

The Incidence of *Trypanosoma cruzi* in *Triatoma* of Tucson, Arizona*

by

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Trypanosoma cruzi was first reported in the United States in 1916 by KOFOID and McCULLOCH (4). They found a flagellate in an assassin bug, *Triatoma protracta*, collected in San Diego, California. Since the authors failed to infect young white rats upon which infected bugs fed and because no trypanosomes were detected in the blood of wood rats from nests containing infected bugs, the trypanosome was named *Trypanosoma triatomae*. Later, KOFOID and DONAT (3) established that *T. triatomae* is in reality *T. cruzi*.

Infected bugs, other than from California, were next collected near Tucson, Arizona and were examined by KOFOID and WHITAKER (5). PACKCHANIAN (9) reported the first naturally infected bugs from Texas, and WOOD (23) reported on the infected *Triatoma* of New Mexico.

Mammals and *Triatoma* in the southeastern United States have also been found to harbor *T. cruzi*. Of 1,584 mammals examined in Georgia (1) 103 were positive. Mammals were found infected in northern Florida (6) and Maryland (13, 14). The trypanosomes from these mammals are antigenically and morphologically the same as *T. cruzi* from South America (15). YAEGER (25) found infected *Triatoma* from areas in Louisiana where infected mammals had been collected. OLSEN *et al.* (8) reported infected opossums and raccoons from east-central Alabama as well as infected *Triatoma* from the same area.

In 1955 the first natural case of Chagas' disease was reported in the United States by WOODY and WOODY (24). They found trypanosomes in the blood of a 10 month old child from Corpus Christi, Texas. A second case was

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also reported in 1955 (7). The patient was a 6 month old infant from Houston, Texas hospitalized for meningitis, and trypanosomes found in the cerebrospinal fluid were identified as *T. cruzi*.

This paper reports the infection rates of *T. cruzi* in *T. rubida ubleri* and *T. protracta* from areas near Tucson, Arizona.

MATERIALS AND METHODS

The most important vertebrate hosts in Arizona are species of the pack rat, *Neotoma*. The vectors are blood sucking reduviids, *Triatoma rubida ubleri* and *T. protracta*, usually found in rat dens and other small mammal burrows, and *T. recurva*, found mainly in the burrows of the ground squirrel, *Citellus* (10).

Because of the difficulty of obtaining specimens of *T. recurva* this survey was limited to the two species found in rat dens. Areas supporting *Neotoma* were located, numbered, and marked on a topographical map. The general ecology of each area and the density of the rat dens were noted.

Dens were selected at random and carefully torn apart. Each rat den was numbered and the following observations made: type of den (construction materials, location, etc.), mammals and reptiles present, triatomes present (species, instar, and sex if adult), and any additional observations of apparent significance. The triatomes from each den were placed in separate containers and transported to the laboratory.

In the laboratory the bugs were dissected by inserting a dissecting needle just anterior to the pygidium and pulling to the posterior. This pulled the last segment of the abdomen away bringing with it the hind gut in which the metacyclic trypanosomes are found. The hind gut was then macerated in a drop of physiological saline solution and inspected microscopically for motile trypanosomes. Twenty microscopic fields at 430 \times were observed before the sample was considered negative.

Attempts were made to infect mice to complete the life cycle. This was done to find the leishmaniform stage as a further indication that the trypanosomes were *T. cruzi*. Young white mice were selected and inoculated intraperitoneally with physiological saline solution mixed with fecal material containing metacyclic trypanosomes. Blood samples were taken from the tails of the inoculated mice and examined microscopically. Laboratory raised, uninfected *T. rubida ubleri* were allowed to feed on an infected mouse. These bugs were dissected on the 20th, 26th, and 42nd days after the feeding. The mouse was then necropsied and tissues removed from the liver, spleen, kidney, and heart and fixed in Zenker's solution. These tissues were sectioned, stained, and inspected for leishmaniform bodies.

RESULTS

From 153 rat dens in 21 areas, 657 Triatomas were collected and dissected. Of these, a total of 65 were infected (9.9 per cent). Of the 657 insects, 524

were *Triatoma rubida ubleri* with 39 infected (7.5 per cent), and 133 were *T. protracta* with 26 infected (19.5 per cent). The average infestation was 4.3 bugs or 3.4 *T. rubida ubleri* and 0.9 *T. protracta* per den (Table 1).

It should be noted (Table 2) that with the exceptions of 10 *T. rubida ubleri* from area 2 and 1 *T. rubida ubleri* from area 20 that all other infected bugs were collected from area 17. Since the per cent of infection in area 17 was higher, 43 rat dens were excavated from this area in an attempt to determine the limits of the infection.

TABLE 1

Dens and Triatoma Collected

Area	Dens	Protracta	Rubida	Infected
1	6	0	12	0
2	9	1	37	10
3	13	1	31	0
4	2	0	8	0
5	7	0	3	0
6	2	6	13	0
7	6	0	3	0
8	6	0	0	0
9	5	7	25	0
10	8	6	44	0
11	3	0	0	0
12	5	0	0	0
13	7	13	33	0
14	4	3	8	0
15	13	5	57	0
16	3	0	13	0
17	43	86	195	54
18	2	0	0	0
19	1	0	1	0
20	3	1	19	1
21	5	4	22	0
Totals	153	133	524	65

In considering the results of this study it is important to note that in the month of June few bugs are present in the rat dens. Because of this the comparisons were made from data calculated from the numbers of rat dens that did contain bugs. The number of rat dens in each area and a listing of the infested dens and dens that contained infected bugs is found in Table 3.

From all areas excluding area 17 there were 329 *T. rubida ubleri* and 47 *T. protracta* collected from 55 rat dens, an average of 6.8 bugs, or 6.0 *T.*

TABLE 2

Species and Instars from Dens Containing Infected Bugs

Area	Den N°	Protracta	Rubida
2	5	0	10 (4th)
17	2	1 (3rd)	1 (3rd)
17	3	1 adult	0
17	7	5 (3rd)	1 (3rd)
		12 (4th)	1 (4th)
		4 (5th)	
17	9	0	2 (4th)
17	18	0	3 (4th)
17	23	0	1 (4th)
17	32	0	1 (5th)
17	34	0	4 (4th)
			3 (5th)
17	36	1 (4th)	9 (4th)
		1 (5th)	
17	37	1 adult	0
17	39	0	2 (3rd)
20	3	0	1 (4th)
Totals	13	26	39

rubida ubleri and 0.8 *T. protracta* per den. Of these, 11 bugs (all *T. rubida ubleri*) were infected, an infection rate of 2.9 per cent, or 3.3 per cent of the *T. rubida ubleri*.

In contrast 281 bugs were collected from area 17. There were 195 *T. rubida ubleri* and 86 *T. protracta*. This is an average of 6.5 bugs or 4.5 *T. rubida ubleri* and 2.0 *T. protracta* per den. The remarkable difference between area 17 and the other areas was the overall infection rate of 19.2 per cent. There were 28 infected *T. rubida ubleri*, which is an infection rate of 14.3 per cent, and 26 infected *T. protracta*, or 30.2 per cent. A further indication of the infection rate of area 17 can be obtained by making a comparison of the number of dens which contained infected bugs. From all areas other than 17, a total of 55 dens containing bugs was excavated. Only two of these dens contained infected bugs, which is a percentage of 3.6. On the other hand, 11 of 40 dens in area 17 contained infected bugs (27.5 per cent).

In the spring of 1963 a few persons reported finding adult *T. rubida ubleri* in or about their residences. No *T. protracta* were found. The *T. rubida ubleri* were reported from the 11th of June to the 25th of June, and of 19 adult bugs dissected 2 were positive for *T. cruzi*, a percentage of 10.5. Apparently the adult bugs were attracted to the homes by lights. One bug was found on the

TABLE 3
*Comparison of Rat Dens Containing
 Triatoma*

Area	No. Rat Dens	With Bugs	With <i>T. cruzi</i>
1	6	3	0
2	9	6	1
3	13	7	0
4	2	2	0
5	7	1	0
6	2	2	0
7	6	3	0
8	6	0	0
9	5	2	0
10	8	3	0
11	3	0	0
12	5	0	0
13	7	4	0
14	4	2	0
15	13	10	0
16	3	2	0
17	43	40	11
18	2	0	0
19	1	1	0
20	3	3	1
21	5	4	0
Totals	153	95	13

front door beneath a porch light, while the others were found inside the homes behind curtains, in bed covers, and other areas where they could hide. To their knowledge none of the people in these homes were bitten. However, one family, contacted later in the summer when no bugs were found in their home, explained that they have problems with *Triatoma* each spring, and both the wife and husband have been bitten several times.

Of 9 mice inoculated with rectal material from infected bugs, blood trypanosomes were observed in only one. Motile trypanosomes were observed in the tail blood on the 29th day after inoculation. The infection of this mouse was then further tested by xenodiagnosis as described above. All four *T. rubida nbleri* became infected and metacyclic trypanosomes were observed. Slides prepared from tissues taken from this mouse were carefully examined and leishmaniform bodies were found in the heart muscle.

DISCUSSION

KOFOID and WHITAKER in 1936 (5) made the first report of infected bugs from Arizona. They received *T. ubleri* (= *T. rubida ubleri*) and *T. protracta* from November 1934 to June 1935 from the vicinity of Tucson, Arizona. Of a total of 79 *T. rubida ubleri* and 2 *T. protracta*, 7 *T. rubida ubleri* were infected (8.9 per cent).

WOOD in 1939, 1940, and 1941 obtained *Triatoma* from the Alvarado Mine near Congress, Arizona. He examined a total of 515 *T. longipes* (= *T. recurva*), *T. protracta*, and *T. p. woodi*. Of these, 28 *T. longipes* and *T. rubida ubleri* (5.4 per cent) were infected (17).

In 1945 SCHUCK (11) received 16 *T. longipes* and 2 *T. rubida ubleri*. These bugs were collected from the Tres de Mayo Mine, 15 miles northeast of Nogales, Arizona. Eight of the *T. longipes* and 1 of the *T. rubida ubleri* were infected. She also reported (11) that Davis found 3 of 7 *T. longipes* infected from this same area in 1941.

WOOD in 1949 (18) collected from two localities in Arizona (21.1 miles west of Casa Grande, and 7 miles south of Continental), and presented a summary of collections made by S.F. Wood and F.D. Wood. There had been 828 *Triatoma* collected that were examined for *T. cruzi*, and of these 63 (7.6 per cent) were infected.

GILLILAND and DOWELL in 1949 (2) collected 4 *T. longipes* and found 3 infected with trypanosomes. During 1952 WOOD (19) collected and dissected 10 *T. rubida ubleri* and 4 *T. longipes* from Tonto National Monument, and all were negative. In 1953 WOOD (20) found 1 *T. longipes* from Tonto National Monument positive for *T. cruzi*. In addition 45 *T. rubida ubleri*, 6 *T. longipes* and 4 *T. protracta* from Tonto National Monument and other areas of the state were dissected and found to be negative. During 1955-1956 WOOD (21) found 1 *T. protracta* from Montezuma Castle and 2 *T. protracta* and 1 *T. rubida ubleri* from Tuzigoot National Monument infected of 22 insects examined. In 1957 (22) he collected 10 infected bugs from Globe, Clarkdale, Montezuma Well, Tucson, and Tuzigoot of 23 bugs dissected.

William H. Brown (unpublished report) collected 57 *T. rubida ubleri* and 16 *T. protracta* from the Tucson area. He found 11 or 15.1 per cent of these infected. Seven of the infected triatomes were *T. rubida ubleri* (12.3 per cent), and 4 were *T. protracta* (25.0 per cent).

Including the 657 *T. rubida ubleri* and *T. protracta* of the present study, there have been 1,677 *Triatoma* collected from Arizona that have been examined for *T. cruzi*. Of these 147 or 8.8 per cent were infected.

Although the average number of bugs per den (6.5) in area 17 of this study was comparable to that found in all other areas (av. 6.8), the infection rate with *T. cruzi* was significantly higher, with 19.2 per cent as compared to 2.9 per cent. There was observed a slight difference between the ratio of *T. protracta* and *T. rubida ubleri* in area 17 in comparison to the other areas. In area 17 there was an average of 2.0 *T. protracta* and 4.5 *T. rubida ubleri* per

den, while in all other areas 0.8 *T. protracta* and 6.0 *T. rubida ubleri* were found per den. However, this ratio of bugs per den is not significantly different at the 5 per cent level of probability.

The life cycle of *T. rubida ubleri* is completed in one year in the laboratory (12), and as collections were made it was noted that certain instars were collected in greater numbers at different times during the year, suggesting a seasonal pattern in the field. In May and June the invasion of homes occurred with *T. rubida ubleri* adults, and during this time few insects were found in the rat dens. From 29 apparently active dens excavated in June, only 2 adults and 1 3rd instar *T. rubida ubleri* were collected. The highest number of 2nd and 3rd instars (34 of 93 insects) was collected in July. In August 63.9% (202 of 316) of all insects collected were 4th instars, which dropped to 26.7% (12 of 45) in September as they began to molt to the 5th instar. From October through April nearly all insects found were 5th instars, and during the months of July through April only 1 adult *T. rubida ubleri* was collected. Fifth instars collected in February began to molt to the adult stage in the laboratory on March 26 and continued to molt throughout the month and into April. However, it is possible that the warmer laboratory temperatures would cause the molt to occur earlier than in the field.

Some instars were collected that did not fit this pattern. However, USINGER (12) found that the length of the nymphal period is dependent upon the time and size of the last blood meal. Considering this, if an instar failed to feed, it could become out of sequence with the other instars. In this study *T. rubida ubleri* nymphs remained alive in the laboratory for over 6 months without feeding, and, for the most part, without molting.

It is interesting to note that the instars and adults collected by WERHLE in 1939 (16) fit the above pattern, and that he indicated that adult *T. rubida ubleri* are most numerous in homes during May and June in the Tucson area.

No set pattern was observed for *T. protracta*, and adults and nymphs in various instars were collected throughout the year.

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SUMMARY

Species of *Triatoma rubida ubleri* and *T. protracta* were collected and dissected to determine the percentages infected with *Trypanosoma cruzi* from areas near Tucson, Arizona. Of 657 *T. rubida ubleri* and *T. protracta* dissected 65 (9.9 per cent) were infected. When each species was considered separately, a higher infection rate was found in *T. protracta* than in *T. rubida ubleri* (19.5 per cent compared to 7.5 per cent), even though there were a higher number of

T. rubida ubleri observed in the field. One area was found with a significantly higher infection rate of 19.2 per cent, compared to 2.9 per cent from all other areas.

A seasonal pattern for the occurrence of *T. rubida ubleri* instars during the year was noted. The adults were found in May and June, 1st, 2nd, 3rd and 4th instars developed through the summer months, and by the end of September the majority of *T. rubida ubleri* collected were 5th instars. The 5th instars molted in the spring to the adult stage.

RESUMEN

Ejemplares de *Triatoma rubida ubleri* y de *T. protracta* fueron recogidos y examinados para determinar los porcentajes de infección, con *Trypanosoma cruzi*, principalmente en cuevas de ratas cerca de Tucson, Arizona. De 657 *T. rubida ubleri* y *T. protracta* examinados, 65 (9.9 por ciento) estaban infectados. Considerando cada especie individualmente se nota una infección más alta en *T. protracta* (19.5 y 7.5 por ciento respectivamente). Sin embargo, un número mayor de *T. rubida ubleri* fue observado en el campo. En una de las áreas se encontró una infección significativamente más alta: 19.2 por ciento en comparación a 2.9 por ciento para todas las demás áreas juntas.

Se observó una relación estacional para los diversos estadios de *T. rubida ubleri* durante el año. Los adultos se observaron en mayo y junio; 1º, 2º, 3º y 4º estadios se encontraron durante el verano, y a finales de setiembre la mayor parte de los ejemplares correspondían al 5º estadio. Estos mudaron en la primavera a adultos.

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