

STUDIES IN SEARCH OF A SUITABLE EXPERIMENTAL INSECT MODEL
FOR XENODIAGNOSIS OF HOSTS WITH CHAGAS' DISEASE.
2 – ATTEMPTS TO UPGRADE THE RELIABILITY AND THE EFFICACY
OF XENODIAGNOSIS IN CHRONIC CHAGAS' DISEASE

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In order to upgrade the reliability of xenodiagnosis, attention has been directed towards population dynamics of the parasite, with particular interest for the following factors:

1. Parasite density which by itself is not a research objective, but by giving an accurate portrayal of parasite development and multiplication, has been incorporated in screening of bugs for xenodiagnosis. 2. On the assumption that food availability might increase parasite density, bugs from xenodiagnosis have been re-fed at biweekly intervals on chicken blood. 3. Infectivity rates and positives harbouring large parasite yields were based on gut infections, in which the parasite population comprised of all developmental forms was more abundant and easier to detect than in fecal infections, thus minimizing the probability of recording false negatives. 4. Since parasite density, low in the first 15 days of infection, increases rapidly in the following 30 days, the interval of 45 days has been adopted for routine examination of bugs from xenodiagnosis. By following the enumerated measures, all aiming to reduce false negative cases, we are getting closer to a reliable xenodiagnostic procedure.

Upgrading the efficacy of xenodiagnosis is also dependent on the xenodiagnostic agent. Of 9 investigated vector species, Panstrongylus megistus deserves top priority as a xenodiagnostic agent. Its extraordinary capability to support fast development and vigorous multiplication of the few parasites, ingested from the host with chronic Chagas' disease, has been revealed by the strikingly close infectivity rates of 91.2% vs. 96.4% among bugs engorged from the same host in the chronic and acute phase of the disease respectively (Table V), the latter comporting an estimated number of 12.3×10^3 parasites in the circulation at the time of xenodiagnosis, as reported previously by the authors (1982).

Key words: chronic Chagas' disease – reliability of xenodiagnosis – efficacy of xenodiagnosis – xenodiagnostic agent

Xenodiagnosis as a diagnostic method in Chagas' disease has been well documented in the literature. There are some who question the value of this method, or who view it as cumbersome and impractical for field surveys. Divergent results obtained raised serious doubt about the vector reliability as a xenodiagnostic agent. But, while there is an awareness of its impracticability in field surveys, there is also an awareness of its usefulness elsewhere; in the evaluation of drug efficiency against the disease and in the isolation of parasite strains.

In most cases workers predicted that efficiency of xenodiagnosis could be increased by the use of large numbers of bugs per patient as recommended by Meckelt (1964); Schenone et al. (1974), Cerisola et al. (1974); by the exposure of patients to repeated xenodiagnosis,

as demonstrated by Castro et al. (1983) and by using vectors of epidemiological significance in the area where the patient lives, as advocated first by Dias (1940) and thereafter by many others: Ryckman (1965); Little et al. (1966); Zeledon (1974) and by Minter et al. (1978). Therefore preference has been given to *Triatoma infestans* in Argentina, Brazil and Chile, while in Venezuela the bug of choice has been *Rhodnius prolixus*. But conflicting evidence concerning the importance of mutual adaptation between the vector and the parasite had led to questioning the interpretability of results obtained.

The question also arose as to whether the great numbers of bugs used could be substituted by the use as xenodiagnostic agents vectors capable of promoting more accelerated multiplication of the parasite than the domiciliated species. And furthermore, as to whether it would be possible to upgrade the efficacy and reliability of the test by altering and/or improving procedures currently used.

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In hope to answer these and other questions, a study on a large scale, under strictly standardized conditions, including 9 different vector species and 7 isolates of *T. cruzi*, has been undertaken. In order to develop an experimental background for the study of xenodiagnosis in chronic Chagas' disease, the test has been first studied in the acute stage of the disease. Results obtained were reported in 1982. In this paper we describe follow-up experiments focussed on the infection rates among bugs of the same 9 species, upon simultaneous feeding on hosts which had recovered from overt infections with Y strain of *T. cruzi*, and whose blood was shown to have remained essentially parasite-free for periods of time varying from 7 weeks to 2.8 years.

MATERIAL AND METHODS

Triatominae: Bugs used for xenodiagnosis were essentially those described by P. Szumlewicz in 1976. 1 – *Triatoma infestans* (Klug, 1834) highly domiciliated, practically no longer found in the wild; *Rhodnius prolixus* Stal, 1859, though considered strictly domiciliated, has been traced by Gamboa (1963) in natural habitats in palm trees; *Triatoma dimidiata* (Latreille, 1811), domiciliated in Costa Rica, Equador and Belize, has been reported by Gomes-Nunez (1969) to interchange between sylvatic and domestic habitats. 2 – *Triatoma brasiliensis* Neiva, 1811, *Triatoma pseudomaculata* Corrêa & Spinola, 1964, *Triatoma sordida* (Stal, 1859) and *Panstrongylus megistus* (Burmeister, 1835), all essentially sylvatic, some already in process of adaptation to human dwellings, others with incipient colonization in human houses maintained under control following successful eradication of *T. infestans*. 3 – *Triatoma rubrovaria* (Blanchard, 1834) and *Rhodnius neglectus* Lent 1954, considered strictly wild, the former entering human houses occasionally, the latter with domiciliary tendencies in regions of agricultural activities. The origin and history of these laboratory reared insects was described by the authors previously (1982).

Mammalian hosts: The authors took advantage of laboratory infected guinea-pigs used in a previous study (1982) in search of an experimental insect model for xenodiagnosis. The animals had been inoculated ip with 0.2 ml of mouse blood containing $\pm 13.9 \times 10^4$ trypomastigotes. All had developed parasitemia within 9-12 days which reached a peak of 980 ± 884.6 parasites in 5 cmm of blood at an interval varying from 18 to 35 days of infection.

Six of these animals which had recovered from the acute infection that lasted from 31 to 48 days and whose blood was shown to have

remained essentially parasite free for periods of time varying from 7 weeks to 2.8 years were used for xenodiagnostic tests.

Xenodiagnosis: Fourth instar nymphs, of each of the 9 vector species, starved during a period of 2-3 weeks after transition from the third stage, were fed on the guinea-pigs according to the following scheme: groups of bugs of each species were fed on 6 guinea-pigs in 6 batches on two different occasions, at intervals of 11 and 15 months after infection. Eight months later, groups of clean bugs of the same species were fed on 3 available guinea-pigs (3 died after the first 2 tests) in 3 batches at an interval of 22.8 months of infection. At intervals of 30.5 and 33.8 months of infection groups of clean bugs of each species were fed on the last 2 guinea-pigs (one died after the third test) in 2 batches. These guinea-pigs died shortly after the fifth xenodiagnosis.

Bugs usually became engorged within 25-30 min. The well fed specimens only were pooled by species in glass jars and kept for examination on 3 or 4 different occasions throughout the period of 60 days of infection. During this period, bugs were fed on chicken blood at biweekly intervals. Examination of bugs for gut infection and parasite yields was as described previously by the authors (1982).

RESULTS

Infectivity of bugs fed on guinea-pigs with chronic infections by *T. cruzi*: Vector infectivity has been recorded as numbers of insects showing at least one parasite in 50 microscopic fields (40x10) out of batches of randomly selected 8 (usually) specimens from groups which had engorged from the same donors. This may be misleading because the possibility of finding parasites when more than 50 fields are examined cannot be ruled out; so the procedure followed with insects apparently free of parasites was more rigid; 200 fields were examined unless metacyclics in any developmental stage were found earlier.

For a complicated system such as the vector-parasite relationship in Chagas' disease, in which fluctuation of results obtained seems to be a constant phenomenon, the only way of analysing recorded data is through overalls computed for each xenodiagnosis separately and/or for pooled results of combined data from all successive tests. The former also enables us to deal with the question as to whether bug infectivity was affected by time elapsed between the experimentally induced infection into the vertebrate host and its transmission to the invertebrate host through xenodiagnosis.

TABLE I

Interspecific variations shown by the proportion of infected bugs after a single feed on guinea-pigs with chronic Chagas' disease

Number of donors used	Infection in bugs days	Percentage of positive bugs from 5 consecutive xenodiagnostic tests (no. infected/no. fed)								
		<i>R. prolixus</i>	<i>R. neglectus</i>	<i>P. megistus</i>	<i>T. infestans</i>	<i>T. brasiliensis</i>	<i>T. sordida</i>	<i>T. psmaculata</i>	<i>T. rubrovaria</i>	<i>T. dimidiata</i>
328 days of infection in donors										
6	15	25.0(2/8)	12.5(1/8)	50.0(4/8)	75.0(6/8)					
	30	37.5(3/8)	87.5(7/8)	75.0(6/8)	75.0(6/8)	37.5(3/8)	50.0(4/8)	37.5(3/8)	37.5(3/8)	0 (0/8)
	45	75.0(6/8)	50.0(5/10)	75.0(6/8)	50.0(4/8)	37.5(3/8)	75.0(6/8)	50.0(4/8)	50.0(4/8)	50.0(4/8)
	60	25.0(2/8)	62.5(5/8)	62.5(5/8)	37.5(3/8)	75.0(6/8)	25.0(2/8)	62.5(5/8)	62.5(5/8)	87.5(7/8)
	Overall	40.6(13/32)	50.9(18/34)	65.6(21/32)	59.4(19/32)	50.0(12/24)	50.0(12/24)	50.0(12/24)	50.0(12/24)	45.8(11/24)
454 days of infection in donors										
6	15	62.5(5/8)	87.5(7/8)	100 (8/8)	37.5(3/8)					
	30	62.5(5/8)	100 (8/8)	62.5(5/8)	62.5(5/8)	0 (0/8)	87.5(7/8)	80.0(4/5)	100 (5/5)	
	45	25.0(2/8)	100 (8/8)	87.5(7/8)	75.0(6/8)	25.0(2/8)	37.5(3/8)	40.0(2/5)	100 (5/5)	
	60	37.5(3/8)	77.8(7/9)	75.0(6/8)	0 (0/8)	25.0(2/8)	50.0(4/8)	60.0(3/5)	100 (6/6)	
	Overall	46.9(15/32)	90.9(30/33)	81.3(26/32)	43.8(14/32)	16.7(4/24)	28.3(14/24)	60.0(9/15)	100 (16/16)	
685 days of infection in donors										
3	30	62.5(5/8)	100 (8/8)	100 (8/8)	100 (8/8)	62.5(5/8)	50.4(4/8)	87.5(7/8)	87.5(7/8)	
	45	50.0(4/8)	75.0(6/8)	100 (8/8)	100 (8/8)	37.5(3/8)	25.0(2/8)	62.5(5/8)	75.0(6/8)	
	60	62.5(5/8)	75.0(6/8)	100 (8/8)	37.5(3/8)	12.5(1/8)	62.5(5/8)	87.5(7/8)	62.5(5/8)	
	Overall	58.3(14/24)	83.3(20/24)	100 (24/24)	79.2(19/24)	37.5(9/24)	45.8(11/24)	79.2(19/24)	75.0(18/24)	
915 days of infection in donors										
2	15	62.5(5/8)	75.0(6/8)	100 (14/14)	37.5(3/8)					
	30	50.0(4/8)	87.5(7/8)	100 (13/13)	25.0(2/8)					
	45	25.0(3/12)	100 (8/8)	100 (18/18)	20.0(2/10)					
	60	25.0(3/12)	84.6(11/13)	100 (20/20)	0 (0/12)					
	Overall	37.5(15/40)	86.5(32/37)	100 (65/65)	18.4(7/38)					
1000 days of infection in donors										
2	30	87.5(7/8)	37.5(3/8)	100 (8/8)	0 (0/8)	50.0(4/8)	100 (8/8)	87.5(7/8)	87.5(7/8)	0 (0/8)
	45	25.0(2/8)	50.0(4/8)	100 (8/8)	0 (0/8)	62.5(5/8)	100 (8/8)	100 (8/8)	87.5(7/8)	75.0(6/8)
	60	75.0(6/8)	62.5(5/8)	100 (8/8)	12.5(1/8)	75.0(6/8)	100 (8/8)	87.5(7/8)	85.7(6/8)	75.0(6/8)
	Overall	62.5(15/24)	50.0(12/24)	100 (24/24)	4.2(1/24)	62.5(15/24)	100 (24/24)	91.7(22/24)	87.0(20/23)	50.0(12/24)
Pooled percentages										
	15	50.0(12/24)	58.3(14/24)	86.7(26/30)	50.0(12/24)					
	30	60.0(24/40)	82.5(33/40)	88.9(40/45)	52.5(21/40)	37.5(12/32)	71.9(23/32)	72.4(21/29)	75.9(22/29)	0 (0/16)
	45	38.6(17/44)	73.8(31/42)	94.0(47/50)	47.6(20/42)	40.6(13/32)	59.4(19/32)	65.5(19/29)	75.9(22/29)	62.5(10/16)
	60	43.2(19/44)	73.9(34/46)	90.4(47/52)	15.9(7/44)	46.9(15/32)	59.4(19/32)	75.9(22/29)	75.9(22/29)	81.3(13/16)
	Overall	47.4(72/152)	73.7(112/152)	90.4(160/177)	40.0(60/150)	41.7(40/96)	63.5(61/96)	71.3(62/87)	75.9(66/87)	47.9(23/48)

From data presented in Table I it is obvious that, although feeding of bugs on chronic donors evokes a clear positive reaction in all vector species, interspecific differences expressed by the proportion of positive bugs were common. The overall infection rates presented by pooled results from all 5 tests, each with triplicate measurements at least, suggest the following order of vector infectivity: *P. megistus* with 90.4% of positives followed by *T. rubrovaria*, *R. neglectus* and *T. pseudomaculata*, almost identical seconds, with infectivity rates of 75.9%, 73.7% and 71.3% respectively. The lowest proportions of infected bugs ranging from 40% to 47.9% were exhibited by *T. infestans*, *R. prolixus* and *T. dimidiata*.

Data summarized in Table II provide further support for this order of bug infectivity. Of a total of 60 tests, utilizing 503 specimens of the first 4 species, 34 (56.7%) exhibited infection rates ranging from 76% to 100%. In the group of the last 3 species, only 4 tests, 9.5% out of 42 with 300 bugs examined, revealed comparable proportions of positives.

The image of bug infectivity variations gains a plausible interpretation by comparing the biotopes the bugs, integrating the two groups under consideration, habitate (Material and Methods). It suggests that the completely wild species (*T. rubrovaria* and *R. neglectus*) and those essentially sylvatic but in process of adaptation to human dwellings (*P. megistus*, *T. pseudomaculata* and eventually *T. sordida*) form the group of high responders, while the domiciliated species are in the group of low responders to the chronic infection by *T. cruzi*. Further experiments are indicated to confirm infectivity rates and parasite yields in *T. sordida*.

On the interference of aging of the chronic infection in infectivity rates among bugs from xenodiagnosis: R. prolixus, used in xenodiagnosis of guinea-pigs infected 328 or 915 days previously, demonstrated comparable infection rates of 40.6% and 37.5% as shown by results summarized in Table I. Similarly *R. neglectus* fed on chronic donors 328 or 1000 days prior to xenodiagnosis produced almost identical proportions (50.9% and 50%) of positives, irrespective of the number of donors tested. Likewise, *P. megistus* displayed 100% of infected specimens from 3 successive xenodiagnosis done at intervals of 685, 915 and 1000 days after infection of donors.

It is therefore concluded that the reaction of bugs to the chronic infection is unrelated to the time elapsed from infection of the vertebrate host to infection of the invertebrate host used in xenodiagnosis.

Nonetheless fluctuating rates of positives have been demonstrated among results from successive xenodiagnosis; *T. infestans*, *T. brasiliensis*, *T. sordida* and *R. prolixus*, with rises and falls rather than more stable rates, with a tendency to increase, as displayed by the high responders to infection: *P. megistus*, *T. pseudomaculata*, *T. rubrovaria* and *R. neglectus* (Table I).

Parasite densities in bugs fed on guinea-pigs with chronic infections by T. cruzi: The reason for incorporating parasite yields in screening bugs for xenodiagnosis is to identify a xenodiagnostic agent capable of promoting both fast development and high multiplication of the low numbers of parasites ingested from the chronic host.

TABLE II

Interspecific variations demonstrated by the frequency of tests with equal ranges of infectivity among bugs fed once on guinea-pigs with chronic Chagas' disease

Vector species	No. of tests	No. of bugs per test (total)	No. & (%) of tests with infectivity rates ranging as:					
			0%	1-25%	26-50%	51-75%	76-99%	100%
<i>P. megistus</i>	18	8-20(177)	0	0	1(5.6)	5(27.8)	1(5.6)	11(61.1)
<i>T. rubrovaria</i>	12	5-8(87)	0	0	2(16.7)	3(25.0)	4(33.3)	3(25.0)
<i>R. neglectus</i>	18	8-13(152)	0	1(5.6)	3(16.7)	5(27.8)	5(27.8)	4(22.2)
<i>T. pseudomaculata</i>	12	5-8(87)	0	0	3(25.0)	3(25.0)	5(41.7)	1(8.3)
<i>T. sordida</i>	12	8(96)	0	2(16.7)	4(33.3)	3(16.7)	1(8.3)	3(25.0)
<i>T. brasiliensis</i>	12	8(96)	1(8.3)	3(25.0)	4(33.3)	4(33.3)	0	0
<i>T. infestans</i>	18	8-12(150)	4(22.2)	3(16.7)	5(27.8)	4(22.2)	0	2(11.1)
<i>R. prolixus</i>	18	8-12(152)	0	6(33.3)	4(22.2)	7(38.9)	1(5.6)	0
<i>T. dimidiata</i>	6	8(48)	2(33.3)	0	1(16.7)	2(33.3)	1(16.7)	0

Combined results from 5 xenodiagnosis done at 328 to 1000 day-intervals after infection of guinea-pig. (used data seen in Table I)

TABLE III

Interspecific variations shown by the frequency of triatomine bugs with similar parasite yields developed upon a single feed on guinea-pigs with chronic Chagas' disease

Infection in bugs days	Percentage of positive bugs with parasite counts above 11 (no. infected/no. fed)								
	<i>R. prolixus</i>	<i>R. neglectus</i>	<i>P. megistus</i>	<i>T. infestans</i>	<i>T. brasiliensis</i>	<i>T. sordida</i>	<i>T. psmaculata</i>	<i>T. rubrovaria</i>	<i>T. dimidiata</i>
328 days of infection in donors									
15	0 (2/8)	* (1/8)	25 (4/8)	0 (6/8)	14.3(7/8)	0 (2/8)	33.3(3/8)	20 (5/8)	0 (3/8)
30	0 (3/8)	85.7(7/8)	83.3(6/8)	0 (6/8)	33.3(3/8)	75.0(4/8)	0 (3/8)	66.7(3/8)	0 (0/8)
45	33.3(6/8)	60 (5/10)	50 (6/8)	50 (4/8)	66.7(3/8)	66.7(6/8)	100 (4/8)	50 (4/8)	0 (4/8)
60	0 (2/8)	80 (5/8)	80 (5/8)	0 (3/8)	66.7(6/8)	100 (2/8)	60 (5/8)	80 (5/8)	0 (7/8)
Overall	15.4(13/32)	77.8(18/34)	61.9(21/32)	10.5(19/32)	42.1(19/32)	64.3(14/32)	53.3(15/32)	52.9(17/32)	0 (14/32)
454 days of infection in donors									
15	0 (5/8)	0 (7/8)	0 (8/8)	0 (3/8)					
30	0 (5/8)	100 (8/8)	60 (5/8)	0 (5/8)	0 (0/8)	57.1(7/8)	0 (4/5)	40 (5/5)	
45	0 (2/8)	100 (8/8)	100 (7/8)	16.7(6/8)	50 (2/8)	33.3(3/8)	100 (2/5)	40 (5/5)	
60	0 (3/8)	42.9(7/9)	100 (6/8)	0 (0/8)	50 (2/8)	100 (4/8)	66.7(3/5)	50 (6/6)	
Overall	0 (15/32)	63.3(30/33)	61.5(26/32)	7.1(14/32)	50 (4/24)	64.3(14/24)	44.4(9/15)	43.8(16/16)	
685 days of infection in donors									
30	40 (5/8)	37.5(8/8)	75 (8/8)	62.5(8/8)	20 (5/8)	25 (4/8)	28.6(7/8)	14.3(7/8)	
45	50 (4/8)	33.3(6/8)	62.5(8/8)	37.5(8/8)	66.7(3/8)	50 (2/8)	20 (5/8)	33.3(6/8)	
60	0 (5/8)	33.3(6/8)	100 (8/8)	66.7(3/8)	0 (1/8)	100 (5/8)	71.4(7/8)	60 (5/8)	
Overall	28.6(14/24)	35 (20/24)	79.2(24/24)	52.6(19/24)	33.3(9/24)	63.6(11/24)	42.1(19/24)	33.3(18/24)	
915 days of infection in donors									
15	0 (5/8)	0 (6/8)	64.3(14/14)	0 (3/8)					
30	0 (4/8)	14.3(7/8)	38.5(13/13)	0 (2/8)					
45	0 (3/12)	50 (8/8)	44.4(18/18)	0 (2/10)					
60	33.3(3/12)	0 (11/13)	40 (20/20)	0 (0/12)					
Overall	6.7(15/40)	15.6(32/37)	46.2(65/65)	0 (7/38)					
1000 days of infection in donors									
30	0 (7/8)	33.3(3/8)	62.5(8/8)	0 (0/8)	75 (4/8)	87.5(8/8)	57.1(7/8)	42.9(7/8)	0 (0/8)
45	0 (2/8)	25 (4/8)	50 (8/8)	0 (0/8)	60 (5/8)	75 (8/8)	100 (8/8)	85.7(7/8)	0 (6/8)
60	0 (6/8)	20 (5/8)	75 (8/8)	0 (1/8)	33.3(6/8)	50 (8/8)	85.7(7/8)	83.3(6/7)	0 (6/8)
Overall	0 (15/24)	25 (12/24)	62.5(24/24)	0 (1/24)	53.3(15/24)	70.8(24/24)	81.8(22/24)	70 (20/23)	0 (12/24)
Pooled percentages									
15	0 (12/24)	7.1(14/24)	38.5(26/30)	0 (12/24)	14.3(7/8)	0 (2/8)	33.3(3/8)	20.0(5/8)	0 (3/8)
30	8.3(24/40)	57.6(33/40)	60.0(40/45)	23.8(21/40)	41.7(17/32)	65.2(23/32)	28.6(21/29)	36.4(22/29)	0 (0/16)
45	23.5(17/44)	58.1(31/42)	57.4(47/50)	30.0(20/42)	61.5(13/32)	63.2(19/32)	78.9(19/29)	54.5(22/29)	0 (10/16)
60	5.3(19/44)	29.4(34/46)	68.1(47/52)	28.6(7/44)	46.7(15/32)	78.9(19/32)	72.7(22/29)	68.2(22/29)	0 (13/16)
Overall	9.7(72/152)	42.9(112/152)	58.1(160/177)	21.7(60/150)	48.9(47/104)	66.7(63/104)	58.5(65/95)	50.7(71/95)	0 (26/56)

* Omitted because of small sample size but included in Overalls. Overalls computed for bugs with parasite counts above 11.

Therefore the magnitude of parasite density was determined for all bugs which became infected after a single feed on the chronic guinea-pigs. Parasite counts in 50 microscopic fields were classified in 5 density ranges with an assigned qualitative score of low (1-5), moderate (6-10), dense (11-100) and very dense (≥ 101).

On the assumption that use of bugs in which parasite yields are high decreases the number of false negative results and the contrary, when the yield of parasites is poor xenodiagnosis will fail to show positives, only those demonstrating parasite yields above 11 in 50 fields were utilized in computing overalls used in the analysis of data summarized in Table III. Nonetheless data related to bugs with low (1-5) and moderate (6-10) parasite counts are also shown in Table IV.

The intensity of the positive reaction in bugs to *T. cruzi* derived from chronic hosts shows a large degree of interspecific variability. While the proportion of bugs with dense and very dense parasite populations varied in *T. dimidiata*, *T. infestans* and *R. prolixus* from a low of zero to a high of 15.4%, it ranged from 42.1% to 77.8% in the remaining 6 species, although all fed on the same donors at the same time, 328 days after infection (Table III). Furthermore, the parasite yields do not remain stable in bugs throughout the 5 xenodiagnosis. The proportion of bugs with parasite counts above 11 in 50 microscopic fields decreased slowly in *R. neglectus* from a high of 77.8% to a low of 25%, as the interval between infection and xenodiagnosis lengthened to 1000 days, and disappeared completely in *R. prolixus* and *T. infestans* under similar conditions. By contrast, in the majority of the remaining vector species the proportion of bugs with parasite yields above 11 peaked at 1000 days after infection. Thus, the observed falls in parasite counts displayed by certain species are not due to aging of the chronic infection in the donor, but to some other mechanisms in which the vector species may be involved. One of those may be food availability, the effect of which is examined in the next paragraph.

On the interaction of food in the vector-parasite relationship: The ups and downs observed in the pattern of parasite density displayed by different vector species, which have been exposed simultaneously to the same infection, might be generated by different causes. One of these could be food, either by way of its direct assimilation by the parasite, or through its participation in host specific components. Another cause could be a build up of a toxic metabolic end product which caused premature death of the parasite. Therefore, an experiment was made aiming to measure the effect

of supplementary feeds given to the bugs during the period following their infection by the blood of the vertebrate donor.

Groups of clean fourth instar nymphs, starved during a period of 2-3 weeks since transition from the preceding stage, were fed once on guinea-pigs infected 454 days previously. The well engorged bugs from each species were divided into 2 equal batches, thus forming 2 groups each representing 8 vector species. One was fed on chicken blood at biweekly intervals following infection, the second was kept under starvation for 60 days, during which randomly chosen (usually 8) specimens from each group were examined for parasite yields at 30, 45 and 60 days of infection.

Fig. 1 shows the effect produced by the supplementary feeds given to infected bugs on the parasite density. In 5 of the 8 vector species herein explored, the proportion of bugs with heavy infections, as assessed by parasite counts among the re-fed, has been found to be around 3 times that seen in starved bugs. While the behaviour of these 5 species offered reasonably convincing evidence of the important part played by additional food, given to bugs from xenodiagnosis, on the population dynamics of the parasite, that of the other 3 species remained virtually unaffected by the supplementary feeds. *T. rubrovaria*, for example, presented a high proportion of specimens with heavy infections without supplementary feeds being involved.

The lack of alterations in parasite densities in *R. prolixus* and *T. infestans* (Fig. 1) when brought together with the low proportions of heavily infected bugs (Table III) shown by the overalls of pooled results (from a low of zero to a high of 21.7% vs. 42.9% to 66.7% in the remaining species) may suggest the existence of an antitrypanosomal substance or a factor inhibiting multiplication of the parasite in the domiciliated vector species *R. prolixus*, *T. infestans* and *T. dimidiata*.

On the effect of duration of infection in the vector on the population dynamics of the parasite: On the assumption that the use of xenodiagnostic agents in which parasite yields are high decreases the number of false negative results and that on the contrary, when parasite yields are low, xenodiagnosis will fail to show positives, bugs from each xenodiagnosis have been examined on 4 occasions, at 15, 30, 45 and 60 days following infection.

Results summarized in Table IV indicate that parasite density is characterized by two distinct phases: it is low in the first 15 days of infection in the bug and increases rapidly in the following 15 days. At 45 days of infection it fluctuates

TABLE IV

Data enabling to select the most appropriate timing for assessment of parasite density in bugs fed on guinea-pigs with chronic Chagas' disease

Density ranges	Percentages of positive bugs with the corresponding parasite densities seen in column 1 (no. positive bugs)								
	<i>R. prolixus</i>	<i>R. neglectus</i>	<i>P. megistus</i>	<i>T. infestans</i>	<i>T. brasiliensis</i>	<i>T. sordida</i>	<i>T. psmaculata</i>	<i>T. rubrovaria</i>	<i>T. dimidiata</i>
*15 days in bug									
1-5	100	85.7	50.0	83.3					
6-10	0	7.1	11.5	16.7					
11-100	0(12)	7.1(14)	38.5(26)	0(12)			not done		
101-250	0	0	0	0					
251-unc.	0	0	0	0					
Overall	0	7.1	38.5	0					
30 days in bug									
1-5	83.3	30.3	17.5	66.7	50.0	34.8	66.7	54.5	
6-10	8.3	12.1	22.5	9.5	8.3	0	4.8	8.1	
11-100	8.3(24)	51.5(33)	40.0(40)	23.8(21)	16.7(12)	30.4(23)	23.8(21)	36.4(22)	** (0)
101-250	0	6.1	5.0	0	25.0	34.8	4.8	0	
251-unc.	0	0	15.0	0	0	0	0	0	
Overall	8.3	57.6	60.0	23.8	41.7	65.2	28.6	36.4	
45 days in bug									
1-5	70.6	38.7	31.9	65.0	30.8	26.3	10.5	22.7	80.0
6-10	5.9	3.2	10.6	5.0	7.7	10.5	10.5	22.7	20.0
11-100	23.5(17)	48.4(31)	44.7(47)	30.0(20)	38.5(13)	47.4(19)	57.9(19)	27.3(22)	0(10)
101-250	0	3.2	4.3	0	15.4	5.3	15.8	13.6	0
251-unc.	0	6.5	8.5	0	7.7	10.5	5.3	13.6	0
Overall	23.5	58.1	57.5	30.0	61.5	63.2	78.9	54.5	0
60 days in bug									
1-5	94.7	58.8	19.1	71.4	46.7	15.8	18.2	22.7	92.3
6-10	0	11.8	12.8	0	6.7	5.3	9.1	9.1	7.7
11-100	5.3(19)	26.5(34)	42.6(47)	0(7)	20.0(15)	31.6(19)	31.8(22)	54.4(22)	0(13)
101-250	0	0	19.1	28.6	13.3	5.3	13.6	9.1	0
251-unc.	0	2.9	6.4	0	13.3	42.1	27.3	4.5	0
Overall	5.3	29.4	68.1	28.6	46.6	79.0	72.7	68.2	0
Pooled percentages									
1-5	86.1	48.2	27.5	70.0	42.5	26.2	32.3	33.3	47.0
6-10	4.2	8.9	14.4	8.3	7.5	4.9	8.1	13.6	13.0
11-100	9.7(72)	37.5(112)	41.9(160)	18.3(60)	25.0(40)	36.1(61)	37.1(62)	39.4(66)	0(23)
101-250	0	2.7	8.3	3.3	17.5	16.4	11.3	7.6	0
251-unc.	0	2.7	8.3	0	7.5	16.4	11.3	6.1	0
Overall	11.7(60)	48.0(98)	61.9(134)	27.1(48)	50.0(40)	68.9(61)	59.7(62)	53.0(66)	0(23)

Overalls of combined results from 5 xenodiagnosis (Table III) computed for bugs with parasite counts above 11 only; *not included in overall of pooled percentages;

**no positives

tuates around the level reached previously in some species, while in others, it continues to rise until the cut off time.

It is concluded, therefore, that the optimum timing for examination of bugs engorged from donors infected with the Y strain of *T. cruzi* is 45 days. There may be however a somewhat shorter optimum period, somewhere between 30 and 45 days following infection of bugs, which was not covered by the experimental regime.

A comparison of results from xenodiagnosis in guinea-pigs with acute and chronic Chagas' disease: The results herein described together with those reported previously (1982) have been integrated in a scheme (Table V) which clearly shows the various factors thought to operate in the vector response to infection by *T. cruzi*.

In order to make results comparable, the sequential examination of bugs engorged from hosts with acute and chronic infections should

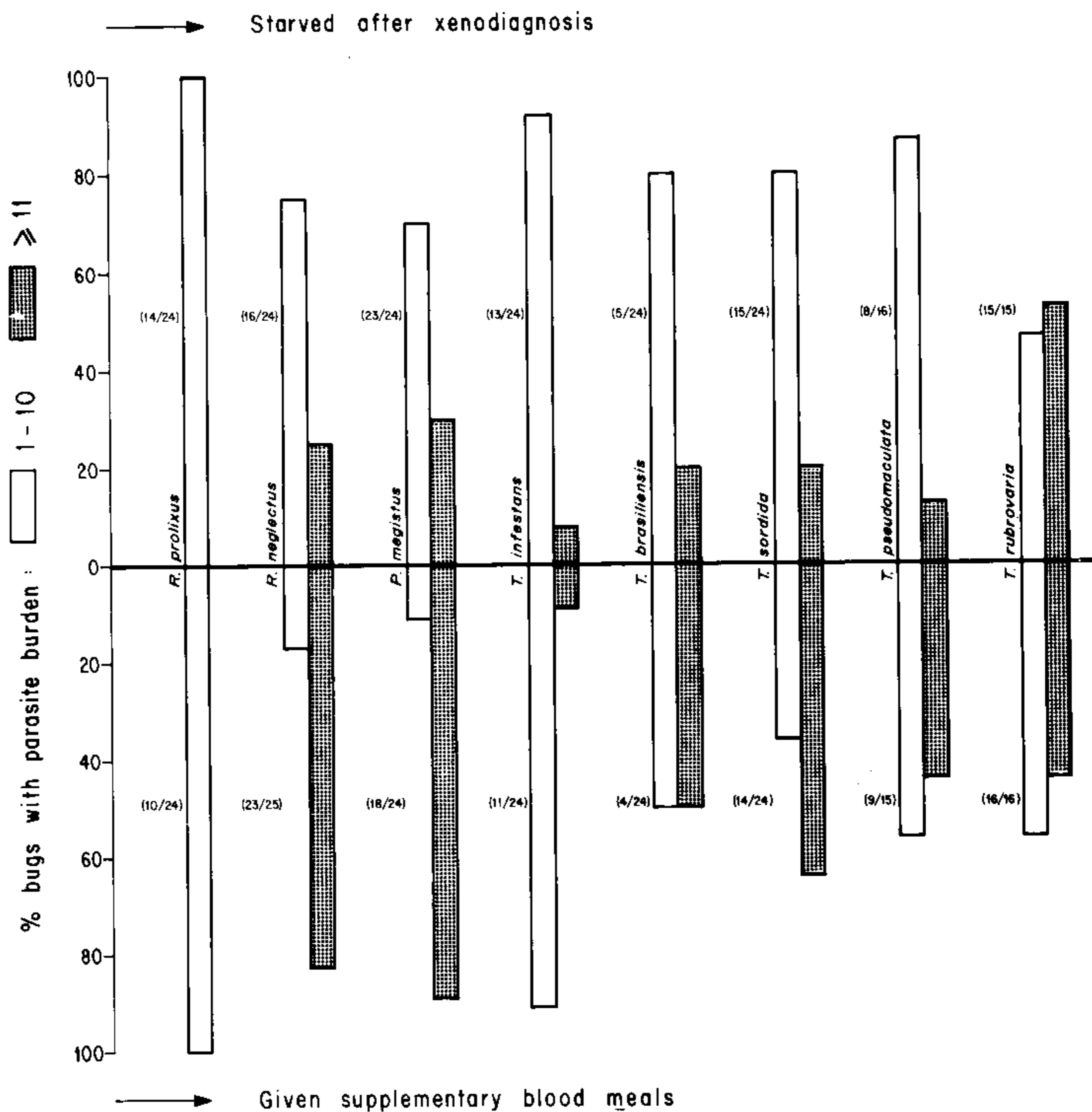


Fig. 1 - The effect of refeeding bugs engorged from hosts with chronic Chagas' disease on parasite densities.

be made at the same time after infection. Therefore, in computing overalls characterizing infectivity rates and intensity of infection in bugs engorged from acute donors, use was made of observations covering a period of 60 days of infection in bugs, extracted from the earlier study of this series (1982). These overalls appear to conflict with those analysed in the previous study. The differences observed might be due to the fact that the 1982 figures utilized in the analysis of results have been based on long lasting observations, up to 300 days of infection in the bug, while the overalls herein used have been covering only 60 days of infection in the bug.

Owing to the apparently complete lack of parasites in the peripheral circulation of the chronic host, one might have expected that the infectivity among bugs from xenodiagnosis would be at least drastically reduced as compared with that in bugs engorged from the same donors prior to clearance of the acute infection. But this was not the case. There was a striking similarity in the pattern of bug responsiveness to both phases of the infection in the same hosts. The main difference was the more vigorous reaction in bugs engorged from the acute donors, infectivity rates ranging from 53.4% to 97.9% in these latter vs. 38.1% to 91.2% in bugs engorged from the chronic donors (Table V). Similarly, the intensity of infection monitored by the proportion of positive bugs with parasite yields above 11 in 50 microscopic fields, ranked from a low of zero to a high of 68.9% among bugs engorged from chronic

donors vs. a range from 18.2% to 91.3% among those fed on acute donors. There are however some surprises; why *T. sordida* and *T. brasiliensis* demonstrated greater proportions of bugs with dense parasite populations (68.9% and 50% respectively) after feeding on chronic donors than those engorged from acute donors (46.4% and 38.1%), is not clear. But by and large, the contrary has been found to be a rule.

As bugs engorged from hosts with acute infection, those fed on chronic hosts tended to dissociate in high and low responders to infection. The highest prevalence of infection and the major proportion of bugs with heavy infections (Table V) have been observed among the completely wild (*R. neglectus*, *T. rubrovaria*) and essentially sylvatic (*P. megistus*, *T. pseudomaculata*, *T. sordida*) vector species, while the lowest have been found in the domiciliated vector species (*R. prolixus*, *T. infestans*, *T. dimidiata*). It seems, therefore, that each distinguished species of good or poor responders to infection by *T. cruzi* maintains its individuality in detecting chronic infections much as in the acute infections.

The further conclusion reached from the analysis of results shown in Table V is that interspecific variations of infectivity rates are completely unaffected by the size of the infective blood meal ingested either from the acute or the chronic donor.

Furthermore, no influence has been observed in infectivity rates and intensity of infection attributable to mutual adaptation of the local

TABLE V

Comparison of vector reactions to acute and chronic infections with *T. cruzi*

Vector (biotope)	* Mean weight of blood intake per bug (mg)	% infected bugs (no. fed)		% bugs with parasites above 11 (no. positives)	
		* Acute infection	**Chronic infection	*Acute infection	** Chronic infection
<i>R. neglectus</i> (wild)	53.2 ± 7.49	97.9(48)	76.6(128)	89.4(47)	48.0(98)
<i>P. megistus</i> +(sylvatic)	118.1 ± 46.09	96.4(56)	91.2(147)	70.4(54)	61.9(134)
<i>T. psmaculata</i> +(sylvatic)	54.5 ± 10.81	95.8(24)	71.3(87)	91.3(23)	59.7(62)
<i>T. rubrovaria</i> (wild)	65.6 ± 35.09	94.4(18)	75.9(87)	70.6(17)	53.0(66)
<i>T. sordida</i> +(sylvatic)	47.1 ± 9.71	93.3(30)	63.5(96)	46.4(28)	68.9(61)
<i>T. brasilien.</i> +(sylvatic)	95.0 ± 40.46	80.8(26)	41.7(96)	38.1(21)	50.0(40)
<i>R. prolixus</i> (domestic)	94.2 ± 16.80	67.3(52)	46.9(128)	31.5(35)	11.7(60)
<i>T. infestans</i> (domestic)	140.3 ± 21.16	53.4(58)	38.1(126)	48.4(31)	27.1(48)
<i>T. dimidiata</i> (domestic)	264.8 ± 147.08	61.1(18)	47.9(48)	18.2(11)	0 (23)

*Extracted from P. Szumlewicz & Muller, 1982; each value represents the overall of results from triplicate examination of bugs at 30, 45, 60 days after infection; **Extracted from Tables I, III; each value represents the overall of pooled results from 5 xenodiagnosis with triplicate examination of bugs at 30, 45 & 60 days after infection; †Essentially sylvatic, some already in process of adaptation to, or with incipient colonization of human homes.

parasite to the local vector species, a widely acknowledged concept in interpreting interspecific variations among infectivity rates in bugs engorged either from acute or chronic donors.

DISCUSSION

So far we cannot explain why infection occurs in bugs fed on chronic hosts, apparently free of peripheral parasitemia, as shown by frequent examination of blood smears and/or by subinoculation of clean animals (P. Szumlewicz, 1977). Neither do we know why, in a single batch engorged at the same time on the same chronic host, some bugs become infected and others do not. Nor do we know the reasons for the large amount of variability often observed in the number of parasites surviving at one point of time within the experimental sample of insects which have been exposed at the same time to the same infection. Because answers to these questions are not available as yet, the interpretation of results from xenodiagnosis still remains a speculative problem with very little precision.

Since the main problem in evaluating the degree of success achieved by xenodiagnosis is in the detection of ultra-low parasite numbers in the chronic host, considerable effort has been extended in the elaboration of procedures used to uncover very low parasite densities in bugs used in xenodiagnosis. In particular, the precautions taken in this study in detecting small numbers of parasites were the most rigid currently known to us and also much more vigorous than those used by other investigators.

It would be out of place to mention all the measures which have been already described by the authors in 1982, rather attention will be drawn to some less obvious yet very important procedures which are required for an efficient and reliable xenodiagnosis. These are the following:

1. While the rates of infection among bugs fed on Chagasic hosts have been based on fecal infections in which the metacyclic trypomastigotes have been sought, the proportion of positive bugs in the present study was based on gut infection, in which the parasite population was more abundant and easier to detect, thus minimizing the probability of including in the number of non infected bugs false negative results.

2. A previous study in search of an insect model for xenodiagnosis (1982) implies the integration of parasite density which is not by itself a research subject and should not be a determinant parameter in xenodiagnosis but it is a component which will be of help in the

choice of a proper xenodiagnostic agent. So far the proportion of positive bugs has been the key parameter in the selection of promising candidates as xenodiagnostic tools. The detection of only one parasite in the insect is sufficient to classify it as positive, but difficulties may be encountered in locating the single parasite. Therefore it is thought that, an experimental insect model, capable of promoting fast development and vigorous multiplication of low numbers of parasites ingested from the chronic host, should be given priority in the selection of the proper candidate for xenodiagnosis. Such an insect would leave less room for false negative results, thus upgrading the reliability of the test.

3. Since vector-species present a broad range of host-parasite relationship, providing sometimes strong indication of individualism, it was felt that the reaction to infection can not be evaluated by studying just a few vector species derived mostly from artificial biotopes, like *T. infestans* and *R. prolixus*. While little or no attention has been given to other indigenous vectors, the simultaneous screening of the infective potential in completely wild, essentially sylvatic, with tendencies of colonizing human dwellings and domiciliated vectors, herein described, offered the chance of attracting a broader spectrum of vector capability to act as a xenodiagnostic agent.

4. Unpublished experiments demonstrated that prolonged starvation of infected bugs is deleterious to the parasite and may become critical to its multiplication and persistency, thus leading to negatization of otherwise positive bugs. Therefore insects from xenodiagnosis kept for examination at intervals exceeding the first 15 days after their infective blood meal, have been fed on chicken blood at biweekly intervals.

5. Comparison of results from xenodiagnosis in the same host during the acute and chronic phases of the disease enabled us to focus attention on the overall aspect of the infection by *T. cruzi* and to see whether the product of xenodiagnosis could be treated as a logical and reproducible dynamic system. Furthermore, by monitoring the infection in the host for a period of 33 months approximately, it was possible to demonstrate that it is not a matter of chance that infection transferred from the host to the bug assumes different characteristics, which are repeatedly observed in some and not in other vector-species.

The course of infection in the bug has been followed during a period of 2 months in 5 separate xenodiagnosis made with different bugs which had engorged from the same donors at the same time and were examined on the same

occasions following infection (Tables III & IV). By examination of the bugs when the parasite yield is large renders false negative results less probable.

In the host which survives the acute infection immune mechanisms are effective in reducing the number of parasites present in the systemic circulation, but they are unable to cope completely with the infection. Thus parasites either protected by mechanisms associated with the synthesis of molecules which mimic those of the host, or by hiding in sites difficult to discover, will be harbored by chronically infected individuals throughout their life. For example, infective parasites have been discovered by Viens et al. (1973) in the kidney capillaries of mice which recovered from an acute infection by *T. musculi* one year previously.

There is still a lack of definite evidence about the mechanism that brings out the parasites from the hiding places as is apparently demonstrated by bugs from xenodiagnosis. The question is raised whether or not the stimulus for their reemergence in the circulation derives from the bug. A factor like stress may alter the host parasite relationship, resulting in the appearing of parasites in the peripheral circulation. Furthermore, since the stimulus triggering this process is strictly associated with the contact between the bug and the infected host, the existence of an attractant derived from the bug should not be overlooked. If such exists, it is unlikely that it will be available in other diagnostic tests which do not involve the direct contact between the host and the bug.

The parasite that successfully established itself in the bug persisted for varying periods of time before decay started in some species but not in others. While the proportion of bugs with dense parasite population in *T. dimidiata*, *R. prolixus* and *T. infestans* varied from a low of zero to a high of 30%, it ranged from 54.5% to 78.9% in the remaining vector species at 45 days of infection (Tab. IV).

Different mechanisms could be responsible for the spontaneous elimination of parasites which die out or linger in numbers too small to be detected; it can be caused by aging of the parasite ("time dependent death") or by the lack of certain nutrients in the gut of the bug ("nutrient dependent death").

The former is hardly acceptable because the infection of all vector species and their examination were time matched. Furthermore, exceptionally long living specimens of *P. megistus* have been found loaded with parasites (uncountable), just prior to death, at 546 days after a single feed on albino mice with an acute infection Luz & Borba (1969) reported that *T. in-*

festans kept eliminating infective parasites at an interval of 812 days after infection. Since to perpetuate throughout the entire life of its invertebrate host seems to be a feature of *T. cruzi*, it precludes death caused by its age.

As for the "nutrient dependent death", it may be generated by lack of food that provides the nutrient elements necessary for direct assimilation by the parasite, which possesses the enzymatic capacity to produce its own nutrients, as demonstrated by Urbino & Crespo and by Urbino & Azavache (1984), and/or for participation in the synthesis of *T. cruzi* specific proteins, described by Astolfi Filho et al. (1984).

Bugs engorged from chronic donors became infected, but the heavy infections were lost with time in certain species, thus indicating that at the very beginning the living forms of the parasite may rely upon nutrient reserves stored by the parasite prior to penetration into the bug. These nutrients may suffice until the parasite reaches the stage of multiplication during which the first supply brought in from the donor might not be adequate.

Our results on the effect of additional feeds offered to the bug do not permit speculation on any specific nutrient derived from the normal blood meals, but there is no doubt that it accelerates the multiplication of the parasite, thus increasing its yield in the bug. It is thought therefore, that the fluctuating rates observed among results from successive xenodiagnosis (Table III), some including alternative rises and falls rather than the more steady rates seen in *P. megistus* or in *T. sordida*, might be a reflection of food interaction in the host-parasite relationship. By increasing of food availability, the rates of bugs with large parasite yields rises while it drops with lack of food. The more steady level observed in certain species may serve as an example of a parasite population remaining in a state of dynamic equilibrium, constant or periodic recruitment of nutrients approximating losses.

However the interaction of additional feeds in the vector-parasite relationship does not seem to be a general phenomenon. Fig. 1 shows the strikingly stimulating effect of the supplementary blood meals on the parasite density as compared with the yield of those lacking the additional meals. Five of the 8 species tested showed at least a 3-fold higher proportion of bugs with dense parasite populations than those kept under starvation. While the behaviour of these 5 species offered reasonably convincing evidence of the important part played by additional food given to the bug in the population dynamics of the parasite, that of the remaining

3 species did not. For example, *T. rubrovaria* was found to make up a high proportion of specimens with heavy parasite burdens without additional feeding being involved, possibly due to its very slow development.

The lack of a food effect on the parasite yields in *T. infestans* and *R. prolixus* is a puzzling phenomenon because these species contain the adequate environment which permits the establishment and maintenance of the infection by *T. cruzi*, although of low intensity (from 1 to 5 parasites per 50 fields). The absence of any demonstrable effect due to additional feeds in these 2 species brought together with the overalls of pooled results from 5 successive xenodiagnosis (Table III), showing the low proportions of bugs with parasite counts over 11 in *T. dimidiata*, *R. prolixus* and *T. infestans* (from zero to 21.7% vs. 42.9%-66.7% in the remaining species), makes it unlikely that lack of nutrients was responsible for the decay of parasites in these species. It raised questions, however, about the existence of an antitrypanosomal substance in these species, similar to that found by Croft et al. (1982) in the haemolymph and in the extract of *Glossina*, capable of agglutinating *Trypanosoma b. brucei*.

As for the behaviour of *T. cruzi* in triatominae, D' Alessandro & Mandel (1969) reported that 18% of *R. prolixus* naturally infected with *T. rangeli* and 12% of those infected with *T. cruzi* lost the infections in 3 months.

In the current study, however, the reduction of parasite yields in the 3 domiciliated species was high but not complete. By 60 days after infection, bugs with dense parasite populations disappeared (Table IV), leaving from 71.4% to 94.7% positives with very low parasite counts (1-5). Therefore, the existence of a factor which impairs parasite multiplication, thus reducing the effect of refeedings offered to the domiciliated bugs on the population dynamics of the parasite seems possible.

It is therefore recommended not to resort to the use of the domiciliated vectors as xenodiagnostic agents, although they are of major epidemiological importance in the transmission of the disease to man and domestic animals.

In contrast with the poor responsiveness to infection observed in the group of domiciliated vectors (percent of positives ranging from 40% to 47.9%) was the good responsiveness (varying from 71.3% to 90.4%) displayed by the group of completely wild and essentially sylvatic bugs of *R. neglectus*, *T. rubrovaria*, *T. pseudomaculata* and *P. megistus* (Table I).

The existence of a close relationship between infectivity of the vector and the biotope inhabits has been first reported by the authors

in 1982. Paradoxical as it seemed, the main assumption underlying the responsiveness of the vector species to acute infection has been that wild and essentially sylvatic bugs were the high responders while the domiciliated were the low responders. Although the reasons for this have not been determined, it has been fully confirmed in the current experiments, thus suggesting it to be a constant phenomenon that has failed to arouse interest in previous investigators. Perhaps the reasons for the lack of comment on this phenomenon is that emphasis has mainly been given to the simple link of efficiency of xenodiagnosis and the size of the infective bloodmeal ingested by the bug, and to the greater susceptibility of indigenous bugs to infection by parasites of their own geographical area. Conflicting evidence concerning this latter led to question the interpretability of results obtained from experiments. For example, comparison has been made of the infectivity rate produced by a Mexican strain of *T. cruzi* in the indigenous wild bug of *T. protracta* by Ryckman (1965) and by Little et al. (1966), or in *Dipetalogaster maximus* from México by Barreto et al. (1978) with that observed in the domiciliated *T. infestans* from South America. It is believed, that had a sylvatic bug of those herein described been used instead of the domiciliated *T. infestans*, the results of the comparative experiments might have been different. Data summarized in Table V provide support for our view; of a total of 56 specimens of *P. megistus* and of 58 of *T. infestans* engorged simultaneously from guinea-pigs with acute Chagas' disease and refeed on three occasions on chicken blood, 96.4% of the former but only 53.4% of the latter were found positive by 60 days after infection.

The enigma of low infection rates in *T. infestans* has been difficult to explain. This could not be related to the quantity of trypanosomes ingested because the estimated number, given by the authors in a previous paper (1982), was approximately 14.6×10^3 vs. 12.6×10^3 in *P. megistus*. Neither could it be associated with the widely acknowledged mutual adaptation of local vectors with local parasite isolates because *T. infestans* was received from the endemic area of Chagas' disease in the State of São Paulo where *T. cruzi* Y strain has been isolated, while *P. megistus* was derived from the State of Ceará, a diverse and distant geographical area.

As for the concept of the bugs biotope involvement in its reaction to infection, no reference has been found as yet to confirm or refute our hypotheses stressed in the previous paper (1982) of this series, that the originally zoonotic *T. cruzi* might find in the domiciliated vector an unsuitable prey species for its survival, while

the ferine ecology encountered in the sylvatic bugs favored its fast development and vigorous multiplication. The question of the association of parasite population dynamics with the biotope, the vectors habitat, will remain open to debate until results from xenodiagnosis with bugs of the same species, derived from natural and artificial biotopes, are available for analysis.

The biological parameters on the basis of which the grouping of vector species as low or high responders to infection were remarkably similar in species within the groups. There were, however, substantial differences between these groups, indicating that the reaction of bugs to infection is not a species specificity but rather a type of responsiveness characteristic for an assemblage of species with different morphologies but similar ecologies.

The parallelism between infectivity rates (from 71.3% to 75.9%, Table I) observed in *T. pseudomaculata*, *R. neglectus* and *T. rubrovaria* might be taken to imply that there is a corresponding similarity in the environments the parasite finds in these bugs. The lack of host specificity exist also in the assemblage of low responders, the proportion of positive bugs ranging from 40 to 47.9 in the domiciliated *T. infestans*, *T. dimidiata* and *R. prolixus* (Table I).

Although this study uncovered 4 promising candidates for xenodiagnostic purposes, *P. megistus* with the highest infectivity rates (91.2%) coupled with large parasite yields, as seen in Table V (61.9% of positives harbouring either dense or very dense parasite populations), merits top priority as a xenodiagnostic agent. Its extraordinary capability to support fast development and vigorous multiplication of the few parasites ingested from the host with chronic Chagas' disease, has been demonstrated by the remarkable parallelism between infectivity rates of 91.2% vs. 96.4% among specimens engorged from the same host in the acute phase of the disease, when it had an estimated number of 12.3×10^3 parasites in circulation at the time of xenodiagnosis, as reported by the authors in a previous paper (1982).

In view of this finding, the use of infectivity rates among bugs engorged from patients with chronic Chagas' disease in evaluation of the patients parasitemia, as described by Castro et al. (1983), cannot be accepted unreservedly. The major objections against it are: 1-that infectivity rates and intensity of infection, expressed by parasite density, are not necessarily related to the number of parasites present in the blood meal engorged from hosts with chronic Chagas' disease (Table V) and 2-that these parameters reflect the interaction of the parasite and the bug used as a xenodiagnostic

agent. While the former is necessary to establish the infection in the bug, the infectivity rates and intensity of infection are related to the capacity of the bug to support the development and multiplication of the parasite, thus indicating that xenodiagnosis is merely a qualitative method aiming to detect small numbers of parasites carried by the host with chronic Chagas' disease throughout his life.

Since some vector species which are suitable for xenodiagnosis may not be conveniently produced by conventional laboratory means, further experiments to make available greater numbers of vector species adequate for xenodiagnosis, are indicated. This might contribute to the selection of an adequate xenodiagnostic agent which would also meet economic and practical criteria.

The practical significance of the findings described will have to be confirmed by establishing whether they are also applicable to other strains or isolates of *T. cruzi* and to different geographical populations of the same vector derived from artificial and natural biotopes. Results obtained in this sense will be reported in a forthcoming paper.

RESUMO

Estudos em busca de um inseto modelo experimental para xenodiagnóstico em hospedeiros com doença de Chagas. 2. Tentativas para aumentar a credibilidade e a eficiência do xenodiagnóstico na doença de Chagas crônica — Assumindo que a otimização do xenodiagnóstico poderia apresentar um passo primordial na estratégia que visa a minimização de casos falso negativos, tanto nos levantamentos longitudinais da doença de Chagas, quanto na sua quimioterapia, apresentamos um estudo sobre a reconstrução do xenodiagnóstico, dando maior ênfase à dinâmica da população parasitária no barbeiro utilizado no xenodiagnóstico. Conseqüentemente, com o objetivo de levantar a credibilidade do xenodiagnóstico foram tomadas as seguintes medidas: 1. A densidade da população parasitária que em si não é um objetivo de investigações, mas retratando nitidamente a evolução e multiplicação do parasita, foi incorporada na triagem de insetos para o xenodiagnóstico. 2. Assumindo que a realimentação do inseto infectado é capaz de triplicar a densidade da população parasitária, este foi realimentado de duas em duas semanas em galinhas. 3. Na avaliação da resposta do inseto a infecção por *T. cruzi* foi medida no tubo digestivo completo no lugar da de fezes obtidas por compressão do barbeiro. 4. Sendo a densidade da população parasitária nos primeiros 15 dias após a infecção bem baixa, porém crescendo rapidamente nos 30 dias posteriores, adotou-se o intervalo de 45

dias para o exame do inseto alimentado no hospedeiro com a doença crônica.

A eficácia do xenodiagnóstico parece estar associada com o inseto utilizado como seu agente. Das 9 espécies vetoras exploradas neste sentido o inseto da espécie *Panstrongylus megistus* mostrou-se o mais promissor, devido ao seu elevado índice de infectividade (91.2%), estando a maioria destes (61.9%) com elevadas cargas parasitárias. Destaca-se também este inseto pela sua extraordinária capacidade de sustentar a rápida evolução e boa multiplicação de poucos parasitas ingeridos do hospedeiro na fase crônica. Isto está bem ilustrado na comparação do seu índice de positivos de 91.2% com este de 96.4% encontrado entre os insetos alimentados no mesmo hospedeiro com a infecção aguda, quando o número estimado de parasitas na sua circulação foi de 12.3×10^3 por ocasião do xenodiagnóstico.

Palavra-chave: doença de Chagas crônica – credibilidade do xenodiagnóstico – eficácia do xenodiagnóstico – agente do xenodiagnóstico

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