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THE CHAGAS' DISEASE RESEARCH UNIT

IN SALVADOR, BAHIA

CONTENTS

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I. INTRODUCTION

South American trypanosomiasis, which includes Chagas' disease in man, is a continental problem of proper concern to the Pan American Health Organization and its member states. While Chagas' disease affects millions of people in South America it should not be forgotten that zoonotic trypanosomiasis occurs among wood rats in California, racoons in Maryland, and occasionally in man in Texas.

The London School of Hygiene and Tropical Medicine, until recent years, had little or no practical association with Chagas' disease research, consequent upon its historically-oriented predominant interests and expertise in those Near and Far East diseases that affect the millions of inhabitants of the former British Empire. Times have changed, however, and the School more recently has looked for a more even global balance of its interests and activities.

For these and other reasons a small group of scientists from London came to the State of Bahia in the latter part of 1971, with the financial support of the Wellcome Trust, to participate in a program of research initiated by Prof. A. Prata (formerly Fundação Gonçalo Moniz and Federal University of Bahia; now Faculty of Health Sciences, University of Brasília).

Progress Report (1971-1974) of a collaborative program between the Gonçalo Moniz Central Laboratory, the London School of Hygiene and Tropical Medicine, and the Pan American Health Organization, with financial support of the Wellcome Trust, London.

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The "São Felipe Project" began in 1965 as a cooperative longitudinal study, mainly clinical in nature, that also included the Nucleo de Pesquisas da Bahia (Dr. Italo A. Sherlock and staff), part of the Federal Health Ministry research organization, FIOCRUZ.

It was not intended that the London Group would become involved in purely clinical aspects of Chagas' disease--already well covered by the Brazilian workers--but would concentrate on the nonclinical fields of vectors, parasites, and general ecology of the disease, in order to help build up the background epidemiology necessary to a better understanding of how, when, and why people became involved in the natural cycle of Trypanosoma cruzi transmission.

More recently a tripartite agreement has been signed between the London School of Hygiene and Tropical Medicine, the Brazilian host institution (now the Gonçalo Moniz Central Laboratory of the Bahia State Health Foundation), and the Pan American Health Organization, under which the expatriate personnel of the Unit have been appointed consultants of the Organization.

Chagas' disease in São Felipe, as elsewhere, is principally a household problem and the London group therefore based its investigations on a small number of representative individual bug-infested households and their nearby environment. These households were chosen not at random but because it was preferred that families should be as complete and static as possible, to have lived for a long time in their present (bug-infested) home and would permit regular examination of their homes for the presence of bugs.

Some 40 such homesteads, of poor subsistence-level farmers, were investigated and about 3,000 Panstrongylus megistus, the sole domestic vector of the area, collected and studied for evidence of T. cruzi infection, feeding behavior (as evidenced by precipitin identification of recent blood meals by Dr. P. F. L. Boreham, Imperial College, Ascot, England) and other biologic parameters.

Dogs and cats living with 37 families (9 families had dogs, 22 had cats, 6 had both) were examined for infection (by xenodiagnosis) as were a few pigs, donkeys, etc. More than 250 domestic, peridomestic, and sylvatic animals were trapped and examined for T. cruzi infection; the majority of these were opossums and rodents.

Earlier searches of a variety of possible habitats of sylvatic bug species by staff of the Núcleo de Pesquisas failed to reveal the presence of nondomestic triatomine bugs other than the uninfected Psammolestes tertius common in the nests of Dendrocolaptid birds in trees. Nine of 16 nests examined contained P. tertius; all bugs were uninfected. The search for sylvatic bugs was, however, resumed and, thanks to the diligence of Dr. M. A. Miles, two species of sylvatic triatomine bugs were encountered, both associated with the nests of arboreal and semiarboreal opossums, spiny rats, and small rodents living in bromeliad epiphytes, often on jack fruit trees. The jack fruit tree is one of the few large tree species remaining in this part of Bahia, because its huge sweet fruits are highly prized food. The first sylvatic species to be found was Rhodnius domesticus, previously unknown in the State of Bahia and later, Triatoma tibiamaculata was encountered in and near animal nests in arboreal bromeliads. Both species of bugs harbored trypanosome infections considered to be T. cruzi on grounds of morphology and infectivity to laboratory animals. P. megistus were found only in houses and chicken houses.

Some results of the work of the Unit have been presented at the Thirteenth Seminar on Trypanosomiasis held in London in 1972 and at the Ninth Congress of the Brazilian Society of Tropical Medicine, held at Fortaleza, Brazil, in February 1973. Further accounts have appeared in the Report on the Work of the School, 1971-1972 and 1972-1973, published by the London School of Hygiene and Tropical Medicine.

Though a number of associated laboratory investigations have also been undertaken, they have in general taken second place to the field-based studies and will not be considered further in this report.

What follows is an attempt to synthesize and analyze the basic findings to date in regard to the light they throw on the basic ecology of South American trypanosomiasis in the simplest epidemiologic situation, that of a single domiciliated triatomine vector; specifically Panstrongylus megistus in eastern Brazil.

II. THE BUGS AND THE HOUSES

The majority of bug-infested houses in the rural area surrounding the more sophisticated minor township of São Felipe are the homes of poor people and are self-made from local raw materials. They therefore conform to a relatively standard pattern.

Buildings are rectangular (about 10 x 4.5 m) with a living room, one or more bedrooms, and a kitchen. Floors are of mud plaster allowed to dry under palm-fronds that are then removed. Walls are of wattle and daub construction: stout vertical pole have outer and inner lines of thinner poles lashed to them with strips of fibrous bark. The interstices are filled with clods of mud and inner and outer surfaces are smoothed roughly back to the horizontal laths. Load-bearing walls are about 2 m high and 16 cm thick.

Inner partition walls are thinner. The roof is built on a gabled timber frame and the gables themselves are of wattle and daub. Roof material is usually of split palm-fronds; less frequently, of simple tiles. There are usually external doors to living room and kitchen but commonly only the living room has a wood-shuttered window (often only one; infrequently two). Kitchen stoves are wood-fired and either solidly made from stones and earth or consist of a layer of earth supported at waist height on poles.

The wattle and daub construction and the properties of the local soil result in deep cracks appearing along the lines of the underlying timber reticulum: these cracks develop as the mud dries, and later spread to break the mud walls into brick-like blocks that also shrink away from the vertical poles, leaving concentric spaces which can easily be colonized by bugs and from which bugs may move laterally along the extensive horizontal cracks. As wall timbers rot and are eaten by termites and beetles the entire structure becomes a three-dimensional lattice of intersecting channels. Unlike many other domiciliated triatome species, P. megistus is essentially limited to this wall-crevice habitat and appears not to utilize the palm-thatch roof. Houses of better construction, with plastered mud-brick walls usually harbor only light bug infestations, if at all, since the crevice habitat is vastly reduced.

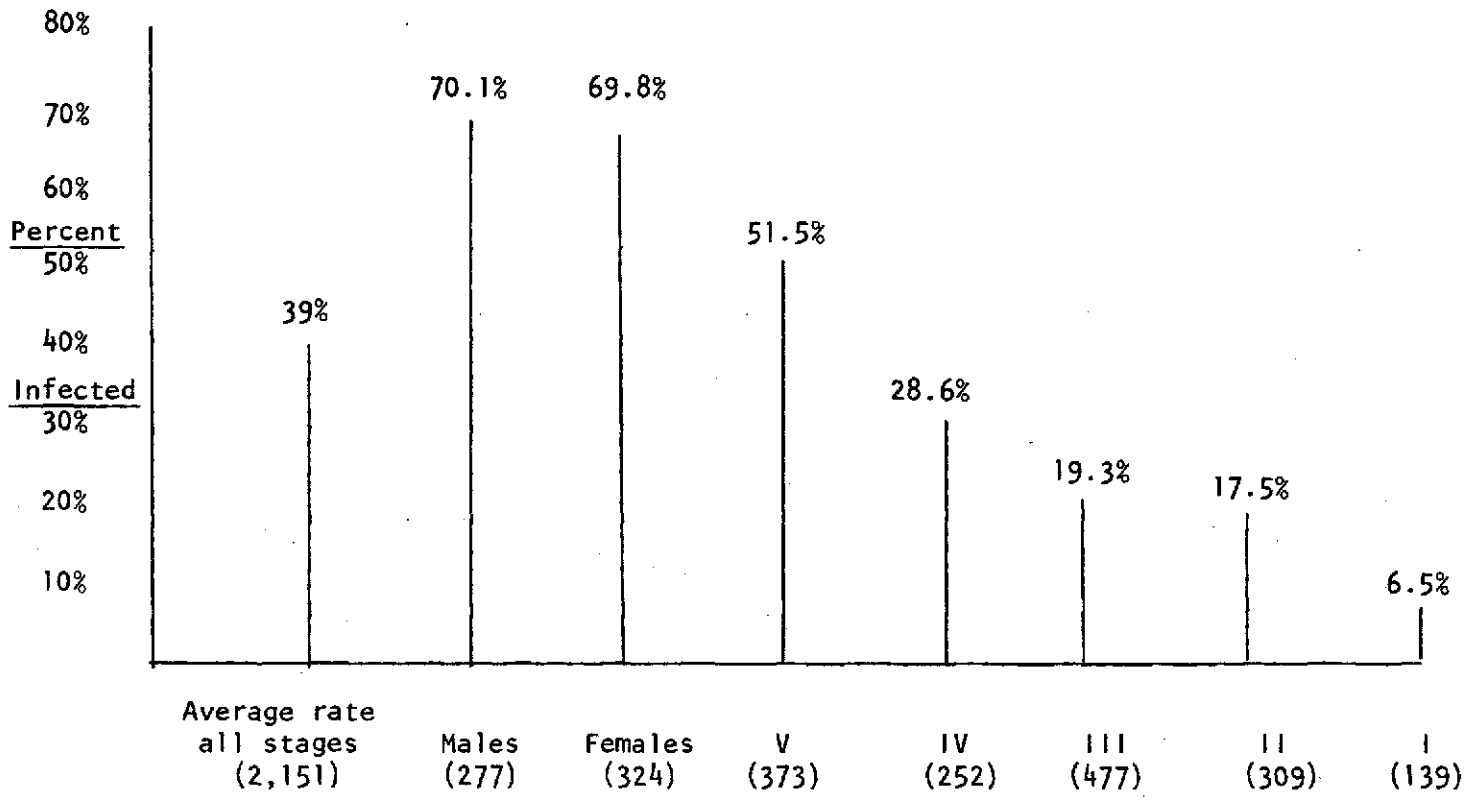
Furniture in these poor houses is rudimentary and beds are usually mat-covered planks built close against the walls, or laid directly upon the floor. Household bug infestations are usually specifically associated with the walls of sleeping areas; sometimes also with those wall areas in other parts of the house where animals--especially chickens--are allowed to sleep in the house or close against the walls outside.

One continuing interest has been to develop a reliable, reproducible sampling method by which household levels of infestation might be assessed and compared. Unfortunately simple box-traps, sticky traps, light-traps, and the like have, so far, proved entirely useless for this purpose with P. megistus and observations are still based on the usual crude method of searching cracks with a torch and catching bugs with forceps. Results were standardized, as far as possible, by expressing the catches on a bugs-per-man-hour basis, yielding a figure referred to later as Apparent Density.

Bugs collected from houses were examined for trypanosome infection by dissecting out the rectum and examining the rectal contents, in saline, with a phase-contrast microscope. Figure 1 shows the results of dissection of more than 2,000 P. megistus of both sexes and all instars: as expected, the infection rate rises steadily from a low level in the first instar to about 70 percent in adults of both sexes. The average infection rate for all stages was 39 percent and the sex ratio of adult bugs was 1.17: 1 in favor of females.

Bugs that contained blood-meal residues had their stomach contents expressed onto filter paper; these were dried and later used for precipitin tests. Figure 2 shows the analysis of 1,373 feeds identified from routine household collections and shows clearly that man is the chief source of food for P. megistus (81 percent) and that the chicken accounts for a further 13 percent of feeds. Feeds from all other sources combined are thus a mere 6 percent. It is seen from Figure 2 that the infection rates of man and bird-feeding bugs are markedly different and that blood-meal residues of mixed origin (representing successive feeds on different hosts) are infrequent, less than 2 percent of the total.

FIGURE 1: The distribution of _T. cruzi_ infection in 2,151 domestic _P. megistus_ from São Felipe, by sex and instar.



P. megistus: stage and number examined.

FIGURE 2. Analysis of blood meals of domiciliated *P. megistus* in São Felipe and infection rates per blood-meal source (mean infection rate, all sources: 35.5%)

BLOOD MEAL IDENTIFICATION	NUMBER OF FEEDS	% TOTAL FEEDS		% INFECTED BUGS PER BLOOD MEAL SOURCE	
Human	1,109	81%		38%	
Avian	177	13%		19%	
Dog	34	2.5%		30%	
"Mammal"*	30	2.2%		50%	
Cat	1	< 0.1%		$1^+/1$	
Rodent	1	< 0.1%		$0^+/1$	
Avian/man	17	1.3%	1.9% (Avian/man to Avian/pig)	42%	47.6% (Avian/man to Avian/pig)
Avian/dog	7	0.5%		43%	
Man/dog	1	< 0.1%		$1^+/1$	
Avian/pig	1	< 0.1%		$1^+/1$	
TOTAL	1,373				

*(The majority of feeds in this class were weak and could not be identified further. A small proportion may have been feeds from mammals for which specific antisera were not available, e.g. opossums).

III. THE DOMESTIC RISK FACTOR (DRF)

The number of bugs caught per man-hour in each house studied, termed Apparent Density (AD), was multiplied by the overall trypanosome infection rate among the bugs from each house, divided by 100 to give a number of convenient size, and was called the Domestic Risk Factor (DRF). The DRF represents a crude assessment of the intensity of current trypanosome challege in each house that may reflect the risk of further individuals becoming infected in future.

The DRF was calculated for 39 infested houses and varied from zero to about 35. Figure 3 shows the AD, infection rate, and DRF for a number of households. The current infection index, on a per capita basis for each family, shown in the final column suggests that a high DRF is often, but not regularly, associated with a high prevalence of *T. cruzi* infection among family members.

Since the DRF is not necessarily stable with the course of time, and because other chance factors are involved in the contaminative transmission of *T. cruzi*, one would not expect the DRF to be an indicator of current human infection. It should also be noted that current infections are cumulative phenomena, acquired over varied (unknown) periods of time. One has no indication of the frequency with which infections occurred in the past; to which the DRF might be more closely correlated.

It is too early to assess whether or not the DRF is a useful prediction of the risk of future individual infections. Attempts to test it by leaving sentinel mice and guinea pigs in houses were inconclusive and it will be necessary to wait until it is possible to compare the rate of appearance of new infections in houses with differing DRF's.

IV. FOCALIZATION OF BUGS IN HOUSES

On two occasions so far we have had the opportunity to completely dismantle old, bug-infested houses and collect every bug we could find,

FIGURE 3. Apparent density and infection rate of *P. megistus* and the Domestic Risk Factor (DRF) of some São Felipe households from which more than 50 bugs were collected.

HOUSE	(1) APPARENT DENSITY (BUGS/MAN/HOUR)	(2) INFECTION RATE	NO. OF BUGS DISSECTED	$DRF = \frac{(1) \times (2)}{100}$	NO. IN FAMILY	CURRENT INFECTION INDEX (+)
Sap. 20	22	11%	66	2.4	4	4.3
Peq. 20A	18	39%	80	7.0	8	3.3
Ferr. 1	32	24%	75	7.7	6	3.8
B.V. 34A	40	21%	160	8.4	6	3.3
Terr. 82	37	31%	149	11.5	3	6.7
Sap. 7	21	70%	74	14.7	10	7.7 (*)
Peq. 18A	30	54%	106	16.2	8	5.3
Terr. 36B	33	54%	58	17.8	6	5.3
Sob. 36	20	94%	92	18.8	11	4.0
Gen. 70	34	64%	177	21.8	9	5.8 (*)
Ferr. 33A	60	58%	201	34.8	3	4.7

(*) - recent acute case

(+) - arbitrary scale per capita based on parasitological, serological and ECG criteria for each family.

noting the location of each and later examining the bugs for T. cruzi infection and source of blood meals. The numbers we collected were unspectacular compared to those recorded for Rhodnius prolixus and T. infestans (several thousands per house) in other endemic areas but a study of infection rate, blood meal source, and localization yielded some interesting results.

Some 80 man-hours were spent in demolishing the first (and most interesting) house. It had been built 9 years before and housed a family of two adults and seven children, six of whom had Chagas' disease. A week after the family moved to their newly-built house, 20 m away from the old one, we received the information that the old house was available. The family chickens were dislodged from their usual roost (along the living room walls) only when we demolished the house and recovered 508 P. megistus of all stages and a large number of eggs and egg shells (est. 500 ± 100). A further 2 adults and 47 nymphs were seen but escaped among the rubble. A total of 398 bugs survived to be examined for T. cruzi infection and blood meals were identified from 296 (a further 27 squashes were negative).

Only three bugs were found in the entire palm-thatch roof; these were uninfected first-instar bugs and were found a few centimeters above a heavily infested bedroom wall.

Secondly, the bugs were located in two physically separate areas of the walls, with a distinctly different infection rate and feeding pattern among the bugs of each area, as shown in Figure 4.

There is thus strong evidence of two separate populations; one associated with the sleeping place of people infected with T. cruzi (six of nine family members were positive to xenodiagnosis) with more than 80 percent of bugs feeding on man. The infection rate among this population was 49 percent. A second bug population, with an infection rate of 10 percent was associated with the sleeping place of the chickens: 97 percent of feeds identified were avian and only 3 percent human. Since chickens are insusceptible to infection with T. cruzi, the low rate of infection in the chicken-feeding bugs suggests that there was little interchange between chicken-feeding and man-feeding groups of bugs. A

FIGURE 4. Focalization and feeding behavior of *P. megistus* encountered when a 9-year-old house at São Felipe was demolished. Six of the nine family members were infected with *T. cruzi*.

WALL SECTOR	NO. BUGS COLLECTED	INFECTION RATE	% FED ON MAN	% FED ON BIRD	% OTHER FEEDS
Outer walls	225	10%	3%	97%	-
Inner walls	280	49%	82%	15%	2% "mammal"
					< 1% avian/man
TOTAL	505 (*)	30%	40%	59%	< 1.5%

*excludes 3 uninfected 1st instar nymphs found in roof.

cat, infected with T. cruzi, lived with the family but no cat feeds were identified. The family had no dogs.

It is worthy of note at this point that the first signs of bug infestation of the newly-built house into which the family moved were observed 6 months after occupation, when two cast skins of fifth instar nymphs were seen, together with the bug feces on some wall surfaces. Since the life cycle of P. megistus normally takes about 1 year, the age of the two molted bugs is likely to have been greater than that of the new house itself, indicating that the bugs had been introduced into the new house from the old one with the family goods and chattels at the time of the move. Two years later the level of infestation in the "new" house seems already comparable to that in the original building.

A second house demolished was a special case: three people had lived in the house, all of whom had a positive IFAT and one wâs also positive to xenodiagnosis. The family owned two chickens, a dog and a cat (the cat positive on xenodiagnosis). During the period of normal occupation the infection rate of bugs caught in the house was 31 percent. Because of the growing dilapidation of the 25-year-old building the family ceased to sleep in the house, although they continued to cook their midday meal there. One chicken refused to abandon the house and roosted on the old kitchen fireplace. Monthly relays of one caged guinea pig and five caged mice were placed under the old beds for 2 1/2 months, (none became infected) and the house was finally demolished, 6 months after the family ceased to sleep there. Figure 5 shows that, when demolished, the infection rate among the 171 bugs collected had fallen to 10 percent, compared to the former level of 30 percent, largely because all bugs younger than the fourth instar were uninfected. In the absence of the sleeping people (who previously provided 87 percent of bug feeds in the house), the solitary chicken had become the chief source of food (84 percent of meals identified). About 7 percent of feeds were from the sentinel guinea pig, two bugs (both infected) had fed on cat and one (uninfected) on rodent--probably on the caged sentinel mice. About 7 percent of feeds were weak and could not be identified further than "mammal".

FIGURE 5. Changes in P. megistus infestation and infection rates in relation to hosts available and bug feeding pattern:

a) when a house was normally occupied and

b) when house demolished after 6 months unoccupied by family

	HOUSE OCCUPIED			HOUSE VACANT 6 MONTHS		
RESIDENT HOSTS AVAILABLE	3 people (xeno: $1^{+}/3$: IFAT $3^{+}/3$) 2 chickens 1 cat (xeno +ve) 1 dog			1 chicken 1 guinea pig 5 white mice	in cases: changed monthly	
P. megistus collected and % infected	149: 31% infected A.D. = 37 : DRF = 11.5)*			171 : 10% infected (at demolition)		
	SOURCE	% TOTAL	% INFECTED	SOURCE	% TOTAL	% INFECTED
Blood meals identified and infecttion rates per blood meal source	Human	87%	41%	Avian	84%	9%
	Avian	10%	18%	"Mammal"	7%	12.5%
	Dog	3.5%	50%	Guinea Pig	7%	11%
				Cat (2)	1.5%	100%
				Rodent (1)	< 1%	0

*A.D. - Apparent Density
DRF - Domestic Risk Factor

These results show that a substantial population of *P. megistus* survived for 6 months in the absence of man, largely supported by one chicken, although "abnormal" hosts (rodent and cat) were used to a greater extent than usual. No bugs fed on the bats and geckoes that were numerous in the walls and gable-ends. No bugs at all were found in the palm-thatch roof.

V. EGG PARASITISM OF *P. MEGISTUS* BY A MINUTE WASP

Telenomus fariai is a microhymenopteran parasite of the eggs of triatomine bugs. It is evidently widely distributed in South America (Venezuela to Argentina) but little is known of its detailed distribution and almost nothing is known of its biology in nature. It has, however, been extensively studied in the laboratory especially by Rabinovich in Venezuela. Eggs that have been parasitized by *Telenomus* or similar wasps, and empty shells from which wasps have emerged, have a characteristic appearance. Mr. T. V. Barrett has recently found that a surprisingly high proportion of *P. megistus* eggs in São Felipe are parasitized: wasps emerging from parasitized eggs have been identified as *Telonomus fariai*.

Figure 6 summarizes findings to date: the cumulative rate of parasitism among nearly 3,600 egg shells collected in 23 houses and chicken houses was a surprising 19 percent, with a range from 6 to 63 percent in 9 houses from which 100 or more shells were collected.

The time of development of the wasps in the bug eggs is about 21 days: from 7 to 12 wasps emerge from each egg, usually with one male (sometimes 2) and from 6 to 10 female wasps per egg.

The figures clearly demonstrate that the activities of these tiny wasps must reduce the potential numbers of *P. megistus* to a considerable extent; of the 300 or so eggs laid by a female *P. megistus* in her lifetime (Zeledon, 1974) about 60 may be lost to hymenopteran egg parasites.

FIGURE 6. Cumulative rates of parasitism of *P. megistus* eggs by *Telonomus fariai* in houses at São Felipe.

Number of shells collected (23 houses)	3,597
Number of shells with evidence of wasp parasitism	680
Mean cumulative rate of parasitism	18.9%
Range of cumulative rate of parasitism (in 9 houses from which 100 or more eggs collected)	6 - 63%
Number of female wasps per egg	6 - 11
Number of male wasps per egg	1 - 2
Time of development in bug egg	Approx. 21 days

VI. ANIMALS AND T. CRUZI

A. Domestic animals

Figure 7 shows the distribution of domestic animals among 31 bug-infested households: most have chickens, pigs, and cats, about half have a horse, donkey, or mule, a third have dogs but few families keep turkeys, cattle, or goats.

Dr. I. Sherlock (Núcleo de Pesquisas da Bahia) carried out a xenodiagnostic survey of 556 dogs in the São Felipe Project area in 1969 and found that 6.1 percent were infected. About 200 pigs and a cow examined were negative.

Cats and dogs. In the households that we studied 12 dogs were distributed among only 9 of 27 houses: one dog was positive to xenodiagnosis (8 percent). 22 of the 27 houses had one or more cats: 8 of the 23 examined (35 percent) were positive to xenodiagnosis. A small number of horses, cattle, goats, and pigs were examined by xenodiagnosis with negative results.

House mice. Of 28 houses in which small-mammal traps were set 15 yielded *Mus musculus*. A total of 20 mice of the 92 caught were found to be infected with *T. cruzi* (21.7 percent): 16 mice were parasitologically positive (fresh blood film and/or xenodiagnosis) of which all but 3 gave significant titers in the indirect fluorescent antibody test (IFAT). Four more mice were regarded as infected on the basis of a positive IFAT, in the absence of a microscopically detectable parasitemia and negative xenodiagnosis. Of 15 houses in which mice were trapped, infected mice were found in 8. Four or fewer mice were trapped in 10 houses. The ratio of infected mice to mice trapped in the other 5 houses was as follows: none of 8 (2 houses), 3 of 6, 4 of 12, and 8 of 33.

The results of the examination of domestic animals, including house mice is shown in Figure 8.

FIGURE 7. Distribution of domestic animals among 31 households at São Felipe.

NUMBER OF 31 FAMILIES WITH:	chickens	turkeys	horses, mules or donkeys	cattle	goat	pigs	dogs	cats
	29	2	16	3	1	25	11	23
TOTAL ANIMALS	± 250	2	19	3	1	37	19	26

FIGURE 8. Mammals and *T. cruzi* infection in São Felipe.

AMBIT	ANIMAL	EVIDENCE OF *T. CRUZI* INFECTION
Domestic	Dog	8% ($1^+/12$) - parasit. + IFAT
	Cat	35% ($8^+/23$) - parasit. + IFAT
	Mouse	22% ($20^+/92$) - parasit. + IFAT - 16 IFAT only - 4
Peridomestic and Sylvatic	Rat	Rural: < 300 m infested houses: 23% ($2^+/13$) Urban: uninfested premises: NIL ($0^+/10$)
	Opossum (*Didelphis azarae*)	Rural: < 300 m infested houses: 30% ($11^+/37$) Rural: uninhabited areas or not associated infested houses: 17% ($4^+/23$) Urban: uninfested premises: NIL ($0^+/4$)

B. Peridomestic and sylvatic animals

Figure 8 also shows the rates of T. cruzi infection among opossums (Didelphis azarae) and among Rattus r. frugivorus in the São Felipe area. Both animals are often trapped near houses and certainly visit them to forage for food at night when bugs are active.

Although numbers of animals examined are small, there is a suggestive difference in the proportion infected among those trapped in urban areas (essentially free of P. megistus) and rural areas within 300 m or less of infested houses. Opossums caught in rural areas remote from infested houses show an intermediate level of infection.

Other marsupials and rodents were trapped near houses with a much lower frequency: these included a semiaquatic opossum (Lutreolina crassicaudata; none of the 7 examined were infected), a carnivorous marsupial (Marmosa cinerea cinerea; none of the 7 examined were infected, and a semi-aquatic rat (Nectomys squamipes aquaticus; none of the 7 examined were infected. A variety of other small mammals were caught in small numbers but none were infected with T. cruzi.

Two species of armadillo (Dasypus septemcinctus and D. novemcinctus) occur in the area but are nowadays uncommon because of extensive hunting. One adult seven-banded armadillo and three juvenile nine-banded armadillos were examined. All were uninfected.

Didelphis azarae and Rattus r. frugivorus are thus the only animal hosts of T. cruzi recognized to date from the São Felipe area.

C. The course of T. cruzi infection in peridomestic animals from São Felipe

The course of natural and induced T. cruzi infections were followed in the laboratory in captive opossums (D. azarae) and Rattus r. frugivorus. Natural infections were invariably chronic, mild, and of long duration with parasitemia generally subpatent by microscopy but positive to xenodiagnosis. Histopathologic changes seen in post-morten material were slight or absent, and no amastigote tissue parasites were seen. Captive opossums and a seven-banded armadillo, when infected with newly isolated human or opossum strains, followed a similar chronic course, without a noticeable initial acute phase. All induced infections thus followed the pattern seen in natural infections of the opossum, D. azarae.

D. Serology of wild animal infections

Filter-paper samples of blood from all wild and domestic animals were collected, dried, and stored. Whole sera were collected and stored at -20°C where possible.

For ease of transport only filter-paper specimens have been subjected to serologic examination in several collaborating laboratories; most specimens have been sent to Dr. C. C. Draper (Ross Institute, London School of Hygiene and Tropical Medicine) for testing by IFAT, direct agglutination and indirect hemagglutination (IHA). Dr. Irving Kagan (Center for Disease Control, Atlanta, Georgia) kindly tested material by direct agglutination, IHA, and Hyland counter-current electrophoresis. Dr. Mario Camargo (São Paulo, Brazil) tested papers by direct agglutination techniques.

With dogs, cats, and rodents IFAT tests gave results that were in general consistent with parasitologic findings. With rodents, direct and indirect agglutination tests also gave results consistent with parasitologic findings. No satisfactory serologic test has yet been found for filter-paper specimens from opossums; immunofluorescence, direct and indirect agglutination techniques, complement fixation, neutralization tests, and counter-current electrophoresis were all tried unsuccessfully. The latex agglutination test (Behringwerke ORIM 09 antigen) gave a high incidence of both false-positive and false-negative results with both opossums and rodents. Skin-tests with a freeze-dried T. cruzi culture antigen were unsuccessful in opossums.

The absence of a satisfactory serologic screening test for infections in wild animals continues to be a hindrance to which no solution is currently in sight. It is to be hoped that immunologists will take up the challenge.

E. T. cruzi and the domestic fowl

The epidemiologic role of the uninfectable chicken remains an enigma; the birds can support largely separate household bug populations in infested houses, but with an infection rate among the bugs very much less than that found among man-feeding bugs encountered elsewhere in the same house. It is not yet clear whether the presence of chickens in houses is beneficial (predation; reduced bug infection rate) or the reverse (support larger total bug populations).

VII. COMMENTS ON T. CRUZI TRANSMISSION IN THE SÃO FELIPE AREA

Investigations to date have shown some unexpected facets of the domestic/peridomestic cycle of transmission, considered later, and that there is at least one extra domestic sylvan cycle that evidently does not involve the domestic vector, P. megistus at al..

Though information on the feral cycle is still fragmentary, it is evident that two species (R. domesticus and T. tibiamaculata) of sylvatic triatomine bugs are in some way involved. Both species appear to occupy an arboreal environment linked to the presence of bromeliad epiphytes on large trees and to various mammals that nest in the same habitat.

R. domesticus has been encountered only once: three bugs (one infected) were found in the nest of an apparently uninfected opossum (D. azarae) in a bromeliad on a tree that, with a number of others, formed a small shady patch of woodland within 100 m of a small uninfested farmhouse. An extensive area of dense secondary forest began a few hundred meters away but searches of epiphytic bromeliads in the forest have so far failed to reveal the presence of further R. domesticus.

Three separate groups of T. tibiamaculata have been found so far, totaling 17 individuals of which 12 were infected, in a habitat generally resembling that in which the R. domesticus were found. T. tibiamaculata were found in or near animal nests in bromeliad epiphytes, on the edge of a secondary forest and in a small patch of woodland that included a number of large jack fruit trees and was close to houses, including one infested with P. megistus less than 50 m away, and close to areas of cultivation.

Of a few arboreal animals so far caught from nests infested by T. tibiamaculata, none were found infected with T. cruzi. The animals examined were three Cercomys (arboreal spiny rats), two rodent-like marsupials (Marmosa cinerea) and three rodents (not yet identified). Thus, the arboreal vertebrate to and/or from which T. tibiamaculata carries T. cruzi is still unknown.

Further work may reveal an arboreal vertebrate host that nests in bromeliad tree epiphytes, but whose habits bring it to the ground either near houses or in areas of cultivation that are close to houses. Didelphis azarae and R. r. frugivorus are two possible links between the sylvatic cycle (or cycles) and the domestic scene where P. megistus is the only vector. Identification of the blood meals from R. domesticus and T. tibiamaculata, once sufficient wild-fed bugs can be found and collected, may be a key to the enigma of whether domestic and nondomestic transmission cycles are interconnected or not; and, if they are, the direction of the association.

The role of extradomiciliary T. cruzi transmission among wild animals remains to be determined, but it seems probable that its impact on the domestic cycle, is unlikely to be very great, once the household cycle is initiated in the first place, by the introduction of bugs and infected people or animals.

Blood meal analysis of P. megistus from São Felipe shows very clearly the intensity of bug-man-bug transmission in the houses: 81 percent of feeds are from man. From other susceptible domestic and peridomestic vertebrates (dogs, cats, house mice, rats, opossums, etc.) P. megistus takes only 6 percent of its feeds, of which only those on dog seem adequate to provide any T. cruzi feedback at all into the bug population. Figure 9 shows the feeding behavior of P. megistus in relation to infection rates in man and animals in São Felipe. Feeds on cats, mice, and rats are so uncommon that these animals may have little or no importance in returning

FIGURE 9. Man, mammals, birds and T. cruzi infection in relation to the feeding behavior of domiciliated Panstrongylus in São Felipe.

% P. megistus feeds	HOST	% infected with T. cruzi
		(*), (**)
81%	Man	27 - 53%
2.5%	Dog	8%
< 0.1%	Cat	35%
< 1.9%	Opossum	23%
< 0.1%	Mouse	22%
	Rat	23%
13%	Avian	NIL (insusceptible)

(*) based on single zenodiagnosis, 213 people.
(**) based on IFAT results, same group of 213.

T. cruzi to new individual bug vectors for onward transmission to further new hosts. T. cruzi infections in cats, mice, and rats are, however, high, as are those in the opossums which, though extradomestic, frequently are caught near houses and certainly visit them for food occasionally or regularly.

Many workers in South America and elsewhere report that dogs, cats, rats, mice, and opossums will eat bugs readily and hence become infected with T. cruzi by the oral route. Carnivorous animals (i.e., cats and opossums in São Felipe) can similarly acquire infection by eating infected rodents. There is convincing circumstantial evidence, from the study of P. megistus blood meals, that either or both of these oral mechanisms of infection are of major importance in the transfer of infection from domestic P. megistus to rodents, cats, and probably opossums. Only in the case of the dog may direct contaminative vector-borne transmission be important.

Mice, rats, cats, and opossums are all active at night, when bugs are also active and easily available as food. Dogs are largely diurnal and relatively inactive at night, when bugs are seeking to feed, so feeding contact by bugs is more easily achieved. But many dogs are shut out of the house at night as watchdogs and so are unavailable to the household bugs for much of the night; these factors may in part account for the lower rate of T. cruzi infection found among dogs in São Felipe than in cats, rodents, and opossums, upon which P. megistus evidently so rarely are able to feed.

Thirteen percent of P. megistus feeds are from the insusceptible chicken which, if roosting in houses, may support a separate bug population with a lower rate of T. cruzi infection. It may be noted that bugs infected previously from mammalian hosts do not lose their T. cruzi infection as a result of feeding upon chickens later in their lives. Populations of chicken-feeding P. megistus living in separate outside chicken houses at São Felipe have been found to be uninfected.

VIII. REGIONAL DIFFERENCE IN THE BEHAVIOR OF PANSTRONGYLUS MEGISTUS

P. megistus in different areas of its extensive range in Brazil has different habits: in southern areas it is primarily a sylvatic species (living mainly in tree holes and animal burrows; less frequently in palm trees and in epiphytic bromeliads), but is nonetheless often found in houses and farm buildings. Further north the species becomes entirely domiciliated and is not found at all in sylvatic habitats; it may be that these differences are of genetic origin rather than purely behavioral.

It is nonetheless instructive to compare the feeding behavior of P. megistus in different areas of Brazil from which feeds have been identified by precipitin tests in comparable numbers. Such a comparison is made in Figure 10, in which the São Felipe data are shown alongside figures for P. megistus collected in houses and farm buildings in the south of São Paulo state, based on the figures of Barretto, 1968. Feeds from bird, rodent, opossum, and pig are prominent; 5 percent of feeds were from dog but only 3 percent were from human origin. Cat feeds are infrequent at 1 percent. The figures suggest that opossums, rodents, and perhaps dogs are significant as reservoir hosts, but, as in São Felipe, cats are of little significance in the transmission cycle of T. cruzi maintained by P. megistus. (It must be noted, however, that in the same area Triatoma infestans is the major domestic vector; with 30 percent of feeds from man, 19 percent from dogs, and 10 percent from cats).

Feeds identified from sylvatic P. megistus collected in natural habitats in the northeast of São Paulo state and in the south of Mato Grosso state are shown in the lower part of Figure 10, the data derived from the work of Barretto, 1968, 1971. Predominant hosts for sylvatic P. megistus in these two areas are opossums, rodents, and birds. The average infection rate among sylvatic P. megistus in São Paulo state is as high as that for the entirely domiciliary populations of São Felipe, where man replaces opossum and rodents as the susceptible host most frequently fed upon by the bugs.

FIGURE 10. *P. megistus* feeding habits in different areas of Brazil

	NORTH BRAZIL (São Felipe) in houses	% feeds	BLOOD MEAL SOURCE	% feeds	SOUTH BRAZIL (NE of São Paul State)(*) in houses and farm buildings
DOMICILIATED BUGS	35.5% of 1,373 bugs infected	81%	Man	3.2%	21% of 804 bugs infected
		13%	Avian	44%	
		2.5%	Dog	5%	
		< 0.1%	Cat	1%	
		< 0.1%	Rodent	14%	
		< 2.0%	Opossum	14%	
		< 0.1%	Pig	10%	
		-	Cow	0.6%	
		-	Armadillo	0.1%	
		1.9% (4 combinations)	Mixed feeds	4.7% (10 combinations)	
	NE of São Paulo State (*)				S. of Mato Grosso State (*)
SYLVATIC BUGS	36.1% of 690 bugs infected	45%	Opossum	69%	9.1% of 175 bugs infected
		22%	Rodent	10%	
		22%	Avian	18%	
		0.7%	Man	-	
		0.2%	Dog	-	
		0.2%	Cat	-	
		1.4%	Armadillo	1.1%	
		3.5%	Bat	2.3%	
		5.2% (5 combinations)	Mixed feeds	0.6%	

(*) data derived from Barretto 1968, 1971)

It is noteworthy that cats are so rarely fed upon by P. megistus in either the northern or southern parts of its extensive range. In areas where household T. cruzi transmissions involves P. megistus as the main or only domestic vector, then the cat clearly plays a much lesser epidemiologic role than is traditionally assumed.

IX. CONCLUDING REMARKS

From the foregoing account it is clear that there are still gaps in our knowledge of the basic household ecology of Chagas' disease in São Felipe and the existence of a sylvan cycle (or cycles), found relatively recently, poses further problems that largely remain to be solved in the future.

However, the studies carried out since 1971 have shown that the application of simple, but comparative methods on a wide biologic front, such as the analysis of bug blood meals in relation to infection rates of bugs and mammals, the careful demolition of infested houses, and the like, can reveal features of importance in the epidemiology of Chagas' disease that might otherwise have been overlooked or misinterpreted if only one or a few aspects had been investigated in isolation.

The methodologies used in the course of the work described, applied in other areas, could add greatly to the present body of knowledge on the epidemiology of Chagas' disease and perhaps suggest, eventually, new methods of prevention and control.

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