

## Studies on *Trypanosoma cruzi* isolated in the United States: A Review\*

by

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In 1960, a WHO study group (42) estimated that there were at least seven million people infected with *Trypanosoma cruzi* in the Americas. The vast majority of these cases are located in the "Chagas' Belt" between northern Argentina and southern Mexico. The acute form of the disease, as described in textbooks, is found predominately in South America, and has a case fatality rate of approximately 10% - the figure being higher in infants. In this area, also, approximately 10% suffer from serious cardiopathy associated with Chagas' disease, the latter figure being only an estimate based on incomplete surveys in these highly endemic areas.

In North America, Chagas' disease is less acute and often asymptomatic. In Mexico, a recent report by TAY *et al.* (32) of the results of 2,383 autopsies in which four cases of chronic myocarditis due to Chagas' disease were found, indicates that chronic Chagas' disease exists in that country. Similar studies by ZELEDÓN (46) in Costa Rica also showed the presence of the infection.

In the United States, infection of *T. cruzi* in animals has been reported from 10 states (Fig. 1). The first and second indigenous cases (2, 39) in man were reported in 1955 in southern Texas. The only case reported since then was a laboratory infection of the Tulahuen strain in 1962 (3).

The finding of human cases of Chagas' disease has been anticipated since 1934 when WOOD (35) identified as *T. cruzi* the flagellates which KOFOID and McCULLOCH (19) had discovered in 1916 in triatomids in southern California.

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Of the 90 species of Triatomidae reported in the Western Hemisphere (33), about 30 have been found naturally infected with *T. cruzi* but only a few have epidemiologic significance. BIAGI and NAVARRETE (5) reported that 26 species and subspecies were found in Mexico, 16 of which were naturally infected. In the United States, nine species or subspecies are on record as carrying *T. cruzi* (43). Since KOFOID and MCCULLOCH (19) found infected *Triatoma protracta protracta* in California in 1916, infected bugs have been found in central and south Arizona and southern New Mexico where *Triatoma protracta protracta*, *Triatoma protracta woodi*, *Triatoma longipes* and *Triatoma rubida ubleri* are the predominant species (36). In the Texas area, the particularly important vectors are *Triatoma gerstaeckeri*, *Triatoma lecticularius* and *Triatoma heidemanni*. Infected *Triatoma neotome* have been found in the southeastern tip of Texas (31). In 1960, infected *Triatoma sanguisuga* were collected in the delta area of Louisiana (44). The most recent addition to the distribution pattern of infected vectors is the Piedmont coastal area in Alabama where OLSEN *et al.* (23), found 6% of 181 *T. sanguisuga* infected with trypanosomes. *T. sanguisuga* has the most northerly distribution of triatomids in the eastern half of the United States.

It is of particular significance that the reported infection rate for triatomids in the United States is about 20% and approaches rates of infection for endemic areas in South America (20.30%) (43).

A variety of mammals are known to harbor the parasite. The number of reservoir hosts discovered (originally mostly rodents) has been extended to 14 species and their geographic distribution expanded eastward and northward (Table 1). In 1956, WALTON. *et al.* (34) found *T. cruzi* in raccoons (*Procyon*

TABLE 1

List of mammals reported to be infected with *Trypanosoma cruzi* in the United States.

Species	Common Name
<i>Neotoma fuscipes macrotis</i>	Dusky footed wood rat
<i>Neotoma albigula</i>	White-throated wood rat
<i>Neotoma micropis</i>	Southern plains wood rat
<i>Neotoma lepida lepida</i>	Desert wood rat
<i>Peromyscus truei</i>	Piñon mouse
<i>Peromyscus boylii</i>	Brush mouse
<i>Mus musculus</i>	House mouse
<i>Didelphis marsupialis</i>	Opossum
<i>Dasypus novemcinctus</i>	9 banded armadillo
<i>Ammoperophilus leucurus</i>	White-tailed antelope squirrel
<i>Procyon lotor</i>	Raccoon
<i>Mephitis mephitis</i>	Striped skunk
<i>Urocyon cinereoargenteus</i>	Grey fox
<i>Bassariscus astutus</i>	Ring-tail cat

lotoz) in Maryland, and at the Communicable Disease Center the flagellates were isolated from 89 of 552 opossums (*Didelphis marsupialis*), 9 of 608 raccoons, 3 of 306 skunks (*Mephitis mephitis*) and 3 of 118 grey foxes (*Urocyon cinereo-argenteus*) (20). Since these findings were incidental in a leptospirosis survey in southwestern Georgia and northwestern Florida, it is assumed that the actual incidence rates may be higher.

With the above evidence of infection in animals in the United States, why has human Chagas' disease not been more commonly reported? Could it be that clinical Chagas' disease is being overlooked in the United States? What are the epidemiologic factors which may prevent the spread of animal infections to man? Do we have inefficient vectors in the United States? Finally, one may ask, are the trypanosomes found in the United States different from those existing in South America?

In connection with the question of "subclinical" disease, WOODY *et al.* in 1961 (40) used the complement fixation test to determine the incidence of human trypanosomiasis in the area in Texas where both infected vectors and a zoonoses exist. They limited the survey to individuals under 18 years of age. Of the 500 people examined, 9 (1.8%) had sufficiently elevated complement fixation titers to be considered positive reactions. All were reported to have been bitten by *T. gerstaeckeri* at some time. Although blood cultures did not reveal the presence of trypanosomes, the serologic findings were considered to be evidence of past infection.

In 1965, WOODY *et al.* (41) examined in the same way sera from a group of 117 persons who had been bitten by *T. gerstaeckeri* and found 3 (2.5%) were positive. *T. cruzi* was not isolated from the serologically reactive individuals, and no clinical evidence of the disease was found. At the Communicable Disease Center, OMINSKY and LATHROP (25) have recently conducted a similar study of residents of an area near San Antonio, Texas where infected *T. gerstaeckeri* have been collected. Sera from 108 individuals, including 48 persons with histories of having been bitten by triatomids, were tested by complement fixation and indirect hemagglutination tests. One serum was positive by complement fixation test. No clinical symptoms compatible with Chagas' disease were found. Isolation of the organism was not attempted. In another study, when sera from 1,909 individuals from different areas in Texas were tested in 1946 (6), a significant serologic titer was found in only one individual, a boy of 8 years. These differences in incidence rates may be due to the populations tested and the antigens employed.

In 1962, FARRAR *et al.* (8) used the complement fixation test to measure antibody levels to *T. cruzi* in Atlanta, Georgia. Antigens were prepared from the Corpus Christi strain of *T. cruzi*, as were those used by WOODY *et al.* (40). Three adult population groups were compared. The first group consisted of 474 sera submitted for syphilis serology in a large city charity hospital; the second, of 449 sera from "upstate" Georgia representing a rural population; and finally, 28 sera from patients with a diagnosis of "diffuse myocardial disease". A rate of 0.4% positively reacting sera was found in each of the first two groups,

and 7.1% in the group of patients with myocarditis of unknown etiology. Neither xenodiagnosis nor blood cultures yielded trypanosomes from any of the six persons with positive serology. Serologic studies in our laboratory are still in progress and sera are being examined from patients with idiopathic myocarditis.

A major problem existing today is the lack of adequate laboratory diagnostic facilities for Chagas' disease. The complement fixation test, certainly the most sensitive and specific available, is not easily performed when only occasional sera are received for testing. Simpler tests must be devised if any large scale survey work is to be done. In our laboratory, a flocculation test with antigen-coated latex particles has shown some reactivity with animal sera. Further work to evaluate this procedure is in progress. A more recent technique, the fluorescent antibody test described by SADUN and co-workers (30) for Chagas' disease, is also available to the laboratory.

To help clarify the ecologic picture of Chagas' disease in the United States, several groups of workers have been conducting surveys of wild life in different parts of the country. A variety of techniques for isolation of flagellates has been employed, but the best method appears to be cultivation of blood and tissues. We have found the method described by YAEGER (45) of agglutinating blood cells with phytohemagglutinin, then concentrating the organisms by centrifugation very practical since it permits the study of 10 cc or more blood from each animal.

HERMAN and BRUCE (13) used a combination of microscopic examination and cultivation techniques in examining the blood samples collected from 2,005 animals in Maryland. They found an infection rate of 2% in raccoons, while no infection was found in 17 other species tested. The particularly interesting finding in this extensive survey was evidence of possible seasonal infection. All but one of the positive raccoons were trapped in the month of November. There was an infection rate of 11% for the 80 specimens sampled in November as compared to one infection in 289 raccoons trapped during the rest of the year. Triatomids have not been readily recovered in Maryland. The possibility of other modes of transmission is suggested by such observations.

In Auburn, Alabama, where another epizootologic study was carried out by OLSEN *et al.* (23), organisms were isolated in an early survey only from opossums. Later they reported 13.8% of 123 opossums, and 14.3% of 35 raccoons collected in east-central Alabama to be infected with *T. cruzi*. These workers relied upon cultivation of blood and tissue for isolation of the parasites. They found one urine culture positive, which confirmed isolations made at CDC from the urine (20).

YAEGER (43), working in Louisiana, and the Alabama group (23) found that in their areas the vector, usually *T. sanguisuga*, was most always located in dead and rotting snags, stumps and trees, or under loosened wood inside hollow oak trees. Only when animal nests were in such dead trees were the triatomids found to be infected. In California, the nests of rats have been reported as good abodes for triatomids. PACKCHANIAN (26), in Texas, recommended examining under loose bark of palm trees.

Since the habitat of the vector partially determines its food supply, these habitat preferences undoubtedly contribute to the variations reported in mammalian hosts of *T. cruzi*. It may also be an important reason why man is not frequently bitten. In two areas of the country (California and Texas) *Triatoma* vectors have been collected under and in houses (25).

Other characteristics of the insects themselves which may affect the transmission of trypanosomes have been studied in California. As early as 1951, WOOD (37) pointed out the significance of feeding and defecating times in the transmission of Chagas' disease. Of the species he originally studied, he reported that *T. rubida* was the most important species for immediate contaminative effect, while *T. protracta* appeared to be a less efficient environmental contaminator. Such data is particularly pertinent in the epidemiology of transmission to humans because only insects defecating during or immediately after feeding can be efficient vectors for Chagas' disease.

In investigating the animal infection cycle, other characteristics such as the number of defecations during feeding and the number of infective parasites in a fecal sample are of significance. In 1960, WOOD (38) reported some of his observations and recommended an "infectivity index" which could be used in identifying various species. He concluded that, in the case of *T. protracta*, the total contaminative capacity of adults was more efficient than that of nymphs; that adult females were twice as efficient as males and fourth instar nymphs; but that the average time after feeding to the first defecation was less for fourth instars (37 min.) as compared to 50 min. for adult females and 52 min. for adult males. He points out that the 2.6 average number of defecations for *T. protracta* is also considerably less than the 11 to 14 reported for *Rhodnius prolixus* and concluded that the greater contaminative effect of the South American vectors is another reason why *T. cruzi* is more successfully transmitted to man in Brazil than in California. OLSEN (24) reported that *T. sanguisuga*, the vector in Alabama, was even less efficient than *T. protracta* in that some of the latter insects do not defecate for two hours after a blood meal.

Of great interest in the study of the *T. cruzi* problem in the United States is the organism, or organisms, isolated. Are these trypanosomes, passed by a moderately efficient vector through various species of mammals for generations, identical with those infecting man and animals in South America? Have the animal passaged strains retained ability to infect man? Is there, in reality, any difference between the so-called strains or should they be identified as "isolates" only? Can the virulence of a strain be permanently modified?

Some data have been collected toward answering these questions. PACK-CHANIAN'S experimental infections (27) of men with a Texas isolate showed the strain had a degree of pathogenicity for man. Certainly the Corpus Christi and Houston strains isolated from the two children in Texas must have been "native". Walton's raccoon strain produced transient parasitemias in domestic animals just as it did in raccoons (7).

GOBLE (9) has compared the infectivity and pathogenicity of the raccoon strain with a highly virulent South American human isolate and three strains

capable of infecting human beings to variable degrees. The work was carried out in dogs, which he believes are more suitable for reproducing the typical pathology of Chagas' disease. Although the raccoon strain produced lower parasitemias than the most virulent human (Brazil) strain, they were equal to or greater than those produced by Corpus Christi, Tulahuen and Houston strains. On the other hand, all dogs infected with the raccoon strain survived as compared to 65%, 67%, 100% and 20% survival rates with the other strains.

At the Communicable Disease Center we have compared 5 opossum, 1 skunk, and 2 raccoon isolates with 3 human strains (21). We could find no morphological differences except that the raccoon strains always produced larger clumps or rosettes of crithidia, more like *T. duttoni*, than do any of the other *T. cruzi* isolates (Figs. 3 and 4).

We maintain the organisms by transferring them every three weeks into fresh Offut's medium, incubated at 25°C (1). The medium is one of the simplest of the modifications of blood medium. The solid phase consists of 10% fresh rabbit blood added to rehydrated blood agar base. The overlay is a buffered saline solution without glucose. On this medium, some strains of *T. cruzi* have showed loss of virulence for mice. The Brazil strain, after many years of unmodified behavior, finally lost its ability to produce heavy parasitemias in mice in the expected two-week interval. The Chilean Tulahuen strain has also lost its ability to kill mice but not to infect them. On the other hand, many other strains have remained unaltered and some have showed apparently increased infectivity.

*T. cruzi* notoriously shows considerable variations in virulence, and methods for measuring these differences present many problems (29). HEWITT and co-workers (14) have been able to standardize experimental infections, particularly by controlling the dosage given subcutaneously, so that quantitative survival time data may be used for measuring chemotherapeutic activity. We first measured only parasitemias and death rates produced in CFW mice by the various isolates. We found that, on this basis, the strains could be roughly divided into 3 groups. Group I, designated as moderately pathogenic, contained only the human Corpus Christi isolate which produced parasitemias in 87% of mice inoculated and 63% of the mice died. Group II, designated as slightly virulent strains, included the human Brazilian strain and three isolates from opossums. Approximately 50% of the mice inoculated with these strains developed parasitemias and less than 10% died as a result of the infection. In Group III, with very low virulence, were placed the strains isolated from a skunk, a raccoon, and an opossum (FR4). These strains produced parasitemias in less than 20% of mice inoculated and less than 10% of the infected mice died.

After two years in culture, the above mentioned strains were retested in weanling CFW mice, and were found considerably more virulent rather than less virulent. Five years later, BAGBY (4) re-examined the organisms and found the infections more like the original ones. He added a strain isolated from a raccoon in Maryland and the Tulahuen strain from Chile, and extended the study by comparing infectivity rates in splenectomized and normal adult male

mice and also in 48-hour-old mice employing the method of PHILLIPS (28) for quantitating his infection dose. He found extremely light parasitemias, and deaths almost non-existent, in the groups of adult mice. Removal of the spleens before inoculation with the flagellates resulted in light to moderate parasitemias, but death rates were not high. The animal strains we had classified in Group II (slightly virulent) were still pathogenic. Only the infectivity of the Corpus Christi strain was markedly enhanced by splenectomy. Inoculation of newborn mice resulted in more readily detected parasitemias with all strains except the two raccoon isolates, which appeared to be non-infective.

The average daily parasitemias of three strains of *T. cruzi* were measured and large differences in the degree of parasitemia were noted between the Tulahuen and FR4 strain. Peak titers of 400,000 organisms per ml for the Tulahuen, 200,000 per ml for the FH5 and 4,000 per ml for the FR4 strain were measured.

The more virulent opossum strain (FH5) and one of the least virulent strains (FR4) were selected for further study and comparison with the Tulahuen strain (which is highly virulent for mice). Practically all organs were invaded by the Tulahuen trypanosomes. The FH5 strain rarely invaded lymph nodes, heart, spleen and lungs, but produced infections in the brain. Practically all of the multiplying forms were in the extracellular matrix of the brain tissue, and were seen in all stages of development and growth, including (in 2 mice) rosettes of crithidia never reported in mammalian tissue. The FR4 was the least invasive. These studies (14) indicated that the animal strains found in the United States do have different tissue tropisms.

Invasion of the cells of the bladder by even the slightly virulent FR4 strain and the presence of both FH5 and Tulahuen organisms in the reproductive organs may indicate that this species has the potential for venereal transmission.

Whether these types of tissue invasion and general virulence of strains are permanent is not known; nor is it certain that there was any real change in virulence during the long period of cultivation in the laboratory. In an attempt to preserve the virulence and identity of *T. cruzi*, some cultures were frozen for storage. All culture samples which had been frozen in 10% glycerine and stored at  $-60^{\circ}$  for over a year and then thawed, cultured, and injected into weanling mice, produced parasitemias. The infectivity for mice was strikingly similar to that produced when the isolates were fresh. We recommend freezing and storage of cultures of hemoflagellates at  $-60^{\circ}\text{C}$  for preservation. We have not detected changes in organisms so stored over periods of 18 months, and the Tulahuen strain, which periodically loses virulence in cultures, was as lethal as when initially frozen (1).

PHILLIPS (28) showed that lost virulence of laboratory strains of *T. cruzi* could be restored by rapid passage of large numbers of trypanosomes through 1 or 2-day-old mice. We have "revived" non-pathogenic Tulahuen cultures in this manner with considerable success. Hays in Auburn, Alabama, has also increased the virulence of the FH6 strain. Whether virulence can be maintained by routine transfer of smaller numbers of organisms is still unknown. This method of enhancement of virulence suggests a genetic "selective" process similar to that

reported for the African trypanosomes using agglutination techniques. It should be noted that the technique of inoculation of mice 2-3 days of age may be a valuable tool, especially when small numbers of trypanosomes are to be studied.

Since we had found only slight morphologic differences among the wild animal strains and the South American trypanosomes, immunologic methods were used for evaluation. We found (22) that when mice were first inoculated with any of the non-pathogenic North American strains, they were protected against the challenging strain of *T. cruzi*-Tulahuen, a South American isolate which is very pathogenic and lethal for mice (Table 2). The protection was apparently less complete when the immunizing strain was more virulent. However, this was undoubtedly due to the effect of superimposed infection on damaged tissues, for when the mice were given more time to gain weight before the challenging inoculation, they were completely protected. (Table 3).

TABLE 2

*Survival of mice initially infected with animal strains of T. cruzi and challenged 30 days later with a lethal strain of T. cruzi-Tulahuen (NORMAN and KAGAN, 1960)*

Initial Infection		Challenge Infection		
Animal Strain	Survival Ratio	Percent Surviving Challenge	Mean Day of Death	
			Experimental	Control
0-FH4	17/20*	100		10.6
0-FH5	20/20	100		12.4
0-FH6	19/20	94.7	6.0	12.3
0-OR21	20/20	80	23.7	12.8
0-FR4	9/10	100		12.5
S	20/20	100		11.1
R	20/20	90	15.0	12.1
M	29/30	83	21.2	10.3

\* No. animals surviving/ No. animals inoculated.

First infections with *T. lewisi* and *T. duttoni* failed to protect the mice from a challenge injection (Table 3). We concluded, therefore, that the animal avirulent strains were immunologically related to the *T. cruzi* isolated in South America. These experiments did not detect differences among the various North America isolates. GOBLE (10) also reported that a previous inoculation with the raccoon strain afforded protection to a dog against infection with the Brazil strain.

Further investigation in our laboratory (16) with one of the more virulent opossum strains (FH5) showed that mice infected only two to three days were not protected against a challenge with a lethal dose of *T. cruzi*-Tulahuen.



TABLE 3

*Survival of animals initially infected with human strains of T. cruzi and other trypanosoma species and challenged 4 to 6 weeks later with a lethal strain of T. cruzi-Tulabuen (NORMAN and KAGAN, 1960)*

Initial Infection		Challenge Infection		
Strain	Survival Ratio	Percent Surviving Challenge	Mean Day of Death	
			Experimental	Control
Human <i>T. cruzi</i>				
Brazil	10/10*	100		13.0
Corpus Christi	10/10	100		12.4
<i>T. duttoni</i>	20/20	0	11.8	11.3
<i>T. lewisi</i> (in rats)	9/10	0	20.7	21.7

\* No. animals surviving/ No. animals inoculated.

Mice infected from 7 to 21 days were partially protected and from 28 days to one year almost completely protected against the challenge infection. The presence of very light residual infections in the mice for at least 18 months suggested that immunity may be dependent on the presence of a residual infection. A "passive transfer" experiment showed that as little as 0.2 ml of plasma from immunized and hyperimmunized mice partially to completely protected other mice (Tables 4 and 5) (17). Recent studies by JOHNSON *et al.* (15) and

TABLE 4

*Survival of male mice infected with T. cruzi-Tulabuen and inoculated with 0.2 ml of immune mouse plasma (KAGAN and NORMAN, 1962)*

Number mice inoculated	Dose	No. Died	Percent Survived	Day of death range	Mean day of death	Mean day of death controls*
7	100,000	7	0%	13 - 20	15.7 ± 1.0	13.1 ± 0.7
10	1,000	8	20%	18 - 26	21.4 ± 1.0	15.3 ± 0.4
20	200	17	15%	14 - 33	20.6 ± 1.2	16.1 ± 0.3
18	100	11	39%	20 - 48	29.5 ± 3.0	17.9 ± 0.3
10	50	6	40%	21 - 43	25.9 ± 1.3	19.5 ± 0.6

\* Mice had been inoculated with normal plasma or diluted whole blood.

TABLE 5

Survival time of CFW male mice infected with *T. cruzi*-Tulabuen and inoculated with 0.2 ml of normal mouse plasma or diluted blood (KAGAN and NORMAN, 1962)

No. mice inoculated	Dosage	Number died	Range	Mean day of death
7	100,000	7	11 - 17	13.1 $\pm$ 0.7
20	1,000	20	13 - 21	15.3 $\pm$ 0.4
30	200	30	13 - 19	16.1 $\pm$ 0.3
29	100	29	16 - 24	17.9 $\pm$ 0.3
19	50	19	16 - 26	19.5 $\pm$ 0.6

GOBLE *et al.* (11), indicate that mice can be protected against infection with a vaccine prepared from killed and disrupted organisms.

By using a sensitive indirect hemagglutination test, we evaluated antisera prepared in rabbits against the various strains of *T. cruzi* with antigens prepared from cultured forms of each isolate. Our results to date indicate that strain differences cannot be detected by the hemagglutination procedure. We did notice that with the antisera prepared against *T. rangeli*, antigens prepared from animal strains were much more reactive than antigens prepared from human strains of *T. cruzi*. Except for a few low responses with antisera prepared against *Leishmania* species, many of the *T. cruzi* antigens were species specific (Table 6).

To test for latent pathogenicity in North American strains, groups of 50 male and 50 female mice infected with the FH5 strains, and corresponding control groups of uninfected mice were observed for almost two years. Weights and survival rates were recorded each month. The results of this experiment are shown in Figure 2. The enhanced pathogenicity for male mice reported by HAUSCHKA (12) and other workers was noted in our experiment, and no difference in female mice for the first 11 months was found. After this time, however, uninfected female mice developed mammary cancers and died sooner than the infected ones (18).

Since none of the infected female mice developed mammary cancers, we repeated the experiment three times, infecting female CFW mice with FH5 and Corpus Christi strains. The results of three experiments are shown in Table 7. In two experiments, significant differences were observed. For the entire group the number of mammary cancers which developed in female CFW infected mice was significantly different statistically from control mice. In a fourth experiment, with a C<sub>3</sub>H strain of mice with a reported high spontaneous mammary cancer rate, significant differences in mammary cancers between infected and non-infected mice were again observed. Forty-seven per cent of the control mice developed tumors and 30 per cent of the infected ones (a statistically significant difference). The basis for the protective relationship between cancer

TABLE 6

Indirect hemagglutination test with hemoflagellate antisera prepared in rabbits and antigens prepared from cultured organisms

ANTISERA \ ANTIGENS	T.c. Corpus Christi	T.c. Brazil	T.c. FH4	T.c. FH6	T.c. FR4	T.c. OR21	T. <i>rangeli</i>	L. <i>donovani</i>	L. <i>tropica</i>	L. <i>brasili- ensis</i>
Tc**—Corpus Christi	1600	6400	3200	1600	6400	12800	1600	100	400	800
Tc—Brazil	800	800	1600	800	1600	12800	6400	200	50	100
Tc—FH4	800	400	1600	1600	1600	25600	6400	100	50	100
Tc—FH6	6400	12800	6400	3200	25600	25600	100	50	50	200
Tc—FR4	400	400	50	800	1600	1600	50	100	200	100
Tc—OR21	6400	3200	200	6400	25600	25600	1600	50	50	50
Tc—Tulahuen	6400	12800	12800	6400	25600	25600	12800	0	100	200
Tc—Maryland	3200	1600	1600	400	3200	25600	25600	50	50	100
Tc—Raccoon	25600	25600	25600	200	25600	25600	400	100	100	800
<i>T. vespertilionis</i>	3200	1600	25600	3200	6400	25600	3200	100	400	100
<i>T. rangeli</i>	400	0	6400	3200	3200	25600	25600	200	50	100
<i>T. duttoni</i>	0	0	50	50	200	12800	400	100	100	100
<i>L. donovani</i>	0	0	0	0	0	400	1600	3200	400	800
<i>L. tropica</i>	200	100	50	800	100	400	50	400	800	3200
<i>L. brasiliensis</i>	0	0	50	100	0	1600	1600	3200	200	3200
<i>L. enriettii</i>	0	0	0	0	0	50	0	200	400	800
Control (Culture medium)	0	0	0	0	0	50	0	100	200	200

\*All sera were tested to a titer of 25,600.

\*\* Tc = *Trypanosoma cruzi*

T = *Trypanosoma*

L = *Leishmania*

TABLE 7

*The development of mammary tumors in mice infected with Trypanosoma cruzi and in non-infected controls.*

Experiment	Infected No. tumors/ No. mice	Controls No. tumors/ No. mice	Significance
I 2/61 - 5/62	7/47	25/50	0.001
II 3/61 - 5/62	13/50	13/50	None
III 3/61 - 5/62	7/50	12/31	0.025
TOTAL	27/147	50/131	0.001

and *T. cruzi* remains unresolved by our experiments.

Review of recent investigations shows evidence of increased interest in and awareness of *T. cruzi* infections in the United States, particularly in regard to its possible role in the etiology of myocarditis in man. Epidemiologic studies have included serologic surveys in man and have extended the areas where the flagellates have been isolated in mammals and Triatominae. A need for rapid diagnostic tests is indicated to expand knowledge in this area. Identification of the organisms has stressed immunologic techniques. At present, North American *T. cruzi* strains, though showing no morphologic or immunologic differences from the South American strains, give evidence of strain differences in pathogenicity and tissue tropism in mice. Three cases of clinical Chagas' disease have been described in the United States and serologic evidence of experience with *T. cruzi* is evident from a number of recent studies. To date there has been no parasitic confirmation of infection in serologic reactive individuals. Research in this area must be pursued to verify the pathogenicity for man of the animal strains described in the United States.

### SUMMARY

Studies on *Trypanosoma cruzi* isolated in the United States are reviewed. In the United States, nine species of triatomids and 14 species of mammals have been found infected with *T. cruzi*. Serologic studies for antibodies against *T. cruzi* in human beings are discussed. The epidemiology of *T. cruzi* infections in the United States differs from the classical pattern encountered in South America since infected insect hosts are found in trees and do not always defecate while feeding on the mammalian host. The infectivity and pathogenicity of "animal" strains of *T. cruzi* have been studied and are reviewed. Most strains are avirulent for mice. The cultural characteristics of several strains have been studied and the course of infection in mice examined. Differences among strains have been found. Immunologic studies have shown that avirulent North American strains of *T. cruzi* protect mice against a virulent lethal South American strain. Attempts to differentiate strains serologically by the indirect hemagglu-

mination test were not successful. Experiments to demonstrate that an infection with *T. cruzi* suppressed the development of mammary cancers in mice indicate that statistically significant differences between infected and uninfected groups of female mice were obtained. The basis for the protective relationship was not determined. *T. cruzi* in the United States is found in animals in the southern part of the country. Whether or not clinical disease in man takes place has not been demonstrated.

## RESUMEN

Los autores revisan la literatura sobre *Trypanosoma cruzi* en los Estados Unidos. En ese país se han encontrado 9 especies de triatóminos y 14 especies de mamíferos infectados con *T. cruzi*. Se discuten asimismo los estudios serológicos realizados en humanos. La epidemiología de las infecciones en los Estados Unidos difiere de la de otros países del continente debido a que los insectos son fundamentalmente extradomiciliares y no siempre defecan cuando se alimentan en el mamífero. Se revisa y se estudia la infectividad y patogenicidad de las cepas de *T. cruzi* de animales. La mayoría de las cepas son avirulentas para ratones. Asimismo, las características culturales de las cepas y el curso de la infección en el ratón han sido observadas, notándose diferencias entre las mismas. Estudios inmunológicos han demostrado que una cepa norteamericana avirulenta protege a los ratones contra una cepa sudamericana que produce infección mortal. Por medio de la prueba de hemaglutinación indirecta, no fue posible mostrar diferencias entre las diversas cepas. Otros experimentos demostraron la acción supresora de la infección por *T. cruzi* en el desarrollo de cáncer mamario en ratones hembras, por comparación estadística con los ratones testigos. No se ha determinado la base de tal acción protectora.

El *T. cruzi* se encuentra con cierta frecuencia en animales principalmente en el Sur de los Estados Unidos. No se ha demostrado aún si la infección humana produce síntomas bien definidos de enfermedad de Chagas como en otros países.

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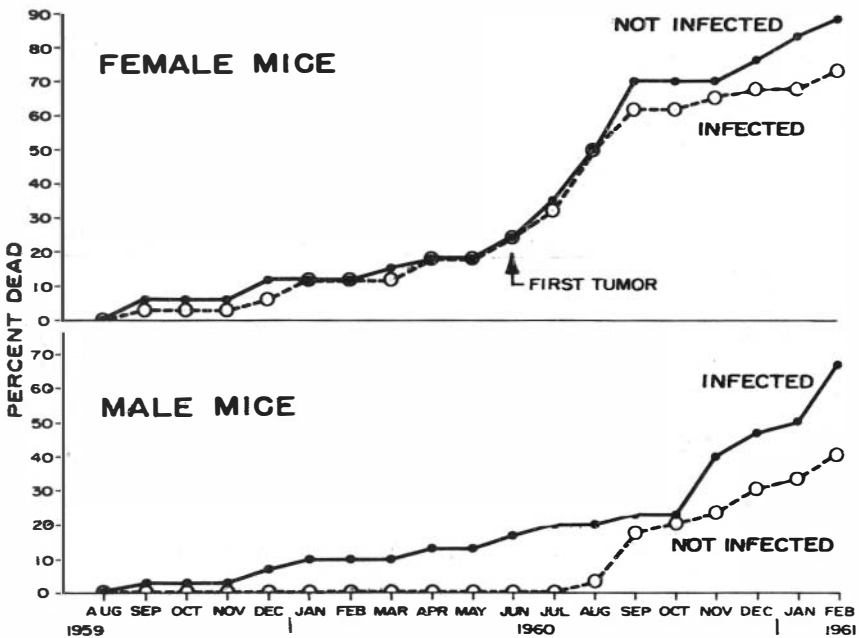
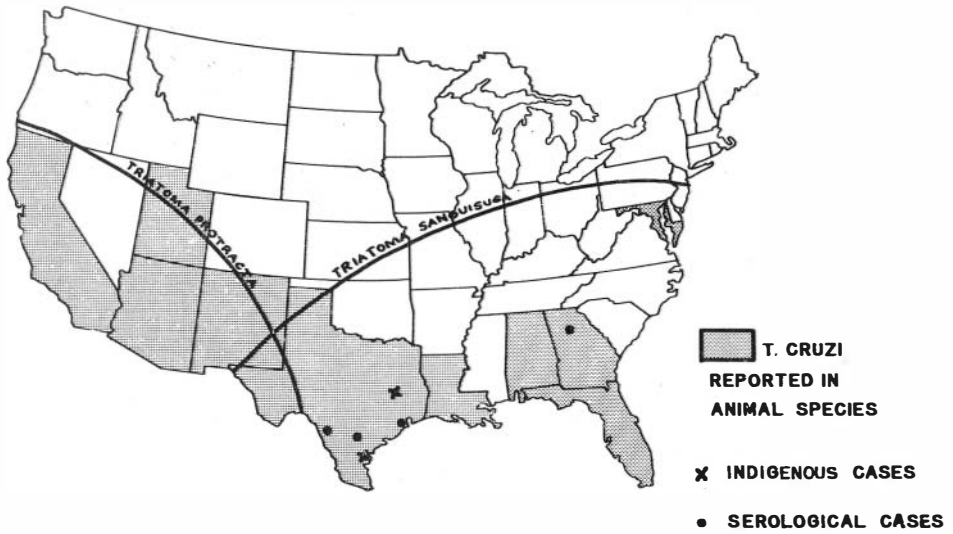
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Fig. 1. The reported distribution of *Trypanosoma cruzi* in the United States and the range of two suitable insect vectors.

Fig. 2. The longevity of male and female CFW mice infected with *T. cruzi* --- FH5 for 2 years.





- Fig. 3. Culture forms of *T. cruzi* — Corpus Christi  
Fig. 4. Culture forms of *T. cruzi* — Raccoon

