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AN EXPERIMENTAL INFECTION OF *Trypanosoma cruzi* IN STRIPED SKUNKS *(Mephitis mephitis)*

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Abstract: Four adult (3 male, 1 female) captive-raised, descented, striped skunks *(Mephitis mephitis)* were infected experimentally with a field strain (Texas-Tulane) of *Trypanosoma cruzi,* originally isolated from a naturally-infected dog. Two skunks were injected intravenously with approximately 4.5×10^6 viable *T. cruzi* trypomastigotes. Two skunks were inoculated *per os* and *per conjunctivum* with 10 ml of phosphate buffered saline containing macerated, *T. cruzi*-infected triatomine intestines and intestinal contents. The skunks had minimal clinical manifestations with no mortalities occurring during 46 days post-exposure. Sera from all skunks were positive at 24 days post-inoculation (PI) by the direct and latex agglutination tests. Blood cultures from the 4 skunks were positive for *T. cruzi* at day 24 PI and 3 were positive at day 46 PI. All skunks had mild to moderately severe chronic granulomatous myocarditis of the atria and ventricles. Typical *T. cruzi* amastigotes were present within myocardial fibers in 3 of 4 skunks.

INTRODUCTION

A wide variety of wild animals has been shown to serve as reservoir hosts of *Trypanosoma cruzi,* the etiologic agent of American Trypanosomiasis or Chagas' disease. Flagellates morphologically indistinguishable from *T. cruzi* have been reported in about 150 mammalian species in the Western Hemisphere.[1,9,17] In various studies in the United States raccoons *(Procyon lotor),*[3,8,12] opossums *(Didelphis marsupialis),* armadillos *(Dasypus novemcintus)* and small rodents[7,14] have been shown to be naturally infected with *T. cruzi.* In a survey for leptospirae in mammals from Georgia and northwestern Florida, "*T. cruzi*-like" organisms were isolated from opossums, raccoons, gray foxes *(Urocyon cinereoargenteus)* and striped skunks *(Mephitis mephitis).*[10] More recently, *Triatoma lecticularius, T. gerstaeckeri,* and *T. sanguisuga* naturally infected with *Trypanosoma cruzi* were found in dog houses in Texas.[13] Thus the potential for a public health hazard exists in the southern United States.[6] Two human cases of Chagas' disease have been reported in Texas.[11,15] The possibility exists that low-grade infections occur, because they are difficult to detect.[4,16]

Since little is known about the disease in the sylvatic reservoir hosts of *T. cruzi,* a study to evaluate the susceptibility, reservoir potential, and pathogenesis of *T. cruzi* in the striped skunk was initiated.

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MATERIALS AND METHODS

Four adult (3 male and 1 female) captive-raised, deodorized, striped skunks *(M. mephitis)* 2.5 years of age were utilized as experimental animals in the study. The skunks were wild-born litter mates that had been captured at 3 weeks of age. The skunks had been held in isolation for over 1.5 years prior to the experiment.

The skunks were kept in individual stainless steel cages and held in an isolation facility from 1 week prior to inoculation until the termination of the project. Urine and feces were collected daily and incinerated. The skunks were offered commercial dried dog food and water *ad libitum*. Ketamine HCl [6] (0.7 ml) injected intramuscularly was used to anesthetize the skunks prior to any handling.

All the skunks were bled via the jugular vein immediately prior to inoculation, at 24 days post-inoculation (PI), and at 46 days PI. A portion (4-5 ml) of the blood was allowed to clot at room temperature, the sera decanted and stored at -20 C. Another portion (2-3 ml) of blood was transferred into an EDTA Vacutainer and later into a Liver-Infusion Tryptose (LIT) medium, a fluid medium derived by one of us (R.G. Yaeger) from an earlier described medium.[5]

Two of the skunks (2794 and 2796) were inoculated by injecting 1.5 ml of infected CD-1 mouse blood containing 4.5×10^6 viable *T. cruzi* trypomastigotes into the jugular vein via a Butterfly infusion set. The CD-1 mice (15 gm) had been inoculated intraperitoneally (IP) 11 days prior to bleeding. A field strain of *T. cruzi* (Texas-Tulane) originally isolated from a naturally-infected dog was utilized in the experiment.

The remaining two skunks (2944 and 2795) were inoculated via the oral-mucosal route by dripping 10 ml of phosphate buffered saline (PBS) containing *T. cruzi*-infected triatomine intestines and intestinal contents onto the oral mucosa and conjunctiva. Nine *Rhodnius prolixus* nymphs which had fed on a CD-1 mouse (with a parasitemia of 5 trypanosomes per high power field in a tail-blood, wet cover glass preparation 16 days post-inoculation IP with *T. cruzi)* were dissected, the hindguts removed and placed in 20 ml of PBS to produce the inoculum.

On day 46 PI the skunks were euthanatized and representative tissue samples of visceral organs were taken at necropsy, fixed in 10% buffered formalin, paraffin embedded, sectioned at 4 μm and stained with hematoxylin and eosin.

The sera were tested for serum antibodies by the latex agglutination test [7] and the direct agglutination test.[2]

Several days after inoculation with blood the LIT media were overlaid on Tobie's blood agar slants. The cultures were incubated at room temperature and examined periodically for 3 months.

RESULTS

Serologic and culture

Sera from all skunks taken prior to inoculation were negative by the latex agglutination test (LAT). All sera taken at 24 days PI and 3 of the 4 taken at 46 days PI were positive in the LAT.

All sera taken prior to inoculation had titers of ≤ 16 by the direct agglutination test (DAT). At 24 and 46 days PI all the skunks had titers of $\geq 16{,}384$. Two of the skunks (2944 and 2796) had titers $\geq 65{,}536$ (Table 1).

The results of the blood culture prior to inoculation were negative. All the blood taken at day 24 PI and cultured was

[6] Parke-Davis & Co., Detroit, Michigan 48232, USA.

[7] Rap/Tex Kit, Behring Diagnostic, Hoechst-Roussel Pharmacy, Inc., Somerville, New Jersey 08876, USA.

TABLE 1. Summary of serologic and culture results of experimental infection of *Trypanosoma cruzi* in striped skunks (*M. mephitis*).

I.D. #	Route of Inoc.	Latex Agglutination			Direct Agglutination			Blood Culture		
		Pre-Inoc.	Day 24	Day 46	Pre-Inoc.	Day 24	Day 46	Pre-Inoc.	Day 24	Day 46
2944	Oral-mucosal	(-)	(+)	(+)	1:16	1:32768	1:65536	(-)	(+)	(+)
2794	I.V.	(-)	(+)	(-)	1:8	1:16384	1:32768	(-)	(+)	(-)
2795	Oral-mucosal	(-)	(+)	(+)	1:16	1:32768	1:32768	(-)	(+)	(+)
2796	I.V.	(-)	(+)	(+)	1:16	1:65536	1:65536	(-)	(+)	(+)

positive, as were 3 of the 4 blood cultures taken at day 46 (2794 was negative).

Histopathologic

All the skunks had a mild-to-moderately severe focal-to-diffuse chronic granulomatous myocarditis of the atria and ventricles. *T. cruzi* amastigotes were present within the myocardial fibers in 3 of 4 skunks. All had lesions attributable to trypanosomiasis, seen as focal infiltrations of lymphocytes and plasma cells as well as reticuloendothelial macrophages. The residual microgranulomas were most prominent in the myocardium and gastric muscularis, the esophageal muscularis and the upper intestinal muscularis, respectively. At 46 days PI the lesions appeared to be resolving and few organisms were identified.

DISCUSSION

Although the skunks failed to show overt clinical signs during the experiment and no gross lesions were seen at necropsy, the serologic, blood culture, and histopathologic results confirmed that all 4 skunks were infected successfully. No apparent difference was observed with regard to the mode of infection with the exeption of skunk 2794, which was inoculated intravenously. At 43 PI, 2794 was LAT negative and blood culture negative. This animal, however, was apparently one of the most severely affected as measured by the residual microgranulomas seen microscopically and at day 46 PI had a DAT of 32,768.

In central Texas striped skunks occupy ground burrows and hollow trees, also a common niche for triatomids in that area. The asymptomatic nature, susceptibility, and prolonged parasitemia of *T. cruzi* in striped skunks as indicated by the experimental infection suggest that *M. mephitis* should be regarded as a potential reservoir host for Chagas' disease in the Southwest.

LITERATURE CITED

1. ADAMS, L.G. and G.D. WILLIAMS. 1979. American Trypanosomiasis. In: *Zoonoses — An Update 1979*. P.R. Schnurrenberger, Ed. Iowa State University Press, Ames, Iowa. (In press)
2. ALLAIN, D.S. and I.G. KAGAN. 1974. An evaluation of the direct agglutination test for Chagas' disease. J. Parasit. 60: 179-184.
3. BROOKS, M.M., L. NORMAN, D. ALLAIN and G.W. GORMAN. 1957. Isolation of *Trypanosoma cruzi*-like organisms from wild animals collected in Georgia. J. Parasit. 43 (Suppl.) 15.
4. CHANDLER, A.C. and C.P. READ. 1961. *Introduction to Parasitology*. 10th ed., John Wiley and Sons, Inc., New York 822 pp.
5. CHANG, S.L. 1947. Studies on haemoflagellates. I. A semi-solid medium and a fluid medium with a solid base for growing various species of *Leishmania* and *Trypanosoma cruzi*. J. Infect. Dis. 80: 164-171.
6. DIAS, E. 1951. Doenca de Chagas nas Americas. I. Estados Unidos. Rev. Bras. de Malariologia. 3: 448-472.
7. FAUST, E.C. 1949. The etiologic agent of Chagas' disease in the United States. Bol. San Panam. 28: 455-461.
8. HABERMANN, R.T., C.M. HERMAN and F.P. WILLIAMS. 1958. Distemper in raccoons and foxes suspected of having rabies. J. Am. vet. med. Ass. 132: 31-35.
9. HOARE, C.A. 1972. *The Trypanosomes of Mammals*. F.A. Davis Co., Philadelphia, Pennsylvania 575 pp.
10. McKEEVER, S., G.W. GORMAN and L. NORMAN. 1958. Occurrence of a *Trypanosoma cruzi*-like organism in some mammals from southeastern Georgia and northwestern Florida. J. Parasit. 44: 583-587.
11. NATIONAL OFFICE OF VITAL STATISTICS: Communicable disease summary for week ending December 19, 1955. U.S. Dept. Health, Ed. and Welf., Washington, D.C.
12. WALTON, B.C., P.M. BAUMAN, L.S. DIAMOND and C.M. HERMAN. 1958. The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. Am. J. Trop. Med. and Hyg. 7: 603-610.
13. WILLIAMS, G.D., L.G. ADAMS, R.G. YAEGER, R.K. McGRATH, W.K. READ, and W.R. BILDERBACK. 1977. Naturally occurring trypanosomiasis (Chagas' disease) in dogs. J. Am. vet. med. Ass. 171: 171-177.
14. WOOD, F.D. and S.F. WOOD. 1941. Present knowledge of the distribution of *Trypanosoma cruzi* in reservoir animals and vectors. Am. J. Trop. Med. 21: 335-345.
15. WOODY, N.C. and H.B. WOODY. 1955. American trypanosomiasis (Chagas' disease): first indigenous case in the United States. J. Am. med. Ass. 159: 676-677.
16. YAEGER, R.G. 1961. The present status of Chagas' disease in the United States. Bull. Tulane Univ. Med. Faculty 21: 9-13.
17. ZELEDON, R. 1974. Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In: *Trypanosomiasis and Leishmaniasis*. Ciba Foundation Symposium 20. Amsterdam, Ass. Sci. Publ., pp. 51-85.

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