

NATURAL INFECTIONS OF *TRITOMA DISPAR* LENT 1950 WITH *TRYPANOSOMA CRUZI* IN PANAMA*

OCTAVIO E. SOUSA AND PEDRO GALINDO

Gorgas Memorial Laboratory, Panama, R. P., P. O. Box 2016,
 Balboa Heights, Canal Zone

Abstract. *Triatoma dispar* Lent has been found for the first time to be naturally infected with *Trypanosoma cruzi*. This is also the first record of the insect species in Darien Province; its range is extended into eastern Panama. The parasite isolated from *Triatoma dispar* was infective for laboratory mice and triatomid bugs (*Rhodnius pallescens* and *R. prolixus*) and showed the morphological and biological characteristics of *Trypanosoma cruzi*. *Triatoma dispar* readily bites man, even in daylight. The evidence suggests that the species may play an important role as the vector of *Trypanosoma cruzi* among arboreal animals, and may be involved in the occasional transmission of Chagas' disease to man in the jungle.

Triatoma dispar was first described by Lent in 1950 from specimens collected in Ecuador and Panama.¹ The holotype is a female from Naranjapata, Ecuador (U. S. Nat. Museum, Washington) previously determined by Usinger as *Triatoma venosa*.² The paratype is also a female, determined by Champion as *Conorrhinus venosus*, from El Volcan de Chiriqui in the Republic of Panama.³ Since the original description, available collecting records indicate that all specimens from Panama are referable to *T. dispar* and no specimen of *T. venosa* proper has been collected in this area.

In addition to the Chiriqui (western Panama) locality, *T. dispar* was found in Central Panama by Galindo and Fairchild.⁴ We now report the presence of this bug in Darien Province (eastern Panama). The species occurs throughout this region in forested areas above 2,000 ft altitude. According to Zeledón *T. dispar* may also extend into Costa Rica in Central America.⁵

Eight species of triatomid bugs have been found in the Republic of Panama.⁶ Several species have been found naturally infected with trypanosomes and are of some importance in the epidemiology of Chagas' disease and distribution of *Trypanosoma cruzi* in this country. The triatomids known to be naturally infected with *T. cruzi* in Panama include the following species:

Panstrongylus geniculatus,⁷ *Rhodnius pallescens*,⁸ *Eratyrus cuspidatus*,⁹ and *Triatoma dimidiata*.¹⁰ The present findings demonstrate that *T. dispar* is also a natural host of *Trypanosoma cruzi* in this area.

MATERIALS AND METHODS

Specimens of *Triatoma dispar* were collected by hand from human bait on a platform, 65 ft above the ground, in the forest canopy. Collection was carried out in the Cerro Quia area in Darien Province near Colombia. This area is a low mountainous mass of igneous rock rising on the right bank and directly south of the upper Tuira River. The top of the mountain is 2,800 ft in elevation. Temperature records taken at ground level, from 3 March to 1 April 1970 indicated a high temperature reading of 85 F at 4:00 PM and a low of 62 F at midnight. The mean daily temperature during the month was 72 F. The annual rainfall in the area probably exceeds 160 inches, as indicated by the vegetation. The entire area consists of tropical humid forest.

The collected triatomid bugs were kept alive and transported, in 8-oz. glass jars lined with plaster of Paris and with loosely packed paper towels, to the Gorgas Laboratory in Panama City. The walls of the jars were wetted to insure high humidity.

Drops of fecal material from wild-caught triatomid bugs were suspended in saline and examined with a compound microscope at 100 to 400× magnification.

Only Carworth Farm white mice were used as experimental hosts. Weanling (20-21 days) and suckling (4-day-old) mice were used for intra-

Accepted 4 December 1971.

* This work was supported by Grant No. He-10689 from the National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014 and by Grant No. AI-02984 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

TABLE 1
Susceptibility of the white laboratory mouse to Trypanosoma cruzi from Triatoma dispar

Route of inoculation	Serial transfer	Inoculum		No. animals	Age (days)	Prepatency (days)	Mortality (at 30 days)
		Size (ml)	No. parasites				
Intraperitoneal	1st	0.1	7,500	1	18	8	*
	2nd	0.2	500	4	19-21	4-5	4/4
Intracerebral	1st	0.02	50	7	4	8-13	7/7
	2nd	0.02	500	16	5	5-7	12/16
	3rd	0.02	500	12	4	7-9	0/12

* Sacrificed for transfer.

peritoneal and intracerebral inoculations, respectively. For preparation of the initial intraperitoneal inoculum, flagellates in bug's fecal material were suspended in saline and counted with the aid of a bright-line Neubauer Counting Chamber.

Heparinized blood was used for the 2nd intraperitoneal serial transfer and for all intracerebral transfers. The intracerebral inoculations were carried out following Martinez-Silva's procedure,¹¹ and 0.02 cm³ was the inoculum size.

Tissues were fixed in formalin, sectioned, and stained with hematoxylin and eosin. Thick and thin blood smears were stained with Giemsa.

Two species of laboratory reared bugs, *Rhodnius pallescens* and *R. prolixus*, were used for xenodiagnosis. Bugs were examined 15, 30, and 45 days after feeding on experimentally infected mice.

Hemoculture procedures using Senekjic's diphasic medium were used to detect and isolate the trypanosomes that developed in experimentally infected mice.

RESULTS

Recently, two collecting trips were made into the Cerro Quia area in Darien Province near Colombia. In March 1970 a collector was making night captures of blood sucking insects on a platform 65 ft high on a "caimitillo" tree (*Bumelia* sp.) in the canopy of the forest, at approximately 2,600 ft above sea level. The collector reported that for 2 consecutive nights several large bugs had attempted to bite him; when a light was focused on them they rapidly retreated under the bark of the tree below the platform. Four adult specimens of *Triatoma dispar* (2 males and 2 females) were collected. The specimens were shipped to the Gorgas Memorial Laboratory in Panama City, and flagellates were detected in

the fecal content of one adult *T. dispar* by microscopic examination.

Panstrongylus rufotuberculatus (Champion) also was collected in this area.

A second trip to the same area in Cerro Quia was made in February and March 1971, for the purpose of collecting *T. dispar*. A total of nine adult bugs was captured between 26 February and 8 March, at 2,400- and 2,600-ft altitude. They were collected with human bait on a platform in the canopy of the forest 65 ft above the ground. Trypanosomatids were found in the fecal content of two bugs.

In order to characterize the organism, trypanosomes from the gut of naturally infected bugs were passaged through a laboratory host. A saline suspension of fecal material containing 7,500 trypomastigotes was inoculated intraperitoneally (0.1 cm³) into a suckling, Carworth Farm white mouse (18 days old). *Trypanosoma cruzi*-like parasites were first seen in the blood of the animal on the 8th day following inoculation. A low level parasitemia, 2 to 3 parasites per mm³, was observed and the animal was sacrificed for blood passage on the 10th day. Serial transfers were carried out by intraperitoneal or intracerebral inoculations.

The intraperitoneal inoculations of 0.2 cc of a blood-saline suspension, containing about 500 flagellates, into each of 4 weanling mice produced low level parasitemias with *T. cruzi*-like trypomastigotes. Xenodiagnosis was performed on the infected mice using *Rhodnius pallescens* and *R. prolixus*. Epimastigotes and metacyclic forms comparable to *T. cruzi* developed in both species of *Rhodnius*.

The intracerebral inoculation of 0.02 cm³ of blood containing 50 to 500 trypomastigotes into 4-day-old suckling mice produced a rising para-

sitemia detectable from the 5th to 9th day. All of the mice used in the first intracerebral passage died between the 11th and the 15th day after inoculation. On the second passage, however, only 12 of 16 animals died of the infection, and these lived a longer time (15-24 days) after inoculation. During the third passage, none of 12 animals died, but all showed low level infections. Evidence indicates that virulence decreases with progressing serial transfer. Histopathological studies revealed amastigotes of *T. cruzi* in the brain and heart of all intracerebrally inoculated mice.

The finding of trypomastigotes in the blood, epimastigotes and metacyclic forms in triatomid bugs and in cultures, as well as the development of amastigotes in the tissues of experimental animals, confirm the identification of *T. cruzi* as a natural parasite of *Triatoma dispar* in Panama.

DISCUSSION

To our knowledge, there is no previous record of *Trypanosoma cruzi* in *Triatoma dispar*. The present findings strongly suggest that the species may play an important role as vector of *Trypanosoma cruzi* amongst arboreal mammals. Galindo and Fairchild recognized the species as highly arboreal in habits, closely associated with *Choloepus hoffmanni*, the two-toed sloth.¹ These authors have also presented evidence that *Triatoma dispar* is attracted to man even in daylight, and recorded the experience of two men who were attacked by nymphs and adults of *T. dispar* while engaged in day-flying mosquito collecting on a tree platform some 45 to 65 ft above the forest floor. Since it readily attacks man when the latter becomes exposed by invading jungle areas, *T. dispar* may also be involved in occasional transmission of Chagas' disease in some forested areas of Panama.

REFERENCES

1. Lent, H., 1950. Nova especie de "Triatoma" Laporte, 1833 (Hemiptera, Reduviidae). *Rev. Brasil. Biol.*, 10: 437-440.
2. Usinger, R. L., 1944. The Triatominae of North and Central America and the West Indies and their public health significance. *Public Health Bull.* No. 288, U. S. Pub. Health Service, Washington, D. C., 81 pp.
3. Champion, G. C., 1899. *Biologia Centrali Americana, Insecta, Rhynchota.*, 2: 209, Tabl. XII, fig. 23.
4. Galindo, P., and Fairchild, G. B., 1962. Notes on habits of two blood-sucking bugs, *Triatoma dispar* Lent, 1950, and *Eratyrus cuspidatus* Stal., 1859 (Hemiptera: Reduviidae). *Proc. Entomol. Soc. Wash.*, 64: 229-230.
5. Zeledón, R., 1952. El problema de la Tripanosomiasis Americana o Enfermedad de Chagas en Costa Rica. Tesis de Grado, Univ. de Costa Rica, Ministerio de Salubridad Publica, *Investigaciones Epidemiológicas* No. 2, Costa Rica, 109 pp.
6. Fairchild, G. B., 1943. An annotated list of the blood sucking insects, ticks and mites known from Panama. *Am. J. Trop. Med.*, 23: 589-591.
7. Clark, H. C., and Dunn, L. H., 1932. Experimental studies on Chagas' disease in Panama. *Am. J. Trop. Med.*, 12: 49-77.
8. Dunn, L. H., 1933. A natural infection of *Trypanosoma cruzi* Chagas found in *Rhodnius pallescens* Barber in Panama. *Am. J. Trop. Med.*, 13: 471-473.
9. Dunn, L. H., 1934. Notes on the Reduviid bug, *Eratyrus cuspidatus* Stal. naturally infected with *Trypanosoma cruzi* Chagas found in Panama. *Am. J. Trop. Med.*, 14: 291-293.
10. Rozeboom, L. E., 1936. *Triatoma dimidiata* Latr. found naturally infected with *Trypanosoma cruzi* Chagas in Panama. *Am. J. Trop. Med.*, 16: 481-484.
11. Martinez-Silva, R., Lopez, V. A., Colon, J. I., and Chiriboga, J., 1969. *Trypanosoma cruzi*: effects of gamma radiation on growth and infectivity. *Exp. Parasitol.*, 25: 162-170.