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NATURAL INFECTION OF *RATTUS RATTUS* BY *TRYPANOSOMA CRUZI* IN PANAMÁ*

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ABSTRACT: In order to define better the animal reservoir of *Trypanosoma cruzi* in some Panamanian communities where Chagas' disease is endemic, we carried out an investigation of the presence of *T. cruzi* in *Rattus rattus*. *T. cruzi* was demonstrated in 57 of 100 wild *R. rattus* trapped in communities of Panamá province, Republic of Panamá. Trypanosomes were seen in thick smears of the peripheral blood of 43 rats, and leishmanial (tissue) forms were found at necropsy in 55 rats. Complement-fixing (CF) antibodies were demonstrated in significant titers in 59 rats, but the results of CF tests did not correlate well with the presence of *T. cruzi* in thick smears and histopathological sections. *R. rattus* appears to be an important reservoir of *T. cruzi* in the province of Panamá, R. P.

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Following Miller's discovery[1] of three cases in man of infection with *Trypanosoma cruzi* in Panamá in 1931, Clark and Dunn reported *T. cruzi*-like trypanosomes in armadillos, opossums, bats, a dog, and a pet squirrel.[2] Since then numerous surveys of wild and domesticated animals have been made in this area, but the published reports make no mention of domestic rats. In considering the relative importance of mammalian reservoirs, Pipkin has written that "it would seem that the opossum offers the greatest potential as a wild animal reservoir in the Panama focus."[3]

De Alencar *et al.*[4–6] and Ferriolli and Barretto[7] have found *Rattus rattus* in Brazil infected with *T. cruzi* by examination of smears from peripheral blood and internal organs, culture of blood, necropsy, xenodiagnosis, and inoculation of blood into mice. Deane includes *R. rattus* in his review of mammals in which *T. cruzi*-like organisms were found.[8] In Trinidad, Downs isolated *T. cruzi* from a rat by intracerebral inoculation of its serum into mice.[9] Beltrán and Perez found *T. cruzi* in the blood of one *Rattus norvegicus* in Mexico City.[10]

Freitas and others using precipitin tests demonstrated rat blood in *Triatoma sordida*, *Triatoma megistus* (*Panstrongylus megistus*), and *Triatoma infestans* collected in Brazil.[11] *T. cruzi*-like parasites were found in 38 of 79 bugs in which rat blood had been demonstrated. Arzube Rodriguez also demonstrated rat blood in the gut of *Triatoma dimidiata* in Guayaquil, Ecuador.[12]

This report describes the prevalence of *T. cruzi* in *R. rattus* trapped during the first 5 months of 1968. The rats live in burrows and other spaces in, under, and near buildings and in the palm-thatched roofs of buildings in very close association with *Rhodnius pallescens*, the chief vector in this area. In January 1968 live trapping was begun in and near houses in the villages of Bonguito, Mendoza, Santa Rita, and Caimito de Capira, agricultural communities endemic for *T. cruzi* located on the Pacific side of the Isthmus in the province of Panamá immediately west of the Canal Zone. Between 10 and 15% of the people in these communities have complement-fixing antibodies for *T. cruzi*, but thick smears and cultures of the peripheral blood are rarely positive for trypanosomes; in less than 0.1% has *T. cruzi* been found. *T. rangeli* is also present in these communities.

MATERIAL AND METHODS

During the first 5 months, 100 *R. rattus* were received and maintained one to a cage in the Laboratory for periods of a few days to 3 months. The rats were bled one or more times by cardiac puncture while anesthetized with ether. Thick smears stained by a modified Giemsa method were examined for *T. cruzi*, and blood serum specimens were examined for complement-fixing antibodies to the parasite by the method of Chaffee *et al.*[13] All the rats died, either being killed inadvertently by cardiac puncture or pur-

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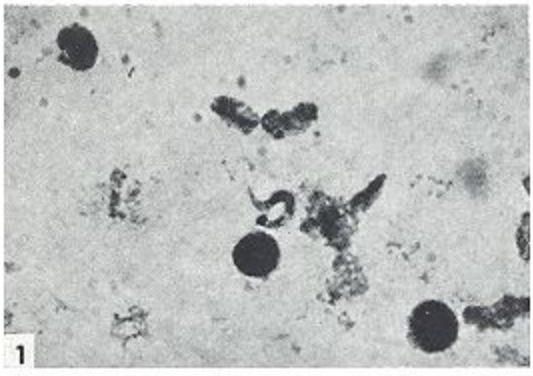

FIGURE 1. Trypanosome consistent in appearance with *T. cruzi* in thick blood film of *Rattus rattus* trapped in Mendoza, Panamá province. (Modified Giemsa stain, oil immersion, × 1,000.)

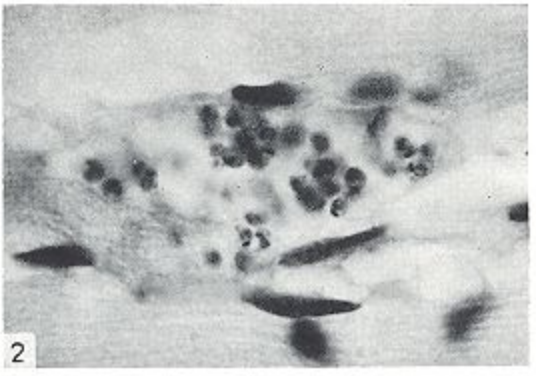

FIGURE 2. Tissue forms, many identifiable as *Leishmania*, of *T. cruzi*, myocardium, *Rattus rattus*. (Hematoxylin-eosin, oil immersion, × 1,000.)

posefully with ether; autopsies were done on all animals. Microscopical examination was done on histologic sections of heart, lungs, brain, intestine, stomach, salivary glands, kidneys, liver, and skeletal muscle stained with hematoxylin and eosin.

RESULTS

Trypanosomes were present in the thick blood smears of 43 rats (Fig. 1). Serial examinations have shown that trypanosomes may be demonstrated by microscopical examination of the blood for as long as 120 days under laboratory conditions that prevented reinfection. Neither *T. rangeli* nor *T. lewisi* were identified in smears of peripheral blood; all trypanosomes seen had the morphologic characteristics of *T. cruzi*.

Tissue forms of *T. cruzi* were present in sections of the heart of 54 rats (Fig. 2). In one animal, organisms were found only in the brain. Parasites were present in the following organs in addition to the heart: the brain (20%), lungs (18%), kidneys (6%), adipose tissue (2%), liver (2%), and skeletal muscle (2%). The tissue forms of *T. cruzi* were present much more frequently in the cerebellum and meninges than in other parts of the brain. In the lung they were most often seen in the smooth muscle of the large pulmonary veins. In the kidney they were found only in the smooth muscle of the pelvis.

Complement-fixing antibodies were demonstrated (3+ and 4+) in the serum of 59 rats. The serum of three was anticomplementary, and serum from 38 rats was negative. The results of the CF tests did not correlate well with the presence of organisms in thick smears and histological sections. Of the 59 CF-positive rats, 45 were CF-positive one or more times and remained so. Seven changed from negative to positive, and seven changed from positive to negative. In the 45 consistently positive rats, organisms were found in 32 (71%); in the seven rats that became positive, organisms were found in two (29%); in the seven rats that became negative, organisms were found in two (29%). Organisms were found in 14 (37%) of the 38 rats that were consistently negative.

DISCUSSION

The most important result of this study is that 57 of 100 *R. rattus*, trapped in an endemic area of Chagas' disease (*T. cruzi* infection in man) in the Republic of Panamá, were infected with *T. cruzi*. De Alencar[6] found 23.5% of *R. rattus* infected in the lower Jaguaribe region of Ceará state, Brazil. In Panamá the percentage of *R. rattus* found to be infected is more than twice that reported by him.

The findings reported here constitute strong, but limited, evidence to support the thesis that *R. rattus* is a major reservoir for Chagas' disease in Panamá province. A prevalence rate of 57% is high for any mammalian species, and the projection of such a rate over a year (our estimate of the life span of a wild rat) suggests a state approaching universal infection. Rates of similar magnitude elsewhere have not been recorded, nor have they been observed previously in Panamá.

The portal of entry of the trypanosome in the rat is unknown. None of the those studied had chagomas or ocular lesions. Rats might be infected with infected feces of bugs through the skin and mucous membranes or by eating infected bugs. The importance of transplacental infection in this host is not known, nor is anything known about transmission of the infection from mothers to offspring.

The high rate of infection may, in part, be attributed to the use of both smears and histopathological examination to demonstrate *T. cruzi*. It might be argued that other trypanosomes might resemble *T. cruzi* in the rat; the confirmation of 41 of 43 identifications in smears by the finding of the tissue forms at necropsy demonstrates that the trypanosome is *T. cruzi*, for it is the only species known to produce tissue forms in the rat.

Since histopathological examination of rats involves considerable time and expense, it should be pointed out that in only one positive rat were tissue forms of *T. cruzi* not found in the heart. For the purpose of surveys to establish prevalence rates in rats, it is likely that very few positive rats would be missed if only the heart were examined.

In the absence of experiments with suitable groups of control and infected rats it is impossible to understand the results of the complement-fixation tests. Negative CF tests in infected animals may occur, and positive CF tests in animals in which parasites were not demonstrated may be the results of self-terminating infections with persistent antibodies, of inadequate search for the parasite, or of nonspecific reactions.

SUMMARY AND CONCLUSIONS

Trypanosoma cruzi was found by examinations of thick blood films and by histopathological examination in 57 of 100 *Rattus rattus* trapped during the first 5 months of 1968 in Panamá province, Republic of Panamá. *R. rattus* appears to be a major mammalian reservoir of *T. cruzi* in relation to endemic Chagas' disease in this area.

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