestic environments but is usually found in natural biotopes (hollow trees). Although data on colonization of human houses by these two species are lacking, Barretto (1976) believes that their domiciliation is not remote. *T. sordida*, essentially sylvatic, tends to reinfest human dwellings maintained under control following successful eradication of *T. infestans*, as reported by Barretto (1976), Forattini et al (1978) and Rocha e Silva, Dias Junior & Gurarita (1969). *P. megistus* although well known to colonize human dwellings in certain areas of Brazil, its domiciliary tendency continues controversial. While Aragão (1961) stated that he has not found "in the literature concerning the *P. megistus* species indication that the species is in process of adaptation to living in homes", incipient colonization of this vector in human habitats in certain regions of the State of São Paulo, maintained under control following eradication of *T. infestans*, has been reported by Barretto (1976) and others quoted by this author and confirmed recently by Forattini et al (1979).

3. Wild insects: *T. rubrovaria*, considered strictly wild has been seen occasionally to enter human houses during summer time. *R. neglectus* is a typical wild insect with domiciliary tendencies in regions of intensive agricultural and cattle breeding activities (Forattini et al, 1979) and in habitats maintained under rigorous control following insecticidal elimination of *T. infestans* (Barretto, 1976).

All colonies were routinely reared at room temperature of 25–28°C and relative humidity of 70 – 80%. Insects were fed on chicken blood at weekly intervals following the procedure described previously by the senior author (1973).

Trypanosoma cruzi: Y strain of Brazilian origin (State of São Paulo), isolated in 1950 from a human infection, maintained in this laboratory since 1974 by syringe passage in albino mice, was used for infection of guinea-pig donors.

Mammalian hosts used in xenodiagnosis: Thirteen white guinea-pigs weighing from 300 to 350g at the start of experiments were inoculated ip. with 0.2ml of infected mouse blood containing $\pm$ 13,9x10^4 *T. cruzi.* All animals developed parasitaemia within 9–12 days which reached a peak of 980 $\pm$ 884.6 parasites in 5cmm of blood between 18 and 35 days after infection. Only nine of these animals were used in xenodiagnosis, the remaining four were set aside for emergency replacement in case of deaths among the former individuals.

Batches of bugs fed on infected hosts: Groups of clean fourth instar nymphs, starved during a period of 2–3 weeks since transition from the proceeding stage, were fed once on the infected guinea-pigs. In order to give equal chances to groups of insects of any of the nine vector species to feed on all guinea-pigs, thus having access to the full range of peripheral parasitaemia from 451 $\pm$ 602.9 to 593 $\pm$ 771.7 *T. cruzi* in 5cmm of blood during the three successive days of feeds, the following scheme was adopted: groups of *T. infestans, P. megistus, R. neglectus* and *R. prolixus* each consisting of 230–250 specimens, were divided in nine aproximately equal subgroups. Similarly the groups of insects (from 70 to 120) of the remaining five species were also divided in nine subgroups. Simultaneous xenodiagnosis of the guinea-pigs was made 18 days after infection with 27 subgroups formed by *T. infestans, T. brasiliensis* and *T. pseudomaculata.* The following day (19 days after infection) simultaneous xenodiagnosis of the same animals was made with a total of 27 subgroups formed by *T. sordida, P. megistus* and *R. neglec-*

tus. The third day simultaneous xenodiagnosis of the guinea-pigs (20 days after infection) was made with 27 subgroups of the remaining species; *T. rubrovaria, T. dimidiata* and *R. prolixus.*

The majority of bugs became well engorged within 25–30 min. Unfed or little engorged bugs were discarded, the well fed, easily distinguished by their dilated body, were pooled by species in individual glass jars, thus forming the laboratory stocks of nine vector species used throughout the study. Maintenance of the experimental bugs for more than one year and the feeding regime on chicken blood at biweekly intervals were as described previously for normal colonies by P.-Szumlewicz (1973), except that the latter have been routinary fed at weekly periods.

Examination of bugs for infection: The technic of bug examination involved cutting up the very rear portion of the abdomen, pulling out the entire digestive tract and grinding it in one or two drops of saline. This suspension was examined microscopically by counting all parasite forms in 50 fields (x400) of two samples for each bug. Quantitation of infection as expressed by both, the proportion of infected bugs and the magnitude of the parasite yields in bugs, started as early as four and eight days after infection in some species, however as a rule, it was done at biweekly intervals during the first 60 days following the single feed on infected animals and monthly thereafter. Examination of bugs initiated with eight specimens of each vector species. The numbers more than doubled later on in testing four species, but diminuished considerably in testing the other five species due to lack of insects. This was also the reason for earlier termination of observations on certain species. Nonetheless, others were followed long enough (240–300 days) to allow observations on the persistence of infection in the aging vectors.

RESULTS

The quantitative assessments of the infective potentialites of the vector species from flagellates found in the alimentary tract are presented as infection rates in Tables I, II, IV and Figure 1, and as parasites densities in Tables III, IV.

Table I shows for bugs of 9 species, fed once only on infected guinea-pigs, that overall infection rates for *T. cruzi* ranged from a relatively low level of 38.2% in *T. dimidiata* to an about optimum level of 97.8% in *P. megistus.* Although these two species represent two genera, we think that recorded differences in infection rates were not associated with generic factors, as evidenced by the following examples: while 93.8% of *R. neglectus* had become infected upon feeding on guinea-pigs, only 53.1% of its congener *prolixus* were positive. Similarly, the percentage of positive bugs in *T. rubrovaria* was 95 but only 51.6% of its congener *infestans* were infected under identical experimental conditions.

The apparent order of vector species infectivity, as based on the overall infection rates summarized in Table IV is as follows: *P. megistus* with 97.8% of infected bugs, *T. rubrovaria* with 95% of positive bugs, a close second followed by *T. pseudomaculata* with 94.3% and *R. neglectus* with 93.8% of positive bugs, almost identical thirds. Other species, *R. prolixus, T. infestans* and *T. dimidiata* exhibited low infection rates; 53.1%, 51.6% and 38.2% respectively, coupled with sharp decreases occurring with aging of infections (Fig. 1). The situation was intermediate in *T. brasiliensis* and *T. sordida* in the sense that the proportions of infected bugs, ranging from 76.9% to 80%, were not as high as in the former four species, nor as low as in the latter three (Table IV).

The same order of infectivity efficiency among the 9 vector species was also suggested by data referred to the frequency of distribution of tests with identical ranges of infection rates among different species as shown in Table II. Of a total of 44 tests performed at different intervals after infection, utilizing *P. megistus, T. rubrovaria, R. ne-*

TABLE I

Rates of infection with Y strain of *T. cruzi* in 9 triatomine species after a single feed on guinea-pigs with acute Chagas' disease

Days after infection	*Percentage of positive bugs at increasing progressively intervals from day 4 to 300 after intake of trypanosomes (no. of bugs infected/fed)*								
	R. prolixus	*R. neglectus*	*P. megistus*	*T. infestans*	*T. brasiliensis*	*T. sordida*	*T. pseudomaculata*	*T. rubrovaria*	*T. dimidiata*
4	37.5(3/8)	100(8/8)	100(8/8)	100(8/8)					
8	87.5(7/8)	100(8/8)	87.5(7/8)	87.5(7/8)		Not done			
15	68.4(13/19)	100(16/16)	93.7(15/16)	68.7(11/16)	100(8/8)	100(8/8)	88.9(8/9)	83.3(5/6)	16.7(1/6)
30	75.0(12/16)	100(16/16)	100(16/16)	75.0(12/16)	87.5(7/8)	90.0(9/10)	100(8/8)	83.3(5/6)	50.0(3/6)
45	50.0(10/20)	93.7(15/16)	89.5(17/19)	40.9(9/22)	80.0(8/10)	91.7(11/12)	100(8/8)	100(6/6)	50.0(3/6)
60	81.2(13/16)	100(16/16)	100(21/21)	50.0(10/20)	75.0(6/8)	100(8/8)	87.5(7/8)	100(6/6)	83.3(5/6)
90	71.4(15/21)	100(19/19)	100(16/16)	47.4(9/19)	75.0(6/8)	100(8/8)	87.5(7/8)	100(4/4)	33.3(1/3)
120	50.0(9/18)	81.3(13/16)	100(16/16)	25.0(5/20)	75.0(6/8)	75.0(6/8)	100(8/8)	100(5/5)	0(0/7)
150	33.3(6/18)	100(18/18)	100(18/18)	50.0(7/14)	50.0(4/8)	75.0(6/8)	100(8/8)	100(3/3)	
180	31.6(6/19)	87.5(14/16)	100(16/16)	10.0(1/10)	71.4(5/7)	50.0(4/8)	87.5(7/8)	100(4/4)	
210	27.8(5/18)	93.8(15/16)	100(14/14)			75.0(6/8)	100(8/8)		
240	27.3(3/11)	78.3(18/23)	100(12/12)			80.0(4/5)	100(8/8)		
300		100(7/7)					83.3(5/6)		
Overall:	53.1(102/192)	93.8(183/195)	97.8(176/180)	51.6(79/153)	76.9(50/65)	84.3(70/83)	94.3(82/87)	95.0(38/40)	38.2(13/34)

Blank-lack of insects.

TABLE II

Showing frequency distribution of tests with identical ranges of infection rates among different vector species

Vector species	*Days after infection of testing bugs (nº of tests)*	*Nº of bugs per test (total tested)*	*Nº & (%) of tests with infection rates ranging as:*					
			0%	*1-25%*	*26-50%*	*51-75%*	*76-99%*	*100%*
P. megistus	4-240(12)	8-21(180)	0	0	0	0	3(25)	9(75)
T. rubrovaria	15-180(8)	3-6(40)	0	0	0	0	2(25)	6(75)
R. neglectus	4-300(13)	7-23(195)	0	0	0	0	5(38.5)	8(61.5)
T. pseudomaculata	15-300(11)	6-9(87)	0	0	0	0	5(45.5)	6(54.5)
T. sordida	15-240(10)	5-12(83)	0	0	1(10)	3(30)	3(30)	3(30)
T. brasiliensis	15-180(8)	7-10(65)	0	0	1(12.5)	4(50)	2(25)	1(12.5)
T. infestans	4-180(10)	8-22(153)	0	2(20)	4(40)	2(20)	1(10)	1(10)
R. prolixus	4-240(12)	8-21(192)	0	0	7(58.3)	3(25)	2(16.7)	0
T. dimidiata	15-120(6)	3-7(34)	1(16.7)	0	4(66.7)	0	1(16.7)	0

glectus and *T. pseudomaculata*, 29 (66%) exhibited infection rates of 100%. Of the 18 exames on *T. sordida* and *T. brasiliensis*, only on four (22%) occasions all were positive. In the group of the remaining three species, *T. infestans*, *R. prolixus* and *T. dimidiata*, of a total of 28 tests only one (3.6%) exhibited 100% of infected bugs. It is also noteworthy that in no instance infection rates declined below 76% within the tested samples of *P. megistus*, *T. rubrovaria*, *T. pseudomaculata* and *R. neglectus*, while infection rates ranging from 1% to 75% were demonstrated in 78% of the total samples from *T. infestans*, *R. prolixus* and *T. dimidiata* examined at different intervals after a single feed on infected donors.

The rate of infections was followed long enough to gain some information about the life pattern of the vector species reaction to *T. cruzi*. Fig. 1 depicts this information indicating that *P. megistus* and *T. rubrovaria* retained infection rates of 100% throughout the observations lasting from 180 to 240 days, and that these oscillated around this level in *T. pseudomaculate* and *R. neglectus* throughout the period of 300 days after infection. The situation was different in *R. prolixus*, *T. infestans* and *T. dimidiata*. Infection rates reached relatively high levels in the former two species during the first eight days after infection and at the end of two months in the latter species, but it decreased gradually thereafter remaining at a very low level in *R. prolixus*, tending to disappear in *T. infestans* and being eliminated in *T. dimidiata* (Fig. 1).

On the assumption that induction of high infection rates with concurrent establishment of dense parasite populations should be considered in the selection of a suitable insect model for xenodiagnosis purposes, the magnitude of parasite burdens was determined for all bugs which had become infected after a single feed on infected donors. Counts of parasite per 50 fields were classified in 4 density ranges from; 1 to 10, 11 to 100, 101 to 250 and 251 to uncountable. The assigned qualitative score to each density range used throughout was: low, moderate, dense and very dense.

The density of parasite populations (Table III) was low and/or moderate in four vector species examined as early as day four following infection but, while in some species it maintained these levels throughout the observations, in others it increased substantially during the following 6 weeks. This can neither be attributed to aging of the infection nor to aging of the insect host. The following examples provide support for this conclusion: *R. neglectus* examined 15 days after infection when still immature (4th instar) or 300 days after infection (as adults) revealed comparable proportions of specimens harbouring parasite populations ranging from dense to very dense. Similarly, *P. megistus* examined 8 or 210 days after infection revealed equal proportions of insects with parasite yields ranging from 251 to uncountable per 50 fields. The situation was different in *R.*

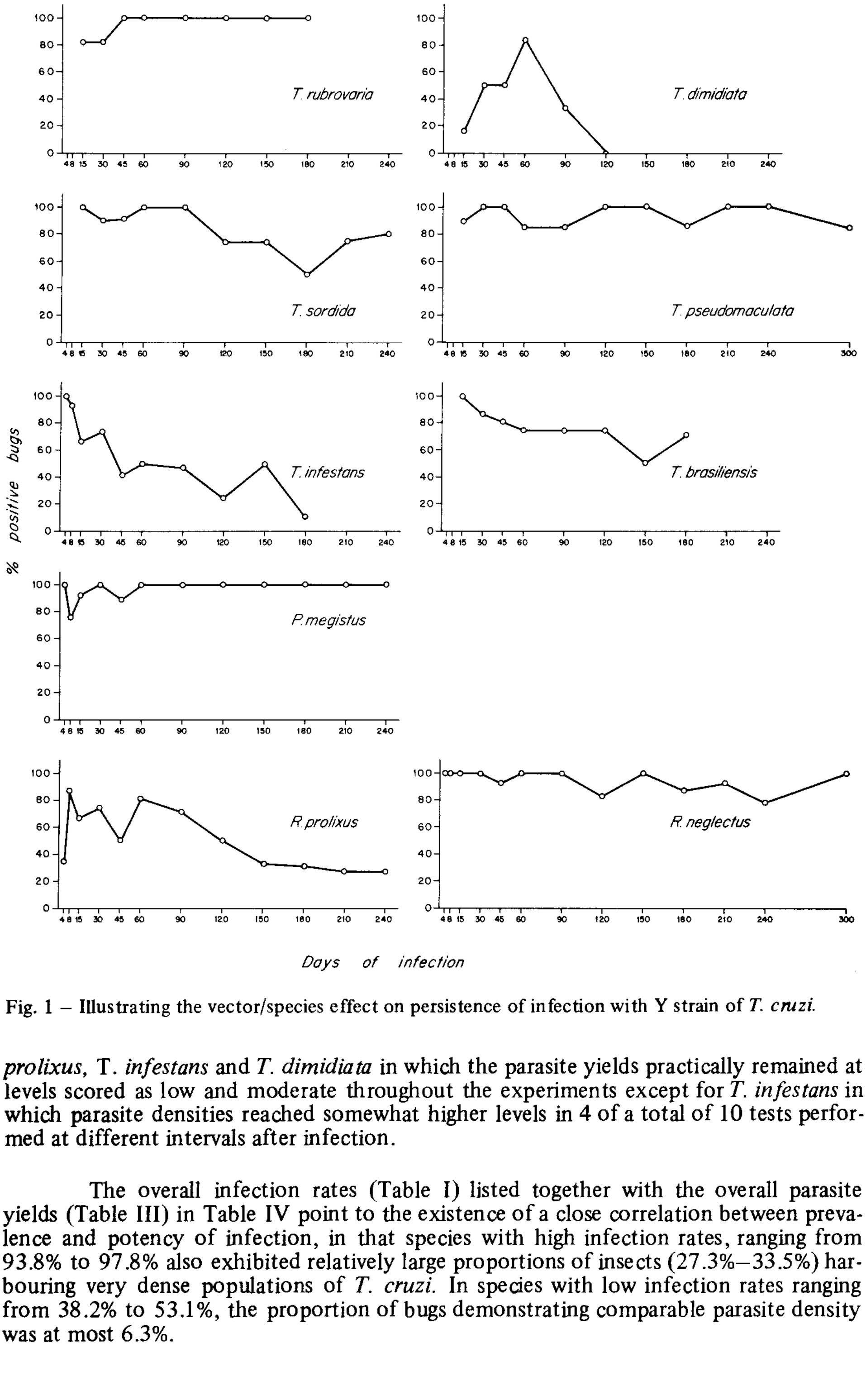

Fig. 1 – Illustrating the vector/species effect on persistence of infection with Y strain of *T. cruzi*.

prolixus, T. *infestans* and *T. dimidiata* in which the parasite yields practically remained at levels scored as low and moderate throughout the experiments except for *T. infestans* in which parasite densities reached somewhat higher levels in 4 of a total of 10 tests performed at different intervals after infection.

The overall infection rates (Table I) listed together with the overall parasite yields (Table III) in Table IV point to the existence of a close correlation between prevalence and potency of infection, in that species with high infection rates, ranging from 93.8% to 97.8% also exhibited relatively large proportions of insects (27.3%–33.5%) harbouring very dense populations of *T. cruzi*. In species with low infection rates ranging from 38.2% to 53.1%, the proportion of bugs demonstrating comparable parasite density was at most 6.3%.

TABLE III

Density of parasites in positive bugs of 9 triatomine species based on counts per 50 fields at increasing progressively intervals after a single feed on guinea-pigs with acute Chagas' disease

Density ranges of T. cruzi	*Days in bug*	*Percentage of bugs with the appropriate numerical values of parasite densities (nº of bugs infected)*								
		R. prolixus	R. neglectus	P. megistus	T. infestans	T. bras.	T. sordida	T. psmac.	T. rubrovaria	T. dimidiata
1-10		100	87.5	62.5	50.0					
11-100	4	0 (3)	12.5 (8)	37.5 (8)	50.0 (8)					
101-250		0	0	0	0					
251-Unc.		0	0	0	0					
1-10		71.4	0	0	42.9					
11-100	8	28.6 (7)	75.0 (8)	85.7 (7)	57.1 (7)					
101-250		0	12.5	0	0					
251-Unc.		0	12.5	14.3	0					
1-10		69.2	6.3	26.7	54.5	25.0	62.5	50.0	0	–
11-100	15	30.8 (13)	50.0 (16)	33.3 (15)	36.4 (11)	37.5 (8)	37.5 (8)	50.0 (8)	80.0 (5)	– (1)
101-250		0	31.2	26.7	9.1	12.5	0	0	20.0	–
251-Unc.		0	12.5	13.3	0	25.0	0	0	0	–
1-10		50.0	12.5	18.8	25.0	71.4	55.6	12.5	80.0	66.7
11-100	30	50.0 (12)	43.8 (16)	43.8 (16)	66.7 (12)	28.6 (7)	44.4 (9)	62.5 (8)	20.0 (5)	33.3 (3
101-250		0	0	25.0	0	0	0	12.5	0	0
251-Unc.		0	43.8	12.5	8.3	0	0	12.5	0	0
1-10		80.0	20.0	29.4	88.9	75.0	63.6	0	0	100
11-100	45	20.0 (10)	40.0 (15)	58.8 (17)	11.1 (9)	0 (8)	18.2 (11)	62.5 (8)	66.7 (6)	0 (3)
101-250		0	6.7	5.9	0	25.0	9.1	12.5	0	0
251-Unc.		0	33.3	5.9	0	0	9.1	25.0	33.3	0
1-10		76.9	0	38.1	50.0	33.3	37.5	14.3	16.7	80.0
11-100	60	23.1 (13)	18.8 (16)	38.1 (21)	50.0 (10)	50.0 (6)	50.0 (8)	42.9 (7)	83.3 (6)	20.0 (5)
101-250		0	12.5	9.5	0	16.7	12.5	14.3	0	0
251-Unc.		0	68.8	14.3	0	0	0	28.6	0	0
1-10		66.7	63.2	31.3	66.7	33.3	25.0	42.9	0	–
11-100	90	33.3 (15)	31.6 (19)	50.0 (16)	22.2 (9)	50.0 (6)	37.5 (8)	42.9 (7)	75.0 (4)	– (1)
101-250		0	5.3	6.3	11.1	0	12.5	0	0	–
251-Unc.		0	0	12.5	0	16.7	25.0	14.3	25.0	–
1-10		88.9	38.5	37.5	40.0	66.7	83.3	37.5	0	–
11-100	120	11.1 (9)	53,8 (13)	31.3 (16)	20.0 (5)	0 (6)	0 (6)	37.5 (8)	40.0 (5)	– (0)
101-250		0	0	0	0	16.7	0	12.5	40.0	–
251-Unc.		0	7.7	31.3	40.0	16.7	16.7	12.5	20.0	–
1-10		50.0	55.6	22.2	71.4	50.0	50.0	0	0	–
11-100	150	50.0 (6)	44.4 (18)	22.2 (18)	28.6 (7)	25.0 (4)	16.7 (6)	75.0 (8)	0 (3)	
101-250		0	0	16.7	0	0	0	12.5	66.7	
251-Unc.		0	0	38.9	0	25.0	33.3	12.5	33.3	
1-10		66.7	42.9	6.3	–	80.0	50.0	28.6	0	
11-100	180	33.3 (6)	50.0 (14)	25.0 (16)	– (1)	0 (5)	25.0 (4)	28.6 (7)	75.0 (4)	
101-250		0	0	18.8	–	0	25.0	0	0	
251-Unc.		0	7.1	50.0	–	20.0	0	42.9	25.0	
1-10		40.0	20.0	14.3			16.7	25.0		
11-100	210	40.0 (5)	46.7 (15)	71.4 (14)			50.0 (6)	12.5 (8)		
101-250		20.0	0	0			33.3	12.5		
251-Unc.		0	33.3	14.3			0	50.0		
1-10		100	22.2	16.7			25.0	12.5		
11-100	240	0 (3)	55.6 (18)	16.7 (12)			25.0 (4)	62.5 (8)		
101-250		0	11.1	25.0			50.0	12.5		
251-Unc.		0	11.1	41.7			0	12.5		

Not done

Density ranges of T. cruzi	*Days in bug*	*Percentage of bugs with the appropriate numerical values of parasite densities (n^{o} of bugs infected)*								
		R. prolixus	R. neglectus	P. megistus	T. infestans	B. bras.	T. sordida	T. psmac.	T. rubrova-ria	T. dimidiata
1-10	300		14.3					40.0		
11-100			42.9 (7)					20.0 (5)		
101-250			28.6					0		
251-Unc.			14.3					40.0		
Overall:										
11 – >250		30.4 (102)	70.5 (183)	74.4 (176)	45.6 (79)	46.0 (50)	51.4 (70)	76.8 (82)	86.8 (38)	23.1 (13)
101 – >250		1.0	27.3	33.5	6.3	22.0	20.0	30.5	28.9	0

Unc. – uncountable; Blank-lack of insects; – Small sample size or lack of positive insects; T. psmac. – T. pseudomaculata.

The results summarized in Table IV, further indicate that blood-meal size, hence the quantity of *T. cruzi* ingested play no part in the above correlations. We found the highest infection rates and the highest parasite yields in *P. megistus*, *T. rubrovaria*, *T. pseudomaculata* and *R. neglectus* although the greatest quantities of blood were ingested by *T. dimidiata* and *T. infestans* which demonstrated the lowest infection rates coupled with poor parasite yields.

In analysing infectivity efficiency, which tends to be in individual vector species of the order seen in Table IV, in association with available information concerning their biotopes described under section "Material and Methods", it is hard to avoid the conclusion that totally wild species and those essentially sylvatic with domiciliary tendencies are superior to highly domiciliated species in prevalence and intensity of infection. This is well illustrated by the following: the infection rate and the proportion of insects with very dense parasite population was 93.8% and 27.3% respectively in the wild *R. neglectus*, while only 53.1% of its domiciliated congener *prolixus* became positive, of which only 1% demonstrated very dense parasite populations. Similarly, the infection rate and the proportion of bugs with large parasite burdens (95% and 28.9% respectively) in the wild *T. rubrovaria* exceeded dramatically those in its domiciliated congener *infestans* (51.6% and 6.3% respectively), in spite of the fact that the latter is known to colonize human dwellings in the same area (State of São Paulo) where the studied Y strain of *T. cruzi* originated, while the former derived from a completely different geographical area (State of Paraná).

Such substantial differences of infectivity efficiency among domiciliated and sylvatic vector species seem to be in line with our notion that domiciliation of the vector implies a change in the nature of interaction between invertebrate hosts and parasites. The magnitude and character of this change was manifested, at least in part, through low infection rates and poor parasite yields in the domiciliated vector species.

DISCUSSION

The first aspect which merits discussion is the modified procedure used for quantitative assessment of the infective potentialities observed among triatomine species. These conveniently expressed by infection rates have routinely been based on fecal infections, in which the metacyclic trypomastigotes have been sought (an essential criterion for vector contaminating efficiency), while in the present study the proportions of insects that became positive upon feeding on infected hosts were based on gut infections, in which the parasite population comprised of all developmental forms was more abundant, easier to detect, hence minimizing the probability of false negative results. This test appeared to us technically more reproducible, less tedious to perform and more reliable.

TABLE IV

Results indicating that interspecific rates of infection and potency of infection are not associated with the blood-meal size

Vector species	**Mean weight ± SD of blood-intake per bug* mg	*Estimated* T. cruzi *intake per bug* $x10^3$	*% of bugs infected* (nº of bugs fed)	*% of bugs with numbers of* T. cruzi *ranging from 101 to over 250 per 50 fields.* (nº of bugs inf.)
P. megistus	118.1 ± 46.09	12.3	97.8(180)	33.5(176)
T. rubrovaria	65.6 ± 35.09	6.8	95.0(40)	28.9(38)
T. pseudomaculata	54.4 ± 10.81	5.6	94.3(87)	30.5(82)
R. neglectus	53.2 ± 7.49	5.5	93.8(195)	27.3(183)
T. sordida	47.1 ± 9.71	4.9	84.3(83)	20.0(70)
T. brasiliensis	95.0 ± 40.46	9.9	76.9(65)	22.0(50)
R. prolixus	94.2 ± 16.80	9.8	53.1(192)	1.0(102)
T. infestans	140.3 ± 21.16	14.6	51.6(153)	6.3(79)
T. dimidiata	264.8 ± 147.08	27.5	38.2(34)	0(13)

*Ten fourth-instar bugs were weighed individually before and after feeding and blood intake obtained by substraction.

Moreover, on the assumption that the parasite burden harboured by the vector gives a remarkable accurate portrayal of parasite development and multiplication, thus representing a useful correlate in the selection of an adequate candidate for xenodiagnosis, the magnitude of parasite populations within all species has been recorded (Table III) and analyzed.

It is perhaps also appropriate to mention precaution taken in order to minimize any contribution of effects distinct from the species effect on the performance of the bug as a xenodiagnostic tool: (1) Only fourth instar nymphs of similar ages were fed on the infected donors, thus excluding the species/instar effect as referred to the phisiological stage of the insect. (2) All insects have been fed on the same guinea-pigs having access to the full range of a moderate parasitemia presented by the infected donors, thus eliminating, on one hand, the contribution claimed by Neal & Miles (1977) and Silva & Nussenzweig (1953) that infection rates are associated with the level of parasitemia in the donor and, on the other hand, minimizing the probability of interspecific differences becoming inapparent due to haevy infections produced by the intake of large parasite loads. (3) All insects were fed on the same infected guinea-pigs, thus eliminating possible differences adherent in the parasite when derived from different biological sources as shown bu Toyé (1974). (4) Also eliminated was any possible influence by factors intrinsic to the parasite of different isolates because Y strain solely has been used throughout. (5) All insects were maintained under the same ambient conditions to ensure that changes in parasite growth in a given species were not attributable to environmental conditions, a phenomenon described by Neves (1971) and Wood (1954).

Results summarized in Tables I and II, apparently confirm that there are differences in the infective potentialities between different triatomine species on one hand, on the other hand it is difficult to avoid the conclusion from Table IV, that neither size of the blood-meals nor the levels of assumed *T. cruzi* intake could be associated with differences in rates, potency and persistence of infections observed among species presently studied. This however may not apply to intraspecific differences of infective potentialities observed by Melo & Chiarini (1980) and Minter, Minter-Goedbloed & Marshall (1978)

among different stadia of the same species or between individuals of the same stage of a given species, as stated by Phillips & Bertram (1967).

Diversity of reactions to *T. cruzi* among different vector species was mostly explained by intrinsic differences in susceptibility to infection, and the possibility of this operating under genetic control has been stressed. That there are differences in vector susceptibility to *T. cruzi* between stadia of the same species and among individuals of the same stage which are heritable has been already reported by Maudlin (1976) and Phillips & Bertram (1967). Whether the same applies to interspecific susceptibility needs to be explored. But most of the species tested so far concern different genera (*Panstrongylus, Triatoma, Rhodnius*) which differ in so many respects that correlation between species susceptibility and genetic constitution seems unacceptable at present. Moreover, with regard to species susceptibility, the suggestion is arguable in itself because of the uncoordinated rises and falls of parasite densities even in species found to support well the development and multiplication of *T. cruzi.* The pattern of the fluctuating parasite population seen in Table III is hard to describe on any other basis than that of conditions on the part of the insect because of its constitution and the viability of components which, if not satisfactory to the parasite, will impair its development and multiplication temporarily or permanently. Circumstantial evidence in support of our view is borne out in demonstration of loads of sluggish and dead parasites in some groups of insects at certain intervals after infection and none in others from the same stock tested thereafter. One should also bear in mind that *T. cruzi* is a harmless commensal with regard to the vector. It is not pathogenic and, according to our unpublished experiments, it does not affect the vital biological parameters which govern the population dynamics of the insect. Thus, the bug can be likened to a living test tube in which the bug provides the environmental conditions, the culture medium and certain chemical factors associated with the nutritional preference of the parasite which seems to be less rigid than generally believed. *T. cruzi* Y strain, for example, was able to establish itself and to multiply vigorously in the digestive tract of bugs of three genera: *Panstrongylus megistus, Triatoma rubrovaria, Triatoma pseudomaculata* and *Rhodnius neglectus.* However, one should bear in mind that each vector parasite system is bound to emphasize an individual set of characteristics, and great care should be taken before conclusions derived from one parasite-insect model can be extrapolated to another parasite-insect system.

Continued claims for the existence of a close mutual adaptation between local vector parasite systems may be received with some skepticism. Our results lead us to question the interpretation that local vector species in areas endemic for Chagas' disease were more readily infected with local strains of *T. cruzi* than were vectors that did not naturally occur in the same area. This was also questioned by Barretto et al (1978) after having demonstrated that *Dipetalogaster maximus* derived from Mexico had been superior to Brazilian *T. infestans* in infection rates produced by Brazilian strains of *T. cruzi.*

The only two vector species which could be considered local in the endemic areas of Chagas' disease in the State of São Paulo, where the studied Y strain of *T. cruzi* has been isolated, were those with almost the highest (93.8% in *R. neglectus*) and the lowest (51.6% in *T. infestans*) infection rates after a single feed on experimentally infected guinea-pigs. While the vector-parasite relationship in the former species might suggest a perfect coadaptation of two local partners, the poor performance by the local *T. infestans* is particularly disappointing, indicating that infectivity efficiency is unrelated to mutual adaptation of vector and parasite. Further support for the latter concept is provided by the strikingly good reactions to infection with *T. cruzi* of vector species derived from diverse and distant geographical areas. *P. megistus* from the State of Ceará, *T. pseudomaculata* from Pernambuco and *T. rubrovaria* from Paraná were found to be superior to the local *T. infestans* in infection rates (Table IV) produced by the indigenous Y strains of *T. cruzi.* Thus, the various hypotheses proposed in previous studies to explain the diversity of reactions by different vector species to infection with *T. cruzi* are not satisfactory and not unequivocal.

To understand the etiology of the interspecific differences in infectivity with the same parasite it was necessary to consider these in relationship to the attendent changes in the vector upon moving from natural to artificial biotopes, particularly to human habitats. Although it is believed that insect populations maintain their integrity following domiciliation, this has not been proved so far. On the other hand it seems conjecturable and hardly acceptable because information given in the last two paragraphs under section "Results" indicate that development and multiplication of the parasite in a given species is influenced by the nature of the biotope it inhabits. Furtheremore, the reaction of infection does not seem to be rigidly restricted to the bug of a given genus or species but to the assemblage of different vector species. While a scanty and sometimes transient infection was produced in the group of vector species widely recognized as domiciliated (*T. infestans, T. dimidiata, R. prolixus*), infection was rich and stable in the essentially sylvatic and completely wild species (*P. megistus, T. pseudomaculata, T. rubrovaria, R. neglectus*). The third group formed by the peridomestic *T. sordida* and *T. brasiliensis* with intermediate infectivity rates points to the importance of gradients of vector species association with artificial biotopes, as a factor involved in vector-parasite relationships.

The most striking feature is that the biological parameters, on which segregation of these vector species in good, poor and intermediate responders to infection with *T. cruzi* was based, were remarkably similar for species within each group, as is well exemplified in Table IV. There were, however, substantial dissimilarities between the groups which differ in ecological features, thus indicating, on one hand, that vector reaction to infection was not a species specifity and suggesting, on the other hand, that it could be related to the kind of biotope the vector species inhabits.

The evidence that prompted us to opt for this alternative which appeared more plausible than those stressed by previous authors, while not firm, is consistent in experiments performed so far. Had the experiments been done on insect samples of the same species derived from natural and artificial biotopes, thus bypassing possible species effects we may have been able to speculate more precisely on the nature of interspecific differences in infectivity efficiency. Unfortunately we were unable to find any report of strictly comparable studies which would permit analysis of vector species responsiveness to *T. cruzi* collectively, by grouping species associated with ecologically similar biotopes and/or demonstrating similar reactions to infection. Nonetheless, our results, while not agreeing with those obtained by other workers, can be reconciled because our hypothesis linking the involvement of species habitat in vector-parasite interrelationships can also be used in support of findings described by others. This seems to be well exemplified by the following: Little, Tay & Biagi (1966) and Ryckman (1965) stated that North American *T. cruzi* developed more readily in the indigenous *T. protracta* than in South American *T. infestans*. Forattini (1973) in a comparison of *R. neglectus* and *T. infestans* in simultaneous xenodiagnosis of naturally infected opossums, showed that the former was superior to the latter in infection rates and recommended it for routine use in xenodiagnosis. More recently Barretto et al (1978) showed that 52% of *D. maximus* from Mexico became positive as compared with 31% of *T. infestans*, in xenodiagnosis of human cases of Chagas' disease. Since *T. infestans* is the sole domiciliary vector used in these experiments while all the others are sylvatic it is not surprising that the latter were superior to the former in infectivity efficiency in all instances, although the comments used to explain this phenomenon are controversial.

It is difficult to understand why wild insect species were superior to domiciliated species in their reaction to infection with *T. cruzi*, but if the hypothesis of Pessoa (1962) that domiciliation of *Triatoma* is due to production of new varieties of insects by mutation rather than by gradual adaptation, the parasite may reach in the domiciliated vector an unsuitable host species for its development and multiplication. If, on the other hand, domiciliation is a gradual adaptation of sylvatic insects to a domestic habitat and to new nutritional sources, the physiological and/or biochemical changes in the insect, if not synchronized with corresponding changes in the parasite, may affect adversely the state

of dynamic equilibrium of the latter, expressed by a gradual decline of infection rates and parasite yields. A further point worthy of consideration is the possibility that the human isolate of *T. cruzi* Y strain was originally zoonotic, survived almost unchanged and therefore encountered in the feric environment of the sylvatic host more favourable conditions for development and proliferation than in the domiciliated vector.

The reasoning above does not necessarily imply that the domiciliated insect lost its contaminating efficiency as a vector of *T. cruzi*, it continues to be the principal source of infections and of major epidemiological importance in the transmission of the disease to man and domestic animals. Nonetheless, it is tempting to suggest that following domiciliation of the vector the environment of its digestive tract becomes somewhat hostile to the parasite, inhibiting its otherwise vigorous proliferation and subsequently limiting its use in xenodiagnosis. And furtheremore, it may not be out of place to recommend not to resort to domiciliated vectors for mass production of in vivo grown parasites for biochemical and immunological studies.

Our interpretation represents an initial approach to interspecific differences in infectivity with *T. cruzi* among triatomine bugs. Additional confirmation, using other isolates of *T. cruzi* (experiments under way in our laboratory), will be necessary before we can safely adopt it. Therefore the problem is subject to alternative explanation and as such must be regarded as tentative.

RESUMO

Em busca de um inseto adequado para xenodiagnóstico em homens e animais com forma crônica da doença de Chagas investigamos, primeiramente, a resposta à infecção aguda com *Trypanosoma cruzi*, empregando nove espécies vetoras: *Triatoma infestans, Triatoma dimidiata* e *Rhodnius prolixus*, todas bem adaptadas a domicílios humanos. *Triatoma rubrovaria* e *Rhodnius neglectus* consideradas totalmente silvestres, *Panstrongylus megistus, Triatoma sordida, Triatoma pseudomaculata, Triatoma brasiliensis*, todas essencialmente silváticas, porém umas com nítida tendência de domiciliação e outras com colonização incipiente de habitações mantidas sob controle após erradicação bem sucedida de *T. infestans*.

As proporções de insetos infectados e as cargas parasitárias nos mesmos, determinadas a fresco no conteúdo do tubo digestivo nos diferentes intervalos após um único repasto em animais infectados indicam que *P. megistus, T. rubrovaria, T. pseudomaculata* e *R. neglectus* constituem o melhor meio natural para evolução e proliferação da cepa Y do *T. cruzi*. Outras espécies em ordem descendente foram: *T. sordida, T. brasiliensis, R. prolixus, T. infestans* e *T. dimidiata*.

Os resultados apresentados na Tabela IV não confirmam a experiência de outros investigadores no que se refere à melhor adaptação do *T. cruzi* a espécies locais de triatomíneos, nem ao volume do sangue ingerido; em outras palavras, a magnitude da carga parasitária ingerida não condiciona a positividade do xenodiagnóstico. Os dados obtidos tendem a apoiar uma hipótese alternativa, vinculando a prevalência, intensidade e a persistência da infecção ao tipo do biótopo associado com a espécie vetora. Enquanto o parasita produziu uma infecção moderada e às vezes passageira no conjunto de espécies domiciliadas, esta foi maciça e persistente em espécies selvagens e nas essencialmente silvestres com tendências domiciliares. O aspecto mais surpreendente é que os parâmetros biológicos, utilizados na segregação das espécies vetoras em grupos com respostas satisfatórias e, vice-versa, à infecção com *T. cruzi*, foram notavelmente similares para as diversas espécies dentro de grupos, sugerindo que o padrão da infectividade do vetor não representa uma característica específica da espécie vetora. Por outro lado, as diferenças proeminentes na prevalência, intensidade e persistência da infecção entre os grupos ecologicamente diferentes, sugere que a domiciliação do vetor implica numa mudança na interação vetor-parasita, a qual se manifesta numa redução substancial tanto de índices de infecção como de cargas

parasitárias nos insetos. As razões deste fenômeno e o envolvimento do biótopo do vetor no seu potencial infectante estão sendo discutidas com respeito à sua utilização na busca de um inseto adequado para o xenodiagnóstico.

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