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SYNANTHROPIC RODENT RESERVOIRS OF *Trypanosoma (Schizotrypanum) cruzi* IN THE VALLEY OF CARACAS, VENEZUELA

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SUMMARY

Direct blood examination and xenodiagnosis of 47 synanthropic rodents (*Rattus rattus*, *R. norvegicus*, *Mus musculus*) captured in the valley of Caracas, Venezuela, revealed trypanosomal infections in 12 *R. rattus*, 10 with *T. lewisi* and 2 with *T. cruzi*. Of the latter the course of parasitemia, the pleomorphism of the bloodstream trypomastigotes, tissue tropism in naturally and experimentally infected rats and mice, host mortality, morphology of fecal parasites in *Rhodnius prolixus* used for xenodiagnosis, and infectivity of the bug feces for NMRI mice, were all characteristic of *Trypanosoma (Schizotrypanum) cruzi*. One rat, with a patent parasitemia, had numerous nests of amastigotes in cardiac muscle and moderate parasitism of the smooth muscle of the duodenum and of skeletal muscle. Mice inoculated with fecal flagellates from the bugs had moderate tissue tropism in the same organs and also in the colon and pancreas. The possible role of *R. rattus* in the establishment of foci of Chagas' disease in Caracas is discussed.

KEYWORDS: *Rattus rattus; Trypanosoma cruzi;* Urban reservoir.

INTRODUCTION

The hemoflagellate *Trypanosoma (Schizotrypanum) cruzi*, the pathogen of American trypanosomiasis (Chagas' disease) is transmitted through fecal contamination by some 100 species of bloodsucking bugs (Hemiptera, Reduviidae, Triatominae); it infects more than 200 mammal species as reservoir hosts, particularly rodents and opossums[2]. The epidemiological significance of synanthropic rodents, especially *Rattus* spp. and *Mus musculus* is little known[2, 11].

In our current investigation of reservoir hosts of *T. cruzi* in the valley of Caracas[6], we have found infected *R. rattus*. The present paper describes infectivity, virulence, and tissue tropism of isolates of *T. cruzi* from these animals.

MATERIALS AND METHODS

"Tomahawk" and "Sherman" live traps, baited with fruits and/or sardines were placed within or near human dwellings in Colinas de Bello Monte, and occasionally in Caricuao, El Cafetal, Las Acacias, Los Chorros, San Roman, and Parque del Este, representative areas of the valley of Caracas[13].

Fresh tail blood from the captured animals was examined at 400 X and in Giemsa-stained smears at 400 and 1000 X[1, 17]. Blood flagellates, when found, were identified[7, 16] and counted[3]. Each animal was xenodiagnosed by allowing 12 3rd – stage laboratory-bred *Rhodnius prolixus* to engorge upon its blood. Feces of these bugs, spontaneously expelled or obtained by dissection, were examined diluted 1:1 in 0.85% saline for up to 45 days after engorgement. Flagellates in positive feces samples were counted by the technique of Brener[3] and Giemsa stained smears were used for the study of morphology[5].

Feces from positive bugs were inoculated i.p into 4 NMRI mice (av wt 15 g) at a rate of 100 metacyclics/g body weight. The course of parasitemia was followed by examination of fresh blood as described above.

The possible presence of *T. rangeli* was checked by examining the hemolymph and salivary glands of xenodiagnostic bugs[4].

Animals naturally infected with *T. cruzi* were sacrificed by anesthetic overdose with Ketaset (Ketamine HCl, Fort Dodge Labs, USA). Samples of heart, skeletal muscle, liver, spleen, colon, duodenum, pancreas, kidney, urinary bladder, lungs, sternum, and skin were fixed in 10% formol, imbedded in paraffin, cut into 5 μ sections, stained with hematoxylin and eosin, and examined at 1000 X. Mice infected by bug feces were examined by the same technique. Parasites detected in the viscera, in stained blood smears, and smears of bug feces were photographed with a Nikon Microflex HPX-35 on Ilford Pan F film.

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RESULTS

Thirty-seven adult and juvenile *R. rattus*, were captured in Colinas de Bello Monte, Los Chorros, El Cafetal, and Las Acacias. One *R. norvegicus* in Caricuao and 9 *Mus musculus* in Colinas de Bello Monte, Caricuao, San Roman, and Parque del Este were also captured. Of the 12 *R. rattus* captured in Colinas de Bello Monte, 10 showed infections with *T. (Herpetosoma) lewisi* on examination of stained blood smears. One of the remaining 2 rats had flagellates visible in fresh blood which had the typical pleomorphism of *T. cruzi*, with a predominance of stout forms (Fig. 1a) and a parasitemia of 20×10^3 flagellates/ml blood. The other rat proved positive for *T. cruzi* by xenodiagnosis.

Stained smears of fecal material from the xenodiagnosis bugs had polymorphic forms resembling *T. cruzi* (Fig. 1b), and when inoculated into mice, infected all of them (strain CO 22). In the mice the prepatent period of the parasitemia averaged 13 days, with a low peak of parasitemia (av 45×10^3 trypomastigotes/ml blood) on day 21 post-inoculation, at which time the mice were dying.

None of the experimental bugs had flagellates in the hemolymph or salivary glands, thus discarding the possibility of *T. rangeli* infection.

Tissue sections from the rat with patent parasitemia (strain CO 84) showed numerous pseudocysts (Fig. 2) with intracellular amastigotes in the heart and moderate parasitism of the smooth muscle of the duodenum and of skeletal muscle. All mice inoculated with bug fecal material (strain CO 22) showed a moderate pattern of tissue tropism in the same organs and also in the colon and pancreas.

DISCUSSION

Natural *T. cruzi* infections in synanthropic rodents (*R. rattus*, *R. norvegicus*, *M. musculus*) have been reported from Texas (USA) to southern Brazil[2]. *R. rattus* is widely distributed, living in sylvatic ecotopes and in human shelters, where they may be abundant and have *T. cruzi* infection rates of up to 30%[15].

This intimate contact with humans has led them to be considered as important reservoir hosts in Venezuela, Colombia, Brazil, Peru, Panama, Costa Rica, and Trinidad[2, 9, 12, 19].

Experimental infections in laboratory animals with strains isolated from reservoir hosts are nearly always light, of long duration, chronic, and not lethal. Parasitemias are subpatent, detecTABLE only by xenodiagnosis and/or blood culture. Tissue parasitism is light or absent[6]. Our isolates from *R. rattus* behaved differently. All mice inoculated with fecal material from bugs used for xenodiagnosis (strain CO 22) showed low parasitemia and tissue parasitism that was not correlated with the death of the animals. They showed erection of the hair, low level of movement, intense urination, and limb paralysis before death, symptoms of a severe *T. cruzi* infection. LAINSON et al. (1979)[8] have reported lethal experimental infections in mice by isolates of *T. cruzi* from sylvatic or peridomestic mammals. In these there were abundant pseudocysts in the tissues, but very few bloodstream trypomastigotes.

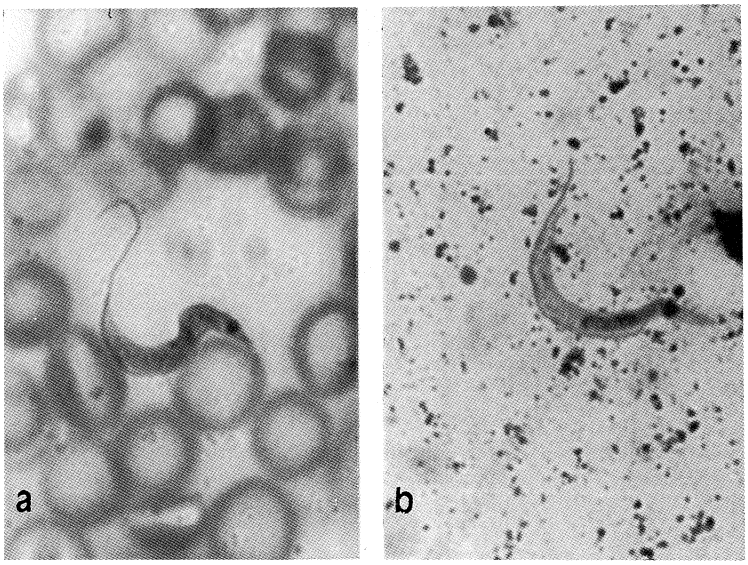

Fig. 1 – Flagellate stages of *Trypanosoma cruzi*:
a) Stout bloodstream trypomastigote from naturally infected *Rattus rattus*. (Giemsa, 1400 X)
b) Metacyclic trypomastigote from feces of *Rhodnius prolixus* used for xenodiagnosis of naturally infected *Rattus rattus*. (Giemsa, 1400 X)

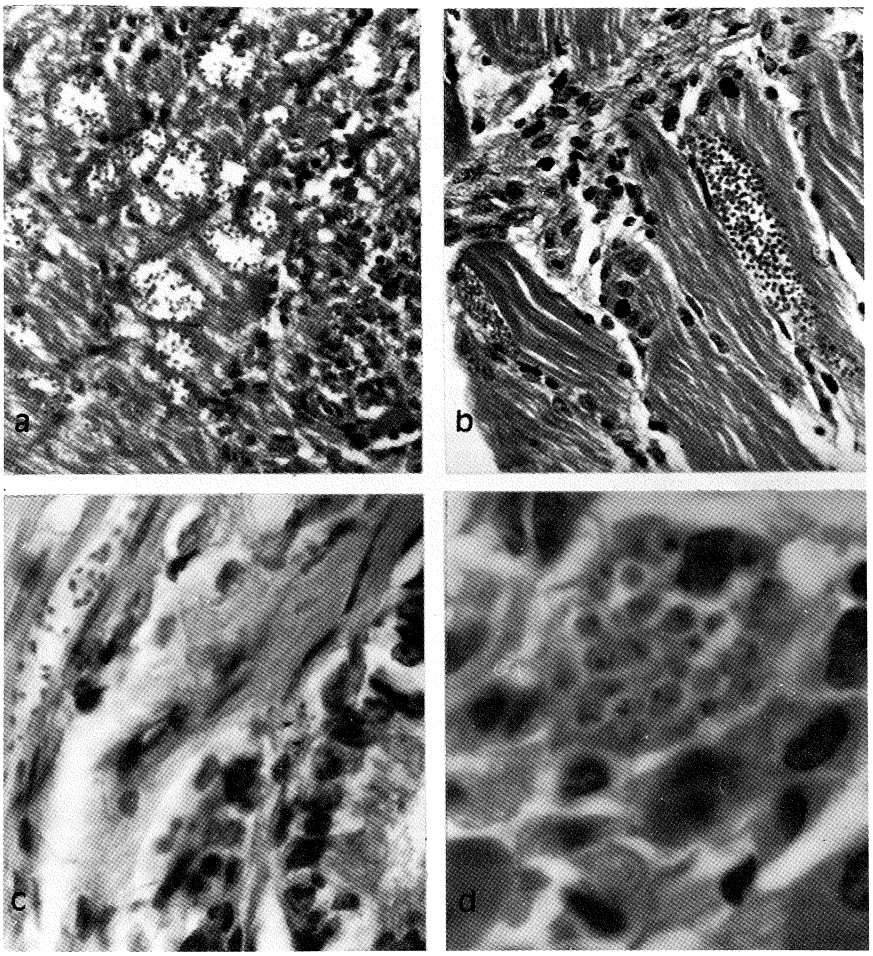

Fig. 2 – Histological sections showing pseudocysts of *Trypanosoma cruzi* with amastigotes in:
a) Cardiac tissue of naturally infected *Rattus rattus* (strain CO 84, Hematoxylin-eosin, 560 X)
b) Skeletal muscle of naturally infected *Rattus rattus* (strain CO 84, Hematoxylin-eosin, 560 X)
c) Smooth muscle fiber from colon of experimentally infected mouse (strain CO 22, Hematoxylin-eosin, 960 X)
d) Acinar cell of pancreas of experimentally infected mouse (strain CO 22, Hematoxylin-eosin, 1400 X)

Natural infections in *R. rattus* and *R. norvegicus* have been generally studied in sylvatic animals[2, 10, 13] or from rural areas[2, 10, 18]; few studies have been made on urban animals[9, 11].

Thirteen species of rodents have been incriminated in Venezuela as reservoir hosts for *T. cruzi*[15], of which *R. rattus*, *R. norvegicus*, and *Mus musculus* live in close contact with humans, thus being important links in the transmission of *T. cruzi* to man and domestic animals.

The few studies of tissue parasitism in experimental animals by isolates of *T. cruzi* from *R. rattus* have shown invasion of heart and brain[18]; our studies have demonstrated cardio- and myotropism. As far as we know, the tissue tropism in naturally infected synanthropic rodents has not been investigated; we have observed many pseudocysts in cardiac muscle and in the smooth muscle of the duodenum, and moderate invasion of skeletal muscle in a naturally infected rat.

Our findings of natural *T. cruzi* infections in urban areas of the valley of Caracas, in the blood of opossums[6] and of rats indicate that natural foci of Chagas' disease exist, and, while humans are not in direct contact with them, there is nevertheless a potencial epidemiological threat[7]. Infected reservoirs and vectors are present[6, 14] in the midst of a large human population of marginal sanitation.

Among the triatome vector – reservoir host complexes, the bug *Panstrongylus geniculatus* has been linked with opossums and armadillos[2]. This hematophagous insect is the only known

vector in the valley of Caracas[13], so that it presumably also feeds on rodents, particularly *R. rattus*.

RESUMO

Reservatórios de roedores sinantrópicos de *Trypanosoma* (*Schizotrypanum*) *cruzi* no Vale de Caracas, Venezuela

Exame direto de sangue e xenodiagnóstico de 47 roedores sinantrópicos (*Rattus rattus*, *R. norvegicus*, *Mus musculus*) capturados no Vale de Caracas, Venezuela, revelaram infecção por tripanosoma em 12 *R. rattus*, 10 com *T. lewisi* e 2 com *T. cruzi*. Dos últimos o curso de parasitemia, o pleomorfismo dos tripomastigotas na corrente sangüínea, tropismo tissular em ratos e camundongos natural e experimentalmente infectados, mortalidade dos hospedeiros, morfologia dos parasitas fecais em *Rhodnius prolixus* usados para xenodiagnóstico e infectividade das fezes do "barbeiro" para camundongos NMRI, foram todos característicos de *Trypanosoma* (*Schizotrypanum*) *cruzi*. Um rato com parasitemia patente, tinha numerosos ninhos de amastigotas no músculo cardíaco e parasitismo moderado do músculo liso do duodeno e do músculo esquelético. Camundongos inoculados com flagelados fecais de "barbeiros" tinham tropismo tissular moderado nos mesmos órgãos e também no cólon e pâncreas. O possível papel do *R. rattus* no estabelecimento de focos de doença de Chagas em Caracas é discutido.

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