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DOMICILIARY REDUVIID BUGS AND THE EPIDEMIOLOGY OF CHAGAS' DISEASE IN PANAMA[1]

(HEMIPTERA: REDUVIIDAE: TRIATOMINAE)

By **Alan C. Pipkin Sr.**[2]

Abstract: A continuing field survey of domiciliary reduviid bugs in Central Panama over a 3-year period indicates that *Rhodnius pallescens* (Barber 1932) is overwhelmingly predominant over other hematophagous Reduviidae frequenting native houses there. *Triatoma dimidiata* (Neiva 1914) mentioned prominently in the literature as an important vector of Chagas' disease in Panama, while occurring next in frequency in the house collections, was comparatively rare. Other species were taken so uncommonly that they can be considered as only occasional visitors or accidental invaders in houses. An average of 36.58% of 3283 bugs (virtually all *R. pallescens*) taken in house collections contained trypanosomes. 32.71% of the total containing *Trypanosoma cruzi* (Chagas 1909) in the feces, while from 4.2–8.1% contained the non-pathogenic hemoflagellate, *Trypanosoma rangeli* (Tejera 1921). The infection rate of the 2 sexes of adult bugs with *T. cruzi* was almost equal, or about 60% in both males and females, while about only 20% of the younger stages were so infected. In an effort to evaluate the potential role of the links in the transmission chain from the infection pool in nature to domesticated bugs living close to the human host, an exploration of peri-domestic and sylvatic ecologic niches was made. *R. pallescens* was found most frequently in chicken houses and pig pens, although only bugs from the former location contained trypanosomes. Out of a dozen strictly sylvatic sites explored, bugs were found only in trees harboring either animals or animal nests. Opossum nests were the most fruitful, yielding 28 bugs from 6 nests, of which 3 were positive for trypanosomes. Experimental laboratory studies on the defecation time after feeding indicated that *R. pallescens*, as compared to *R. prolixus* (Stål 1859) is a tardy defecator. The speculation is made that this delayed defecation may partially account for the apparent inefficiency of *R. pallescens* as a vector of *T. cruzi* in Panama. A precursory survey of small terrestrial and arboreal wild animals taken from forests near the native houses revealed that of 209 animals of 8 different species, 41 of 128 opossums examined, or 32.03%, were infected with *T. cruzi*. None of 43 armadillos examined harbored trypanosomes. Marmosets, anteaters, coatis and sloths were found occasionally infected, but the opossum seems to be the most likely wild vertebrate reservoir.

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The first 3 cases of human trypanosomiasis in Panama were reported by Miller in 1931. Three years later, Johnson & Di Rivas (1933) indicated that the total number of cases in the literature had increased to 19. Clark (1942) mentioned 2 additional cases, bringing the total to 21. Recently, Castro (1955) in reviewing the cases of Chagas'

disease seen at Santo Tomás Hospital in Panama City, described 5 cases not previously mentioned by others, thus bringing the total to 26. Actually, these cases, diagnosed primarily by direct demonstration of the parasite or by clinical symptoms, or both, represent but a small fraction of those occurring in Panama during this period. Johnson & Kelser (1935) reported 48 cases known to be positive for *Trypanosoma cruzi* (Chagas 1909) by complement fixation tests at the Gorgas Memorial Laboratory in Panama. Furthermore, in the current Annual Report (1966) of this institution, mention is made of a series of some 2912 complement fixation tests made on sera from several sources, in which 4.4% (128 cases) were likewise positive. So, in the 34 years since Miller's original report, some 192 human infections, diagnosed either by clinical symptoms, direct demonstration of the parasite in blood films, or by indirect serological means, have been reported in the literature from Panama. It is therefore apparent that although the incidence of human cases is not as high as has been reported from neighboring countries (Pifano 1960 reported, for instance, that 1876 cases have occurred in Venezuela in the preceeding 23 years), it reflects, in Panama, a public health problem of appreciable importance.

The epidemiology of this infection in Panama is but poorly understood, although there have been several reports of natural infection of reduviid bugs with *T. cruzi* in the literature. In 1932, Clark & Dunn published a very careful study, including the finding of "Triatoma" (*Panstrongylus*) *geniculatus* (Pinto 1931) naturally infected with *T. cruzi*. Then, Dunn (1933) discovered *Rhodnius pallescens* (Barber 1929) similarly infected in nature. Shortly thereafter, Rozeboom (1936) found naturally infected specimens of *Triatoma dimidiata* (Neiva 1914) in a local house. Dunn (1934) also reported a natural infection with this same parasite in *Eratyrus cuspidatus* (Stål 1859). Thus, in the short span of less than 3 years following the initial discovery of human cases of American trypanosomiasis in Panama, 4 species of Triatominae were shown to be potential vectors of the disease in nature.

Although these reports established the potential role of the reduviid bugs mentioned here in the transmission of Chagas' disease in Panama, there had been, up until 1960, no organized attempt to actually evaluate the opportunity which these different species have to infect man. There was also virtually nothing known concerning the incidence of natural infection of these bugs with *T. cruzi* and its related species, *Trypanosoma rangeli* (Tejera 1921).

An understanding of the epidemiology of Chagas' disease is based on the elucidation of a number of contributory factors which influence the insect vector-flagellate parasite-vertebrate host complex and its operation in the transmission cycle. Included is such information as: (A) The degree of intimacy of the reduviid species with the human host, (B) The infection rate of the insect vector with *T. cruzi* and related hemoflagellates with which it might be mistaken, and (C) A thorough understanding of the biology of the 3 members of the vector-parasite-host complex, including such ancillary information as: (a) Proof of ability of the suspected insect vector to transmit the infection, at least experimentally, (b) Relative efficiency as a vector, (c) Distribution of the insect vector in nature, and (d) Its relationship with the wild animal population which might serve as a sylvatic pool of infection in nature.

The elucidation of these various contributory factors to the epidemiology of Chagas' disease in Panama was, in fact, the principal goal of the project as a whole.

MATERIALS AND METHODS

(A) *Ecology Of The Survey Areas*

1. *Geography.* Over a 3-year period (1961–64) field collections of reduviid bugs were made from selected locations in Central Panama, and from other more distant rural communities in Eastern and Western Panama. Because of its relatively under-developed state, the Republic of Panama is sparsely populated except for districts adjacent to the Panama Canal and certain districts in the western half of the country which lend themselves to agriculture. Eastern Panama, virtually devoid of roads, is almost entirely unsettled except for a few small towns associated with the lumbering industry and several indian villages located on rivers in this region. Because of the problems of transportation, only spot checks were possible in these outlying areas.

The bulk of the collections reported here were made in continuing surveys over the 3-year period from rural communities of the east-central section of Panama within a 48 km radius just west of the Canal Zone, (FIG. 1) in the Cocle, Panama and Colon Provinces. Attention to these areas was suggested by previous clinical and serological surveys done by Dr Carl Johnson and his associates at the Gorgas Memorial Laboratory during the 1930's and early '40's...areas which were known to harbor cases of Chagas' disease. The Santa

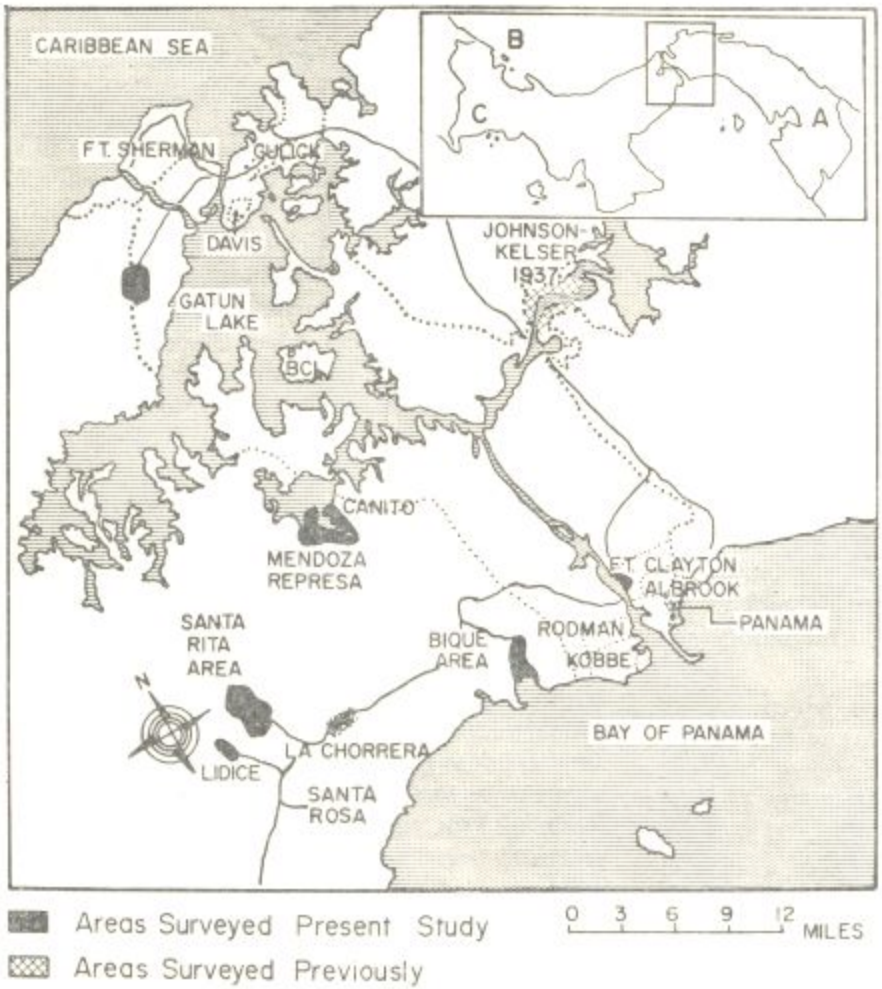

FIG. 1. Panama Canal Zone. The insert shows the Republic of Panama with the outlying areas that were visited indicated.

Rita area, where a majority of the bugs was taken, is a very hilly rural community lying some 32 km west of the Canal Zone in the Cocle Province. It is about 11 km northwest of the little market town of Chorerra, the economic hub of the surrounding area, which is supported largely by marginal agriculture. It has an efficient public health dispensary whose field sanitation staff cooperated generously in the field surveys for reduviid bugs.

Lidice, Santa Rita and Santa Rosa are all in the same general area, (FIG. 1) but are separated by 8–13 km of very hilly terrain lying some 230–300 m above sea level in the foothills of Cerro Campana, which rises to some 730 m. Lidice and Santa Rita are on hard top roads, while Santa Rosa, a mere hamlet of a few houses, is on a dirt road. Mendoza and Represa are located about 16–19 km to the southeast on the backwaters of Gatun Lake, at about 27 m above sea level. Bique is almost coastal, being situated within 1.5 km of the Pacific shores and almost adjacent to the Canal Zone to the southeast. All these communities lie on the Pacific slope of the Isthmus of Panama within a radius of about 32 km of each other. All of them are accessible by road during the dry season, but some only with difficulty by road during the rainy months. Mendoza and Represa, which are served only by dirt trails, can be reached more easily by motor launch after the rains set in.

The more distant communities in the outlying areas (A), (B) and (C) (FIG. 1, insert) of the interior of Panama can be visited by small commercial air transports which operate on a bi-weekly schedule. The Chiriqui Province (C) is on the so-called Inter-American Highway which is still under construction, but usable. El Volcan, the community visited in that area, lies some 600 km west of the Canal Zone and about 80 km interior at an altitude of about 730 m. The Almirante district of the Bocas del Toro Province (B) is about the same distance west of the Canal Zone but is situated on the opposite or Atlantic side of the Isthmus and is low and frankly tropical. El Real (A) in the Darien Province of Eastern Panama, while not coastal, is

located only a short distance inland on the Tuira-Chucanaque River, which is navigable that far from the deeply indented San Miguel Bay. The surrounding countryside is fairly low, and is covered with timber which supports a considerable lumbering industry.

2. *Panamanian communities, their people and houses.* Throughout Panama towns are few and relatively small, most of them having less than 500 people. There are, however, many isolated hamlets or small community centers scattered throughout the country. Associated with such rural centers may be a public school and/or a church and a few country stores. Around them is usually a handful of very modest private dwellings occupied by the staff of the school and owners of the little businesses or perhaps 1 or 2 more affluent owners of nearby farm property. This may be deceiving in that such villages usually serve a considerable rural community back in the hills, for most of the people live on the land which supports them.

Except for the Chiriqui region which has an appreciable number of immigrants of foreign extraction (American, German, Japanese, Chinese, etc.), the interior of Panama is largely peopled by Spanish and Indian-Spanish stock. There are few cash crops other than a little rice, corn and citrus fruit, and some small truck farming. A typical Panamanian hill-farmer in Central Panama lives in a crude 2 or 3-room mud hut with a thatch roof. Most of them are constructed entirely out of materials available at the site, and have only dirt floors, though an occasional house may boast of a wooden deck in 1 or 2 rooms. The windows are often mere openings in the mud walls and only rarely in the homes of the more affluent they may be fitted with rough window frames and wooden shutters, but these are seldom screened. The small farms or "fincas" are actually just clearings in the jungle and are reached by dirt foot trails that become muddy quagmires in the rainy season.

(B) *Collection Techniques Employed in Field Studies*

1. *Domiciliary bugs and their intimacy with man.* Because of the nocturnal feeding habits of reduviid bugs, it is generally agreed that those species which frequent human dwellings, particularly at night when the inhabitants are asleep and quiescent, have the best opportunity to transmit Chagas' disease to man, other things (infection rate, vector efficiency, etc.) being equal. Those species which are living and breeding in close proximity to man, either in his domicile or outside of it but near enough to invite invasion of his living quarters, can more surely be assumed to play an appreciable role in the transmission of the infection to man than do those species which are found to be only occasional visitors. For this reason, primary emphasis in the presently reported survey was given to night collections of reduviid bugs from inside the native huts in an attempt to estimate the degree of intimacy existing between the human population and the different species of reduviid bugs known to occur in Panama. Study of other factors affecting the epidemiology, other than the infection rate of the bugs with hemoflagellates, was undertaken secondarily.

2. *Domiciliary collection methods.* Bug collecting in Panama was particularly trying at times since the density of domiciliary bugs there, as compared to that in neighboring countries, seemed definitely lower. Collections made by this writer in El Salvador yielded several dozen *Rhodnius prolixus* (Stål 1859) from a single house in mid afternoon within an hour, while yields of *R. pallescens* from Panamanian houses with a similar investment of time and effort, day or night, was usually a quarter as great, or sometimes less.

Except for continued and repeated careful searching of likely hiding places, field survey methods consisted of little else than the use of a few basic collection techniques and accessories. Domiciliary collections were made by hand, chiefly at night, since attempts at day collecting were relatively unproductive. Hand flashlights or gasoline lanterns were useful for exploring dark corners of the native huts, although it was surprising how adept the natives were at spotting bugs even in semi-darkness. Use of pyrethrum dusts or aerosol insecticidal "bombs" were resorted to in an effort to flush the bugs from their hiding places in the cracks of the mud walls and from the thatched roof. However, this approach was not very successful with the species of bugs concerned. Most of the bugs were, in fact, captured when they emerged voluntarily from their hiding places either to obtain a blood meal or because they were apparently disturbed by the light reflected into their harborage.

Once caught, the bugs were held in small tin ointment boxes well ventilated by holes punched in top and bottom. The boxes bore labels with space for notation of collection date, collection area, immediate source and the collectors' name. After the initial surveys in a given area, a good proportion of the follow-up collections were made with the assistance of selected individuals in the native

communities. To gain the advantage of a continuing effort, a small bounty was sometimes offered through community representatives, often the native pastor or school teacher or a resident public health worker. This approach insured the enthusiastic cooperation of children and adults alike. Collections were picked up at a pre-set time, usually within a week or less of the previous collection. On occasion, however, because of difficulties of obtaining transportation or due to local weather conditions or the distances involved, the pick-up was unavoidably delayed for a day or more. In the drier months some desiccation of the specimens occurred if the pick-up was late. In such cases there was some mortality among the specimens confined for more than 4 or 5 days, especially if they were not well-blooded which was sometimes the case with the nymphs. To alleviate drying, moist filter paper linings were placed in the boxes. With such precautions, and a word of warning to the collectors to keep the boxes out of the direct sunlight, the loss was considerably less. Life expectancy of the bugs so held was, in fact, surprisingly good. Adults and well-blooded nymphs were usually found in fair to good shape after 4–5 days' confinement during the cooler wet months. Coming in from the field, collection boxes were sometimes stored in a foam plastic refrigerator box with prefrozen canned liquid ice-substitute or CO_2 ice if they were to be hauled for more than a few hours. On reaching the laboratory, the holding tins were opened, their contents recorded and the individual bugs checked as soon as possible for natural infection with hemoflagellates. Viable trypanosomes were often recoverable from bugs that had obviously been dead for several days, as reported previously by Wood (1942, 1944) and de Lucena (1957).

3. *Peri-domiciliary and sylvatic collections.* Collections from ecological niches outside of but closely associated with the houses were undertaken to obtain an estimate of the role which such bug populations might play in the transmission cycle between domiciliary and sylvatic pools of flagellate infection. The methodology was similar to that employed for house collections but applied to ecological niches less intimately associated with the human host. One particularly useful accessory employed in sifting the debris from chicken and bird and animal nests was a large square of yellow oil cloth. When the contents of the nests were spread out on such a contrasting colored background, the moving larvae, nymphs and adults of *R. pallescens* were easily spotted.

(C) *Laboratory Methods for Demonstration of Flagellate Infections*

1. *Infection of bugs with T. cruzi.* Determination of incidence of infection of bugs with *T. cruzi* was routinely accomplished by direct microscopic examination of the bug feces, inoculation of the bug feces into conventional blood agar (NNN) cultures and/or laboratory animals, or both. The bug feces were obtained through 1 or more of 3 approaches: (a) collection of the feces from the walls and bottom of shell vials used as holding containers for the insects, (b) dissection of the posterior alimentary tract of the bugs, and (c) through artificially inducing the bugs to defecate by the application of gentle dorso-ventral pressure on the posterior abdomen. The first of these techniques was used considerably in making defecation timing studies on the bugs. The second approach, use of dissections, was not only the most laborious, but it entailed the sacrifice of the insect specimen. Where there was no reason for preserving the specimen for future study, however, it offers an obvious and fairly easy method of determining presence of flagellate infection, although it is not infallible. In practice, the posterior tip of the abdomen of the bug specimen was clipped off with a pair of scissors, transferred to a drop of saline on a slide and the rectum teased out under a stereoscope. Sounding more difficult than it really is, such a dissection can be accomplished very rapidly with a little practice. The third approach, forced defecation, was considered a somewhat risky procedure at first, but it was later resorted to extensively when experience showed that with due care, there was no apparent deleterious effect on the insects. It was the simplest, perhaps the easiest, and certainly the quickest approach. It should be stressed that examination of bug feces for flagellate infection should be combined with other techniques such as culture methods and animal inoculation. Guedes (1952) found that fecal examination alone revealed about 47% of the flagellate infections in bugs.

Examination of the bug feces for trypanosomes was made first under low magnification (10×) by ordinary transmitted light microscopy. When the characteristic motion of the flagellates was spotted, the microscope objective was switched to high dry magnification (43×) and the light was reduced for observation and identification. Phase contrast microscopy was available for closer observation and for still photography and microcinematography.

2. *Infection of bugs with T. rangeli.* As the survey

progressed, it became apparent in examining the bug feces, that some of the bugs were infected with another somewhat larger polymorphic trypanosome which was identified as *Trypanosoma rangeli*. At the suggestion of Dr R. Zeledon, of the University of Costa Rica, who has done considerable work on this nonpathogenic flagellate parasite of man, a concerted search was made for this species in the reduviid bugs. On his recommendation, examination of the hemocoele fluid from each specimen was carried out according to the following procedure: Using a very fine (22–24 gauge) hypodermic needle fitted to a tuberculin or .25 cc syringe, a few drops of coelomic fluid were withdrawn from the basal (coxal) joint of 1 leg. Examined carefully under high-dry magnification with subdued light, the characteristic long, thin polymorphic crithidia of *T. rangeli* were usually discernible with little searching if they were present.

(D) *Methods for Studying the Biology of the Vector-Parasite-host Complex*

1. *Proof of experimental Infectability of reduviid with T. cruzi.* To establish definitely the potential ability of a reduviid bug to transmit *T. cruzi*, it must be shown that it can (a) acquire the infection by feeding on an animal known to be infected with *T. cruzi*, (b) have the ability to nurture the flagellate infection and support the production of the infectious metacyclic forms in the rectum and pass them in an infectious form in its feces, and (c) achieve actual transmission of the flagellate infection to a known susceptible laboratory animal.

Experiments for testing the experimental infectability of reduviids with *T. cruzi*, and experiments to test actually the ability of the bugs to transmit the infection to clean experimental animals are fundamentally similar in technical approach, differing only in that the former makes use of clean bugs and previously infected mice, while the latter employs clean mice and infected bugs. For the infectability experiments, groups of 6–8 C3H agouty Swiss mice (a strain known to be particularly susceptible to *T. cruzi*), which had been infected previously intraperitoneally with the "Brasil" strain of *T. cruzi*, were individually immobilized in mouse restraining cylinders made of 6.3 mm mesh hardware screen. Each mouse was placed in a suitable wide mouth jar with 4–6 clean nymphs or adults of *R. pallescens* reared in our laboratory and known to be free from flagellate infection. After the mouths of the jars were sealed with cloth netting, they were placed for about 2 hr in subdued light in the temperature-humidity controlled incubator housing the bug colonies. If properly starved beforehand, the bugs usually fed within half an hour or so; however, some nymphs, if previously fed since the last molt, often refused to eat, although most of the adults fed consistently. After about an hour of exposure to the mice, the bugs were examined and those seen to have fed (as evidenced by their bloated abdomens) were isolated in holding jars for further long-term observation over a 6–8-week period. Evidence of flagellate infection was determined by direct microscopic examination of the bug feces.

An important corollary experiment was that of infectability with *local Panamanian* strains of *T. cruzi*. This was carried out in identically the same manner as described for the "Brasil" strain, except for the substitution of Panamanian strains of human origin or strains isolated from the domiciliary bugs.

2. *Proof of ability to achieve actual transmission was* carried out in a similar manner, using known infected *R. pallescens* and clean C3H mice. Again, it was important to use starved bugs to stimulate the bugs to feed, and later, defecate. Defecation usually followed feeding anywhere from a few minutes to as long as an hour in some cases, and the bugs routinely were left in contact with the clean mice for at least an hour.

3. *Relative efficiency of R. pallescens as a vector of T. cruzi.* The relative efficiency of *R. pallescens* as a transmitter of *T. cruzi* is influenced by a variety of factors, some more subtle than others. One of the most important of these is that of defecation time, or the time elapsing following feeding before defecation usually occurs (Wood 1951, 1960; Dias 1956). In contaminative transmission via the posterior route, now generally accepted as the usual method of transmission of *T. cruzi*, this particular biological characteristic is of great practical importance, determining in fact, whether or not transmission will occur. Furthermore, all evidence suggests that it is fairly constant for a given species of reduviid bug.

The determination of defecation time is arrived at through direct observations based on timed experiments on individual bugs. To lend validity to such observations, they are made best on groups of bugs held individually in numbered shell vials for at least an hour after being fed. The holding vials were kept in a tray for convenience of handling, and observations were made at intervals of a minute for evidence of fecal deposits on sides and bottom of the vials. As soon as a given bug was seen to defecate, it was removed from the series and

the time recorded. The average of the resulting defecation times for lots of about 20 bugs gave a fair estimate for a given species. Since species other than *R. pallescens* were extremely rare in our collections, comparisons with other Panamanian species were virtually limited to *T. dimidiata* and even these observations were admittedly meager. More meaningful comparisons were made with *R. prolixus* from our laboratory colonies.

4. *Natural infection of animal reservoirs with T. cruzi.* Isolation of strains of trypanosomes from wild animal reservoirs involved collection of blood samples, usually by heart puncture. The animals, for the most part small terrestrial and arboreal species known to be relatively common in the communities at night, were collected by native trappers on a contract basis and brought in from the field alive in crude cages. The animals were then transferred to larger holding cases and kept for a few days until it was convenient to draw blood specimens on a number of them at one time. This was accomplished most efficiently with the animal anesthetized lightly with chloroform or ether, or in the case of larger animals, with sodium pentothol or a barbiturate. Following application of a topical antiseptic (iodine or cresol) to the chest wall, the cardiac puncture was made with a large (3.1 cm, 18–20 ga.) needle. To avoid clotting of the blood, the syringe (20 cc disposable) was usually flushed thoroughly with an anticoagulant (0.25% heparin or oxalate). The blood was aseptically transferred to centrifuge tubes (rubber stoppered) and the sample concentrated after the technique of Yeager (1960). The sediment was then drawn off, planted in NNN slants or inoculated intraperitoneally into C3H mice, or both.

RESULTS AND DISCUSSION

(A) *Relative Intimacy of Reduviid Bugs with Man in Panama*

1. *Domiciliary collections.* In our search for reduviid bugs in the native houses, it soon became apparent that certain hiding places of *R. pallescens* were more fruitful than others. Furthermore, these places were all peculiarly and intimately associated in some way with the human inhabitants of the houses. They included: *a.* Cracks in the wall *near beds*. *b.* Crevices and folds of *bed clothes*, beneath *sleeping mats*, and in crevices in the *bedstead*. *c.* Behind *clothes* hanging on nails or pegs in the wall. *d.* In the thatch of the roof immediately over the *sleeping platforms* in the loft.

All these places are associated either with *close contact with the human body itself*, or with *clothing* or *bedding* which has been in close contact with the human body. It was assumed, therefore, that the attraction to these places must be olfactory in the same manner that mosquitoes are attracted to people in the dark. Most of them afford easy access to sleeping people. While the intimacy is not quite that seen to exist between fleas or lice with their host, the relationship, although more fleeting and interrupted, is still a relatively intimate one.

In the beds and associated with the sleeping pads in the loft, young nymphs and an occasional adult were found hidden in crevices although the adults more often had a wider spread harborage. The eggs, as a result, were found in all rooms of the house, where they were frequently attached to the thatch overhead in the roofs. During the daytime, adult *R. pallescens* could sometimes be found, in fact, among the thatch of the roof, particularly along the eaves. They were more apparent at night, however, and could be found in cracks in the wall throughout the house. At times they seemed more abundant in the kitchen, where they might have been attracted by the cooking odors or the warmth, or both.

2. *Relative frequency of domiciliary reduviid bugs.* A general summary of the results of house collections of reduviid bugs from the 5 collection areas in Central Panama is presented in TABLE 1.

TABLE 1. Collections of domiciliary reduviid bugs in Central Panama.

Collection Area	Total From Each Area	Number of Bugs Collected by Species*						
		(Rp)*	(Td)*	(Pg)*	(Ec)*	(Cp)*	(Pr)*	(Ph)*
Santa Rita	2660	2649	3	4	1	1	1	0
Mendoza-Represa	339	330	5	2	2	1	1	0
Bique	175	169	6	0	0	0	0	0
Lidice	95	95	0	0	0	0	0	0
Santa Rosa	14	14	0	0	0	0	0	0
Totals	3283	3257	14	6	3	2	2	0
					=27/3283			
Per Cent		99.18%			=0.82%			

(Rp)* = *Rhodnius pallescens;* (Td)* = *Triatoma dimidiata;* (Pg)* = *Panstrongylus geniculatus;* (Ec)* = *Eratyrus cuspidatus;* (Cp)* = *Cavernicola pilosa;* (Pr)* = *Panstrongylus rufotuberculatus;* (Ph)* = *Panstrongylus humeralis.*

Although 6 of the 7 reduviid species previously reported from Panama (Usinger 1944) were encountered among the 3283 bugs taken from houses during the 3-year study, only 4 of these, including *R. pallescens*, *T. dimidiata*, *P. geniculatus*, and *Eratyrus cuspidatus* (Stål 1859), were taken as many as 3 times or more in house collections. The vast

majority, some 99.18% of the bugs, was identified as *R. pallescens*, while only 27 specimens, 0.82%, made up the other 5 species encountered.

Romana (1961), perhaps influenced by Clark & Dunn's (1932) mistaken statement that *Rhodnius prolixus* occurred in Panama, incorrectly implicates *R. prolixus* as a vector of Chagas' disease there. Rozeboom (1936) drew attention to this mistaken identity, saying: "Bugs identified as *Rhodnius prolixus* (by Clark & Dunn, 1932) were encountered in native huts. Bugs *that were the same as these were later described as new* (i.e., a new species) by Barber (1932) and named *Rhodnius pallescens*." While a superficial resemblance between *R. prolixus* and *R. pallescens* may be at first confusing, particularly if they are viewed separately, differentiation of the 2 is easy and is based on solid taxonomic features which are readily seen. The most obvious difference, other than size (*R. prolixus* is slightly the larger of the 2) is the pale buff coloration of *R. pallescens* as contrasted to a distinctly deeper brown of *R. prolixus*. Barber (1932) gives a useful short key to 3 species of *Rhodnius*, and Lent (1948, 1954) considers the taxonomy of all species of the Genus *Rhodnius* exhaustively.

Since *R. prolixus* is known to occur commonly in Venezuela (southeast of Panama), in Guatemala, El Salvador and Costa Rica (to the northwest) and is known to transmit *T. cruzi* frequently in these countries, Romana's acceptance of Clark & Dunn's report is understandable, and it is conceivable that eventually it may be shown to occur in parts of Panama bordering these countries. In the material collected so far, and from a careful survey of the literature, there is as yet, however, no evidence that *R. prolixus* occurs in Panama. Both Dias (1952) and Del Ponte (1958) imply that they concur in this opinion, indicating that the species concerned in Panama is probably *R. pallescens*, not *R. prolixus*.

T. dimidiata was encountered only 14 times during the survey, a fact which lends little support (as far as Central Panama is concerned) to the suggestion by Romana (1961) that it is an important vector of Chagas' disease in Panama. Since as an adult, it is nearly twice the size of *R. pallescens*, and has a much more contrasting coloration, *T. dimidiata* should have been theoretically easier to spot in houses if it had been present. There seems to be more than a little doubt, however, that it is adapted well enough to living close to man even to be considered a domestic species in Panama or elsewhere. Del Ponte (1959) states: "The published observations give the impression that it (*T. dimidiata*) is a species which is poorly adapted to (living) in human dwellings, especially when compared to *Triatoma infestans*." Furthermore, he quotes Zeledon (1952) who considered it "recently adapted" (to domiciliary living) in Costa Rica, and Pifano (1941) who felt at that time that it was principally a sylvatic species in Venezuela.

As to the other 3 relatively uncommon species of reduviid bugs, *P. geniculatus* was found only 6 times, and *E. cuspidatus* only 3 times; so these may be considered as rare or only occasional house invaders in the survey concerned. Clark & Dunn (1932) found *P. geniculatus* plentiful only in cave collections in Panama, although it was apparently sometimes attracted to lights in houses since it was reported by them as having been collected twice outside window screens in the Canal Zone. *Cavernicola pilosa* (Barber 1937) and *Panstrongylus rufotuberculatus* (Usinger 1939) were seen only twice each in our survey, and can thus be thought of as mere accidental visitors to houses in Panama. *Panstrongylus humeralis* (Usinger 1939) was never encountered at all.

The surprising preponderance of *R. pallescens* over all other species encountered was one of the most unexpected and at the same time one of the most interesting findings of the survey. Although it had been previously known from the experience of other workers (Johnson 1943, Galindo 1960) at the Gorgas Memorial Laboratory that this species was relatively common and thus probably instrumental in the epidemiology of Chagas' disease in Panama, the present writer had not anticipated the overwhelming preponderance of *R. pallescens* and the comparative rarity of other species in houses.

This rarity of species other than *R. pallescens* in house collections is enigmatic. One must either assume that all species other than *R. pallescens* are only occasional or chance invaders of human dwellings in Panama, or that some selective influence is operating to discourage or even repel all other species. The first assumption does not seem tenable, particularly in the case of *T. dimidiata*, although it may, as previously suggested, be as yet poorly adapted to living close to man and just occasionally enter houses. The other alternative, operation of a selective influence, while not offered with any proof, seems to the writer to be at least a plausible possibility. The most obvious man-made alteration in the domiciliary environment of the reduviid bugs under study is the periodic (at yearly intervals usually, but occasionally more often) application of residual insecticidal sprays to the inside surfaces of the native huts by the

Panamanian malaria control teams. DDT spray formulations are routinely used, although Dieldren was also applied once during the survey period. The persistence of *R. pallescens* under these circumstances, coupled with the almost complete absence of other species, may suggest a relative resistance of the former species and a susceptibility of the latter, to such chemical control measures.

Whatever is the cause of this apparent imbalance, it is evident that *R. pallescens* is the most common domiciliary reduviid bug in the areas surveyed. Furthermore, the constancy of its occurrence in house collections seems to suggest an intimate relationship with man. This species was taken in all stages of development: egg, nymph, through adult, while only the adults of the other 3 relatively uncommon reduviid bugs were encountered. This array of developmental stages of *R. pallescens*, particularly associated with sleeping areas in the houses, indicates clearly that it is breeding in the houses in close contact with the human host. There is a semi-permanent and continuing, and perhaps even an anthrophilic, relationship with man.

In addition to the collections summarized in TABLE 1, spot-check surveys were made in several more far-flung areas of Panama as previously described. These were uniformly disappointing. At Almirante in the Bocas del Toro Province of western Panama (B on insert, FIG. 1), visits to native houses in the banana and cacao fincas, in the hilly country encompassed in the United Fruit Company holdings there, produced no bugs. A side trip to Cusepin in the Guayme Indian Reservation on the Valiente Peninsula, some 32 km to the southeast, was likewise fruitless. In both locations, the predominant type of housing, crude wooden huts with galvanized iron roofs, offered poor harborage for reduviid bugs. A similar situation was noted at El Real (A on insert, FIG. 1) in the Darien province of eastern Panama. At Yape, a Choco Indian village some 24 km upriver from El Real, Johnson et al. (1937) found 5 of 101 sera positive for *T. cruzi* by complement fixation tests. Our spot-check of the thatched-roof huts in this same village in November of 1962 disclosed no bugs, although the natives had some recollection of having seen them in the past. Results from the Chiriqui district in western Panama were likewise negative.

3. *Effect of seasonal rainfall on domiciliary collections.* Panama has a definite seasonal rainfall which occurs in a long rainy season extending from late May through mid-December. This is followed by a more or less dry season extending from mid-January through mid-May. It was soon realized that there was an apparent discrepancy between the dry season collections and those made during the wet months. This is evident by an examination of the collection totals over an 18-month period from December 1961 through July 1963, shown in TABLE 2, and depicted graphically in FIG. 2, a bar diagram of the same data. Collection totals from both the Santa Rita and Mendoza areas increased dramatically during the rainy season, and gradually declined with the onset of the dry season. It is also evident that there was a gradual build-up in the bug totals toward the end of the drier months, with a build-up ending in a peak for the wet season in August and September in both areas. This build-up associated with the wet months is thought to reflect generally more favorable conditions for the bug populations, including such factors as greater availability of food (blood meals) during these months when the human population out of necessity remained indoors more both day and night, as well as more subtle influences of the climate on the welfare of the bug population both indoors and outdoors. The dry season conversely was thought to provide generally more unfavorable conditions for the bugs, possibly fewer human subjects to feed upon, and an unfavorable environment, both indoors and outdoors, for the bugs.

4. *Peri-domiciliary and sylvatic collections.* Ecological niches immediately associated with human habitations, i.e., "peri-domiciliary" sites, which were thought to provide likely harborage for reduviid bugs near houses, included domestic animal shelters and outhouses. These were chicken houses, pig pens, horse-cow barns, etc., which were usually built adjacent to houses for reasons of convenience or security, or both. Frankly extra-domiciliary, or

TABLE 2. Relation of bi-monthly bug collection totals to seasonal rainfall in major collection areas.

Months:	Dec-Jan	Feb-Mar	Apr-May	Jun-Jul	Aug-Sept	Oct-Nov	Dec-Jan	Feb-Mar	Apr-May	Jun-Jul
Year:	1961–62	1962	1962	1962	1962	1962	1962–63	1963	1963	1963
Area:										
Santa Rita	37	60	148	289	957	471	342	43	18	34
Bique-Mendoza	0	10	42	38	136	25	87	41	34	84
Totals	37	70	190	327	1093	496	429	84	52	118
		Dry Season			Wet Season			Dry Season		

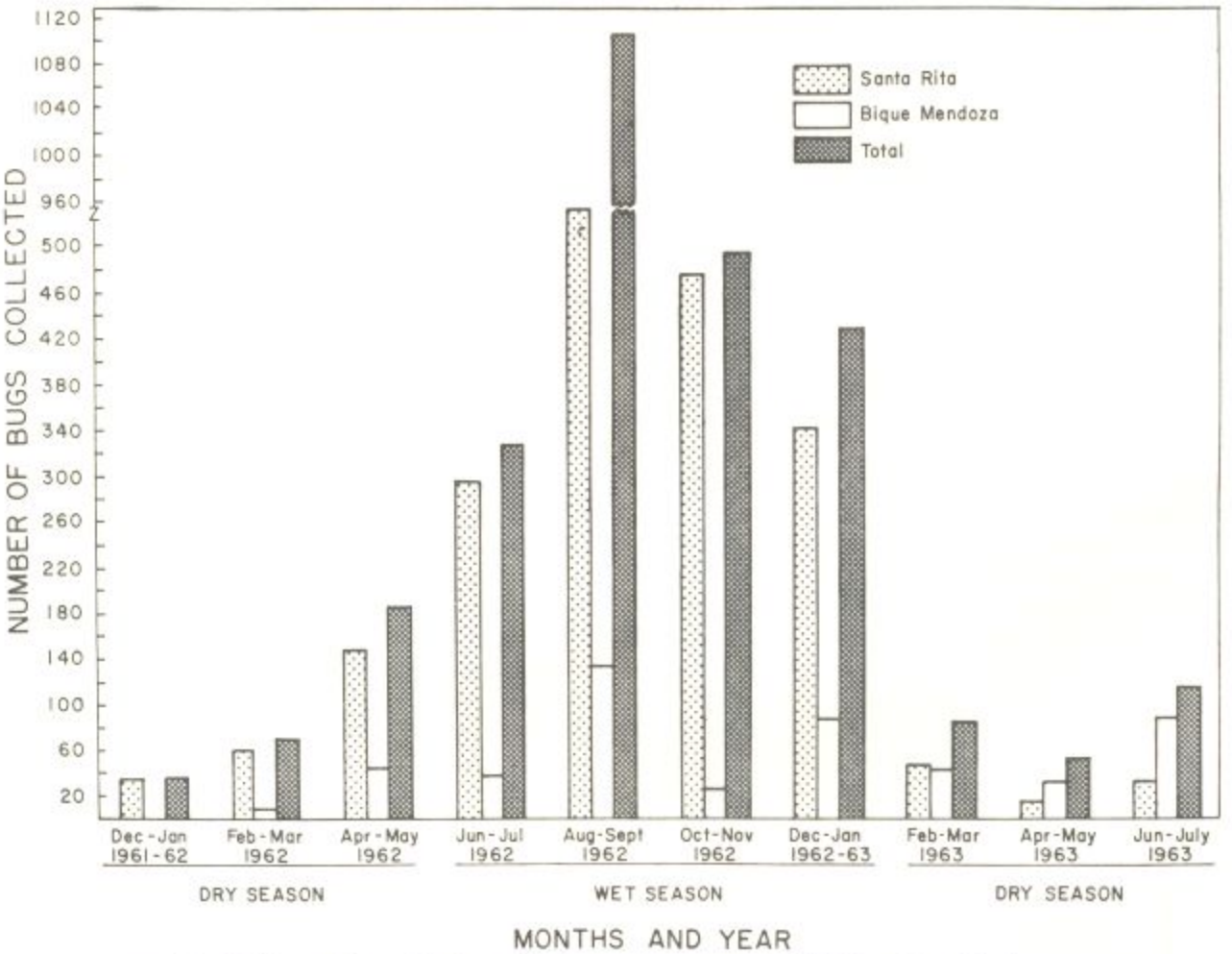

FIG. 2. Relation of bi-monthly bug collection totals to seasonal rainfall in major collection areas.

what perhaps might more accurately be called "sylvatic" ecological niches, included a wide variety of forest sites still further removed from the houses. Some of these, such as fence posts, dead logs and stumps, might be thought of as mere temporary hiding places for the bugs, while others, such as hollow logs or burrows and dens of small animals, would seem to offer more permanent shelter since sharing them with animals offered a source of blood meals.

A list of these collection sites and the results are summarized in TABLE 3. It is apparent that of the peri-domiciliary sites, pig pens and chicken houses were attractive to the reduviid bugs, particularly *R. pallescens*. Of the 2, chicken houses were found to harbor bugs slightly more often (7 of 52 as compared to 4 of 35 checked) and only the chicken house collections yielded any trypanosomes. Since chickens are known to be refractory to *T. cruzi* infections, one can probably assume that these 3 bugs got their infections either from some other animal source, or by cannibalism. Horse barns yielded no bugs.

Of the sylvatic sites, only 3, all trees, which harbored either animals or animal nests, or contained tree holes offering at least temporary harborage for animals, were found to contain *R. pallescens*. Armadillo burrows, which were difficult to explore, were free from bugs although armadillos were flushed from several of these. Bird nests were likewise barren. The opossum nests yielded the greater number of bugs, 28 from 6 nests, of which 3 were positive for *T. cruzi*. While the number of ecological niches explored were considerable, relatively few yielded bugs.

(B) *Hemoflagellate Infection Rate of Domiciliary Reduviid Bugs in Panama*

1. *Previous reports.* Of the "*Triatoma*" (*Panstrongylus*) *geniculata* specimens reported on by Clark & Dunn (1932) all but 2 came from the Chilibrillo caves, which were at that time quite isolated from human dwellings. No infection rate data

TABLE 3. Survey of potential peri-domiciliary and sylvatic ecologic niches for reduviid bugs.

	Ecologic Niche	No. Explored	Niches With Bugs	Bugs Positive
A.	*Peri-Domiciliary Sites:*			
	1. Chicken Houses	52	7 (23)*	3
	2. Pig Pens	32	4 (11)*	0
	3. Cow & Horse Barns	19	0	0
B.	*Sylvatic Sites:*			
	4. Palm Trees (uninhabited)	41	0	0
	5. Palm Trees (with animal nests)	22	0	0
	6. Other Trees (with Sloths)	29	1 (1)*	0
	7. Other Trees (with Opossom nests)	32	6 (28)*	3
	8. Tree Holes	9	1 (3)*	1
	9. Oropendula Nests	36	0	0
	10. Other Bird Nests	18	0	0
	11. Termite Nests	12	0	0
	12. Dead Logs, Stumps	43	0	0
	13. Armadillo Burrows	17	0	0
	14. Caves	2	0	0
	15. Fence Posts	61	0	0
	Totals:	428	19 (66)*	7

Number in parenthesis ( ) is number of bugs collected in this niche.

were mentioned for the 2 specimens caught near houses, but of the 90 taken from the caves, the feces of 10 of 18, or 55.5% of those examined, were infected with trypanosomes. Twenty-one, or 80.8%, of 26 of the 90 bugs were also proven infective when individual saline emulsions prepared from the 26 bugs were injected into 26 guinea pigs intraperitoneally, so it is apparent that the infection rate was high. Dunn's (1933) report on *R. pallescens* was based on only 8 bugs taken from a native hut. While it is impossible to estimate the infection rate among even this small sample since Dunn exposed the bugs to guinea pigs in batches of 2 or 3 at a time, he definitely established the ability of this species to transmit the infection to laboratory animals. Dunn's (1934) report of the natural infection in *E. cuspidatus* was apparently based on a single specimen. Finally, Rozeboom's (1936) initial report on *T. dimidiata* indicates that 3 of 11 domiciliary bugs examined were positive for *T. cruzi* by fecal examination and 1 of these 3 transmitted the infection to a clean guinea pig.

While these earlier reports were admittedly limited in sample size, they did point to the existence of a potential infection pool with *T. cruzi* among the Panamanian reduviid population. No further reports reflecting on the infection rate appear in the literature, although Dias (1952) mentions *R. pallescens*' presence in Panama, suggesting its possible connection with the transmission of Chagas' disease there, but avoids any speculation regarding the infection rate with *T. cruzi* among the local species there.

2. *Present findings.* A consideration of the infection rate with trypanosomes among the domiciliary bugs taken from the 5 collection areas of Central Panama is presented in TABLE 4. Between 27.36% (Lidice) and 48.57% (Bique) of the bugs taken from the houses, with an average of 36.58% (over all), were found infected with trypanosomes. Most of these, or about 32.71% (over all), were identified as *T. cruzi*. In all probability, the latter figure is close to an accurate estimate of the incidence of infection in Central Panama, since it agrees well with the results from the Santa Rita area, where 2660 bugs were examined.

TABLE 4. Infection of domiciliary reduviid bugs with trypanosomes.

Collection Area	No. & % Bugs with Trypanosomes in Feces	No. & % Bugs with *T. cruzi* in Feces	No. & % Bugs with *T. rangeli* in Feces	No. & % Bugs with *T. rangeli* in Hemocoele	No. & % Bugs with *T. rangeli* in Feces and/or Hemocoele
Santa Rita	933/2660 =35.07%	856/2660 =32.19%	79/2660 =2.40%	9/168 =5.35%	86/2660 =3.23%
Mendoza-Represa	152/339 =44.82%	121/339 =35.66%	23/339 =6.78%	15/140 =10.71%	31/339 =9.12
Bique	85/175 =48.57%	66/175 =37.71%	17/175 =9.71%	3/32 =9.37%	19/175 =10.85
Lidice	26/95 =27.36%	26/95 =27.36%	0/95	1/6 =16.6%	1/95 =1.02%
Santa Rosa	5/14 =35.70%	5/14 =35.70%	0/14	0/14	0/14
Totals	1201/3283	1074/3283	119/3283	28/346	137/3283
Per Cent	=36.58	=32.71	=3.62	=8.09	=4.17

Interest in the incidence of *T. rangeli* in the bugs did not develop until later in the survey, but some 346 bugs were examined specifically for this purpose. Of these, 28 or 8.09% were found to harbor this flagellate in the hemocoele. Results from similar examinations based entirely on the fecal examination, gave somewhat smaller infection rates (3.62%) for *T. rangeli*. These 2, when averaged together, give 4.17% infection as based on fecal examination and hemocoele examinations combined.

It is apparent that Santa Rita, Bique and the Mendoza-Represa areas are all plagued with an appreciable population of domiciliary reduviid bugs in which about a third are infected with *T. cruzi*. In our experience, Bique and the Mendoza-Represa areas were more commonly infected with *T. rangeli* (with an average of 10.85 and 9.12%

infection, respectively) than was the Santa Rita area. The Lidice and Santa Rosa areas, supplying only 109 specimens in all, represent so small a sample as to give little meaning to their respective results, either for *T. cruzi* or *T. rangeli*.

3. *Relation of stage of development sex of reduviid bugs to infection rate.* Of the sample of reduviid bugs upon which data relating to sex and developmental stage were kept, as summarized in TABLE 5, some 670 *R. pallescens* were taken from houses. Of these, 210, or 31.3%, were nymphs, and 460 or 68.7 were adults—the larger numbers of adults due in part, in all probability, to the greater difficulty of uncovering the younger bugs, or to the ambulatory habits of the older bugs, or both. Of the adults, the females outnumbered the males almost 3:2 (270:190), the differences again being due to the habits of the bugs, probably, rather than to any real discrepancy in the ratio of males to females produced.

TABLE 5. Relation of stage of development and sex of the bugs to infection rates.

		Nymphs		Adult Females		Adult Males	
		No. Bugs Collected	No. Bugs Positive	No. Bugs Collected	No. Bugs Positive	No. Bugs Collected	No. Bugs Positive
Dec	1962	84	17	100	56	58	39
Jan	1963	3	0	19	14	11	8
Feb	1963	33	5	39	21	22	13
Mar	1963	41	7	12	8	8	4
Apr	1963	10	4	7	4	31	9
May	1963	4	0	13	10	7	6
Jun	1963	29	4	48	21	26	18
Jul	1963	6	1	32	25	27	19
Totals		210=31.3%		270=40.02%		190=28.3%	
Infection Rates			18%		58.8%		60.1%

The infection rate of the 2 sexes of adult bugs was almost equal (58.8% for ♀♀, 60.1% for ♂♂) and the difference is obviously not significantly different. The infection rate for the younger bugs was, however, much lower than that of adult bugs of either sex, and considering the fact that adults outnumbered the young bugs more than 2 to 1, with this low infection rate, it would seem that the younger bugs probably are of less importance in the transmission of the infection. While the infection rate of the 2 sexes of adults was almost equal, the larger numbers of females taken in the house collections would indicate that they have a greater opportunity to come into contact with people than do the less abundant males, and are, therefore, possibly somewhat more important for this reason.

(C) *Biology of the Vector-Parasite-Host Complex*

1. *Proof of infectability of insect vector with T. cruzi. T. cruzi* infections in *R. pallescens* were extremely slow in developing, first evidence of any apparent crithidial population in the mid gut in these bugs being seen at about 2 or 3 weeks, and metacyclic forms were usually not seen in the rectum for 5–6 weeks or more after the blood meal. If the bugs were observed to take a copious blood meal on the infected mouse, trypanosomes were usually evident in the gut for 1–2 days, sometimes longer, but these soon disappeared, to be replaced later by crithidia. The infection rate was moderately high, running between 50 and 75% of those exposed, this varying with the degree of parasitemia in the C3H mice used as a blood source.

There was no obvious difference in the apparent infection rate in *R. pallescens* exposed to standard laboratory strains of *T. cruzi* and those exposed to local Panamanian strains of this organism, either of human or wild animal source. In our experience there was some tendency for the local strains to produce an infection sooner in the bugs than did the "Brasil" strain of *T. cruzi*.

2. *Transmission experiments.* Although Dunn (1933) established the ability of *R. pallescens* to transmit the infection to laboratory animals, he was using naturally infected bugs, and it seemed fitting to run similar tests using strains of *T. cruzi* about which more was known, and bugs that were known to have been originally clean of any flagellate infection. As it developed, however, both our laboratory-reared *R. pallescens*, and naturally infected bugs captured from nature, were found equally capable of transmitting either the "Brasil" laboratory strain of *T. cruzi*, or local Panamanian strains of this organism, to clean C3H Swiss mice. A parasitemia detectable in unconcentrated plain smear was produced in 60–75% of the test mice within 9–16 days. Test runs using local strains of the flagellate were, however, more variable in results than the "Brasil" strain.

3. *Sylvatic infection pool in wild animal reservoirs.* During the last 18 months of the survey, some 209 small terrestrial and arboreal wild animals of 8 different species were collected from more or less forested areas surrounding the native houses in the different communities, although most of these were from the Bique area. These collections are summarized, along with their infection rates with *T. cruzi*, in TABLE 6.

By far the greater number (128 of 209) of the animals collected were opossums. This was due likely not only to the fact that opossums were

TABLE 6. Survey of wild animal reservoirs of *T. cruzi* infections in nature.

Animal	No. Collected	No. Positive	% Positive
Opossum (*Didelphis marsupialis* Linnaeus, 1758)	128	41	32.03
Anteater (*Tamandua tetradactyla* Gray, 1825)	14	2	14.28
Coati (*Nasua narica* Linnaeus, 1766)	4	1	25.00
Marmoset (*Saguinus goeffroyi* Pucheran, 1845)	8	3	37.50
3-Toed Sloth (*Bradypus infuscatus* Wagler, 1831)	7	1	14.27
Porcupine (*Coendou rotschildi* Thomas, 1902)	4	0	0
9-Banded Armadillo (*Dasypus novemcinctus* Linnaeus, 1758)	43	0	0
11-Banded Armadillo (*Cabassous centralis* Miller, 1899)	1	0	0

among the most common small animals, but also to the fact that they were more easily caught. The next most common animal was the armadillo. Fairly easy to catch, it comprised 44 of the 209, all but 1 of which were of the 9-banded variety. Of the 2 animals, however, opossums are obviously closer to man. While the armadillo may occasionally come near houses at night in search of food (root vegetables, etc.), it is essentially a very shy animal and seldom lives adjacent to populated communities. The opossum, on the other hand, builds its nests in trees not too far from houses and has, in fact, been known to live in the loft of peri-domiciliary buildings. It is a bold marauder, frequently raiding chicken houses at night, affording excellent opportunity for contact with domiciliary or peri-domiciliary bugs.

Of the other animals collected, most are seen rarely around houses, although marmosets are sometimes kept as pets and coatis are sometimes captured for food and may be held temporarily in captivity. Sloths, although not obviously shy, seldom build their nests near houses. Anteaters are also definitely wild. It seems unlikely that any of these latter animals are routinely important as peri-domiciliary wild animal reservoirs of the infection.

Opossums proved to be very commonly infected with *T. cruzi*, some 32% being found so. Surprisingly, armadillos were, in our survey, entirely negative. This was distinctly unexpected, since Clark & Dunn (1932) had found them infected, and they are frequently referred to in the literature as an important reservoir of *T. cruzi* in rural sections of other South American countries.

One wonders about the actual importance of the armadillo in the transmission cycle of *T. cruzi* in Panama, since none of the 48 isolations of *T. cruzi* in our animal survey was from armadillos, and since only 1 of 58 *T. cruzi* isolations in a similar survey made at the Gorgas Memorial Laboratory in 1965 was from armadillos. Furthermore, other workers with *T. cruzi* in both Costa Rica and El Salvador have indicated to me that in their experience, the armadillo is almost never infected with *T. cruzi* in their countries. The opossum, on the other hand, was found commonly infected with *T. cruzi* in our presently reported survey, and in the reports (1965) of the Gorgas Memorial Laboratory group. It would seem that the opossum offers the greatest potential as a wild animal reservoir in the Panama focus, particularly in view of its relatively bold nature. Other small animals, possibly marmosets kept as pets, armadillos, coatis, etc., kept temporarily for food, being secondarily important. These, and possibly other small terrestrial and arboreal animals not covered in this survey, represent, with the opossum, an ever present sylvatic reservoir of infection in nature whence the infection spreads via extra-domiciliary reduviid bugs to peri-domiciliary and eventually to domiciliary reduviid populations living more or less permanently intimately associated with man in his domicile.

4. *Feeding and defecation times of Rhodnius species and vector efficiency.* Results of studies on feeding and defecation times of *R. pallescens* and its related species *R. prolixus* are summarized in TABLE 7. These data represent a compilation of results from 10 experimental runs (5 from each species) since it would have been virtually impossible to make accurate observations on feeding and defecation habits of such a large number of insects simultaneously. It might also be pointed out that the data include observations only on bugs that were observed to defecate following a blood meal; those not being observed to feed and subsequently defecate were disqualified. Results may be thought of as reflecting, in considerable degree, the effect of feeding on subsequent defecation habits.

Feeding took place rapidly in both species after the bugs were placed on C3H mice. One hundred and one of 110, or 91.78%, of the *R. pallescens*, and 108 of 122, or 88.9%, of the *R. prolixus* took a blood meal within the first 30 min. of observation, as seen in FIG. 3. Curves for both species follow essentially similar trends, a heavy majority of the bugs feeding early after exposure. It may be assumed, therefore, that both species are almost equally prone to feed shortly after exposure to a suitable host. Furthermore, among *R. pallescens* adults tested, almost equal numbers of males and females (46 and 47,

TABLE 7. Comparison of feeding and defecation habits of *Rhodnius pallescens* and *R. prolixus*. Number of Bugs Feeding or Defecating, by Time Interval in Minutes

R. pallescens:													
Interval:	0 to 10		10 to 20		20 to 30		30 to 60		60 to 120		120 to 150		
Observation:	Fed	Def	Fed	Def	Fed	Def	Fed	Def	Fed	Def	Fed	Def	Cum Totals
Male:	(24		22		5)		(2		0		0)		53
		(2		0		2)		(3		27		4)	38
Female:	(17		21		2)		(3		3		0)		46
		(7		3		2)		(6		7		2)	27
Nymph:	(5		4		1)		(1		0		0)		11
		(1		1		1)		(3		0		0)	6
Total Fed:	(46		47		8)		(6		3		0)		110
Total Def:		(10		4		5)		(12		34		6)	71
Per Cent Fed:	(41.8 + 42.7 + 7.27 = 91.8 %)						(5.45 + 2.77 + 0.0 = 8.2 %)						
Per Cent Def:	(14.0 + 5.63 + 7.04 = 26.67%)						(16.0 + 47.8 + 8.45 = 73.33%)						
R. prolixus:													
Interval:	0 to 10		10 to 20		20 to 30		30 to 60		60 to 120		120 to 150		
Observation:	Fed	Def	Fed	Def	Fed	Def	Fed	Def	Fed	Def	Fed	Def	
Male:	(17		24		4)		(6		0		0)		51
		(14		16		0)		(0		0		0)	31
Female:	(28		21		5)		(5		2		0)		61
		(25		18		3)		(1		0		0)	47
Nymph:	(7		2		0)		(1		0		0)		10
		(3		1		0)		(0		0		0)	4
Total Fed:	(52		47		9)		(11		2		0)		122
Total Def:		(42		35		3)		(1		1		0)	82
Per Cent Fed:	(42.6 + 38.5 + 7.37 = 88.9%)						(9.01		1.60		0.0)		
Per Cent Def:	(51.2 + 42.6 + 2.11 = 95.9%)							(1.21		1.21		0.0)	

respectively) were seen to feed during the initial 30 min. of observation. On the other hand, among *R. prolixus* adults tested, slightly greater numbers (54 and 45, respectively) of females appeared to feed earlier after exposure than did males (FIG. 3). A majority of the nymphs of both species that were apparently destined to feed at all, fed during this same period. So, for all practical purposes, it would appear that except for a slight tendency for greater numbers of females of *R. prolixus* to feed earlier than males of that species, there was no suggestion of a tendency toward early feeding after exposure which could be correlated with sex, stage of development, or species of bug being tested. In general, both sexes of adults, as well as nymphs of the 2 species showed a similar healthy interest in obtaining a blood meal as soon as possible after their fast.

From the cumulative totals to the right side of TABLE 7, it can be seen that of the total numbers of bugs of both species seen to have fed, roughly two-thirds (71 of 110 *R. pallescens*, and 82 of 122 *R. prolixus*) of either species were subsequently seen to defecate at least once during the 150 min. of the observation period. Many, in fact, defecated several times. This tendency toward repeated defecation may, as Dias (1956) points out, have an important bearing on their vector efficiency. To achieve fecal contamination of the puncture wound at all, however, it is obvious that defecation must follow feeding by a reasonably short period, and determination of this time interval is the main purpose of our observations. Results of these observations are plotted in FIG. 4.

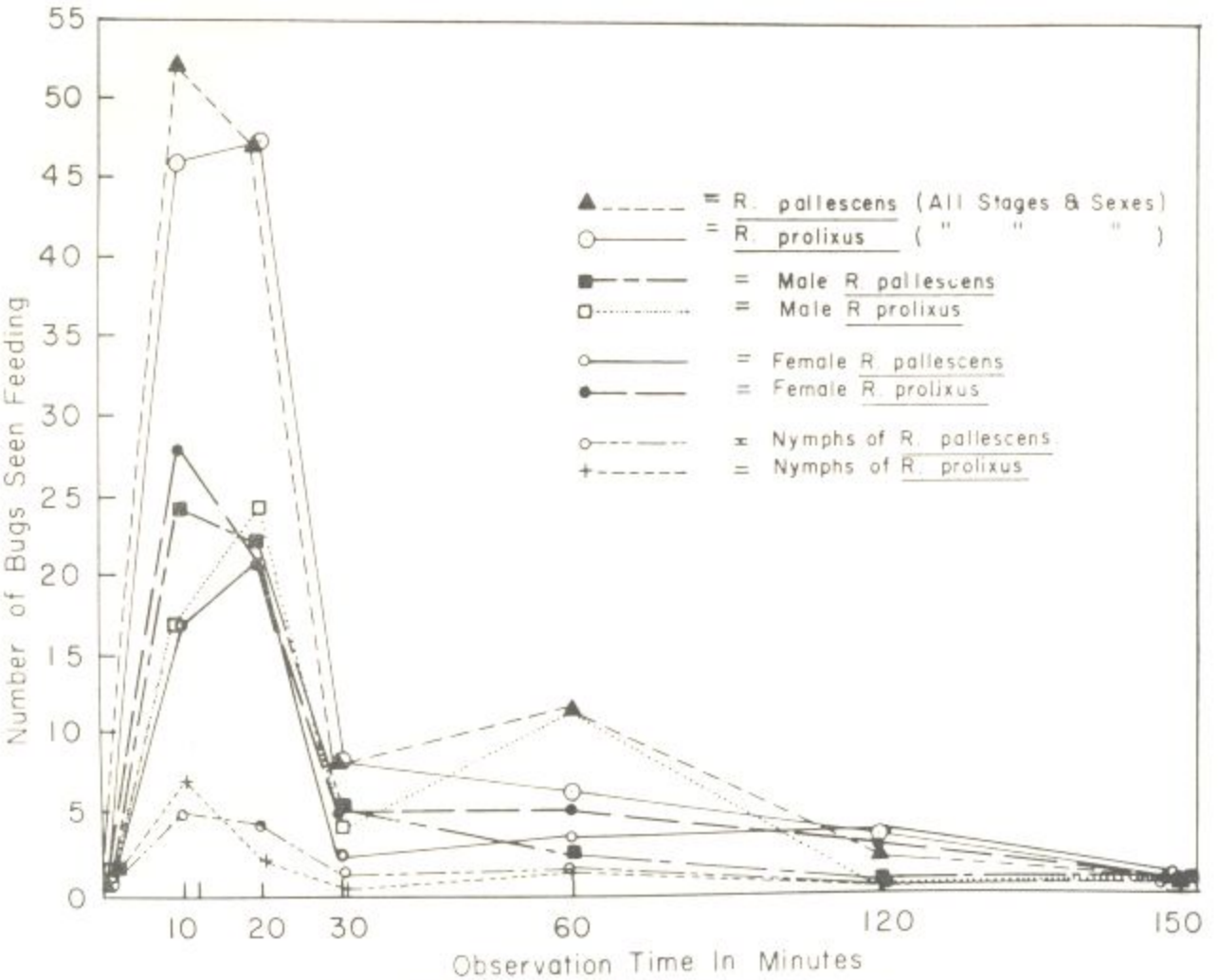

FIG. 3. Feeding habits of *Rhodnius* species after starvation.

Of the 82 *R. prolixus* seen to defecate during the 150-min. observation period, 80, or 95.9%, of those defecating, did so during the first 30 min., supporting previous reports that this species is a relatively rapid defecator. On the other hand, of the 71 *R. pallescens* seen to defecate, only 19, or 26.67%, did so during the initial 30 min. while 42, or 73.33%, defecated later, most of them doing so an hour or more after the beginning of observation (FIG. 4). This suggests that the Panamanian species, *R. pallescens*, is a tardy defecator. While this may not entirely prevent transmission by the contaminative route, it may well decrease the likelihood that the initial puncture wound be contaminated. Only if multiple defecation occurs, and some of these deposits occur soon enough after a subsequent puncture, would contamination and resulting transmission be expected to occur. If the bug should persist in continued delay of defecation after feeding each time, transmission might never occur. What seems more likely, if in fact *R. pallescens* is the most likely vector in Panama, is that transmission does take place but not as consistently as it does with a more rapid defecator, such as *R. prolixus*, so that people may be bitten in single attacks by infected *R. pallescens* without transmission occurring. When the bug is not disturbed in feeding, or has an opportunity to make repeated attacks, in spite of the delay between feeding and defecation, transmission may eventually occur. This, in my opinion, may also explain in part, the relatively low incidence of human infection with *T. cruzi* in Panama.

In a breakdown of the data as to sex and stage of development, among the *R. pallescens* adults tested, the males seemed to retain their fecal load longer than did the females, 31 of 38 males defecating during the last 60 min. of the 150-min. observation period as compared to 15 of 27 females defecating during this same period. Greater numbers of females of *R. pallescens* defecated over a longer spread of time during the observation period

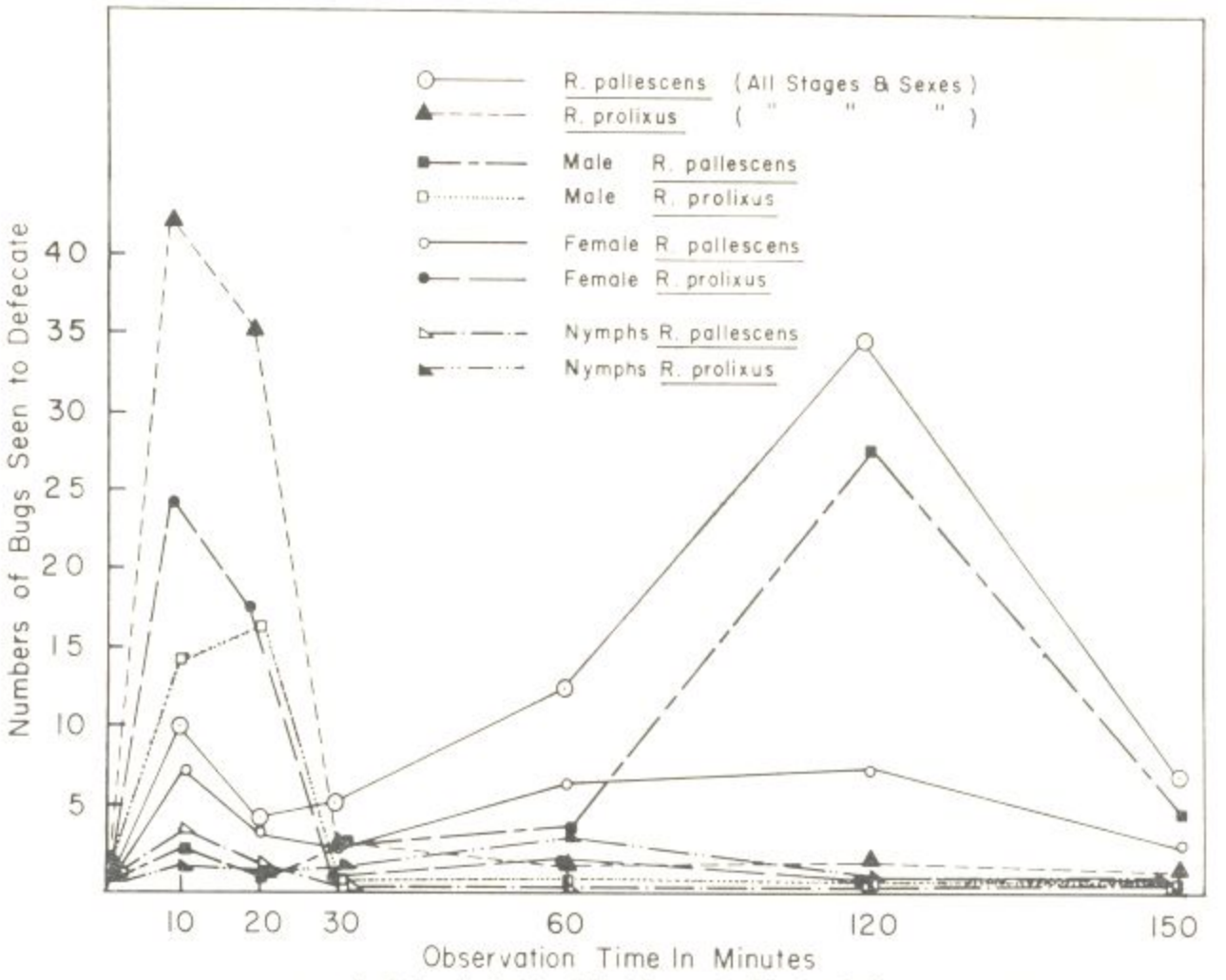

FIG. 4. Defecation habits of *Rhodnius* species following feeding.

than did males of this same species (FIG. 4), where defecation was largely confined to the last hour of the observation period. In a sense, then, the females of this species might present a slightly greater threat as effective vectors by the contaminative route than do the males, since they seem to defecate at least some during the initial 30 min., while the males seldom do (FIG. 4).

Among the adult *R. prolixus* tested, 20 of 31 (about 67%) of the males defecated during the first 30 min., while 41 of 47, or 87.2%, of the females defecated during this period (FIG. 4). While it is apparent that both sexes are relatively early defecators in this species, there would seem to be, as with *R. pallescens*, a slightly greater potential for transmission by females than by males, since they are generally earlier defecators, although more so with *R. prolixus* than *R. pallescens*.

Although our observations on nymphs of both species were necessarily smaller by far than with adults, of those of both species which were observed to have fed, about half of them later defecated, most of them within the first 30 min. of observation.

Because of the relative scarcity of reduviid species other than *R. pallescens* in our collections, and the difficulty in establishing colonies of these, only limited attempts at comparative studies on the feeding and defecation habits of these were possible. Whenever specimens occurred in our collections, if they were alive when brought into the laboratory, such observations were made as soon as feasible. Three *E. cuspidatus* fed within 5, 6.5 and 11.5 min., respectively, after exposure to Swiss mice. Two of them failed to defecate, while the other defecated 68 min. after feeding. Of 4 *T. dimidiata* observed, all fed at 7, 8.5, 13 and 18 min., respectively, after exposure. One defecated at 21 min., one at 26.5, one at 50 min., and one failed to defecate, this agreeing in general with previous descriptions of the habits of this latter species.

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