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Survival of Trypanosoma cruzi in Dead Chronically Infected Mice

Lawrence C. Hinck
Arkansas State University

James L. Stevens
Arkansas State University

Rogers D. Meurer
Merck Institute for Therapeutic Research

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The following summarizes efforts made thus far to protect important Arkansas gray bat caves. Some of these efforts have been previously reported (Harvey, 1975, 1976a, 1976b, 1979, 1980; Harvey et al., 1979).

The very important gray bat hibernaculum located in the Ozark National Forest of Baxter County was gated by the U.S. Forest Service in 1975. The gate was subsequently redesigned to better fit the particular conditions at the cave and a replacement gate was installed during the summer of 1980. Five Buffalo National River caves have been fenced by the National Park Service to protect gray bat (and Indiana bat) colonies. One cave located on a private housing development in Benton County has been fenced by the developer to protect a gray bat summer colony. The U.S. Army Corps of Engineers recently constructed an artificual entrance into a gray bat cave located on Beaver Lake in Benton County, since the natural entrance is sometimes inundated during high lake levels.

In addition to the above measures, the Arkansas Game and Fish Commission, U.S. Fish and Wildlife Service, U.S. Forest Service, and National Park Service have placed warning/interpretive signs at several gray bat caves. Other agencies and organizations involved in the gray bat conservation effort include the Nature Conservancy, Arkansas Department of Parks and Tourism, Arkansas Natural Heritage Commission, National Speleological Society, Cave Research Foundation, Association for Arkansas Cave Studies, and Ozark Underground Laboratory.

Important gray bat hibernacula and summer caves will be monitored for the next several years. Additional measures will also be taken to protect other important gray bat caves. Hopefully, the conservation effort will result in removal of the gray bat from the endangered species list.

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MICHAEL J. HARVEY, Ecological Research Center, Department of Biology, Memphis State University, Memphis, TN 38152. (Present address, Department of Biology, Tennessee Technological University, Cookeville, TN 38505.)

SURVIVAL OF *TRYPANOSOMA CRUZI* IN DEAD CHRONICALLY INFECTED MICE

Trypanosoma cruzi (Chagas, 1909), the cause of Chagas' disease, is a flagellate protozoan found throughout most of Central and South America where it infects approximately 12 million people (World Health Organization, 1960). In the United States this agent has been reported in wildlife mammals in California, Arizona (Kofoid and Whitaker, 1936), Texas (Packchanian, 1941), New Mexico (Dias, 1951), Minnesota (Hedrick, 1955), Virginia (Tromba, 1951), Maryland (Walton *et al.*, 1956), Georgia, Florida (McKeever *et al.*, 1958), Alabama (Olsen *et al.*, 1964), and Louisiana (Yeager and Bacigalupo, 1960). Cited reservoir hosts include such common mammals as the raccoon *(Procyon lotor)*, opossum *(Didelphis virginiana)*, grey fox *(Urocyon cinereoargenteus)*, and striped skunk *(Mephitis mephitis)* (McKeever *et al.*, 1958).

The normal transmission of *T. cruzi* occurs when a bug (Hemiptera:Reduviidae) takes a blood meal and deposits feces containing trypomastigotes on the skin. The parasites enter the bite wound and invade cells of many organs throughout the body as they circulate in the blood for approximately 30 days or longer. Upon entering a cell, the trypomastigote typically loses its flagellum and transforms into an amastigote which reproduces by binary fission. During the acute phase amastigotes produced intracellularly are released by cell lysis and either reinvade new cells or transform into trypomastigotes and reenter the circulation. The cells most often attacked are reticuloendothelial cells of the liver and spleen, cells of cardiac, smooth and skeletal muscle and certain cells of the nervous system. Animals surviving the acute phase typically develop chronic infections in which amastigotes form pseudocysts in the host's tissues producing only limited cell lysis. In the chronic infection, trypomastigotes in the blood are scanty and difficult to demonstrate by microscopic examination.

Epidemiologic studies of zoonoses, such as Chagas' disease, contribute to the basic understanding of the biology of the etiologic agents and provide information relating to both geographic distribution and transmission. Such information can be useful in prevention and control of these diseases which may pose a threat to man, domestic animals and wildlife. Often studies of this nature have required the capture and, in some cases, the sacrifice of potential wildlife reservoirs.

Meurer (1980), in a preliminary study, demonstrated that *T. cruzi* could be detected in dead chronically infected mice by inoculating a modified

Tobie's diphasic medium (Tobie *et al.*, 1950) with a homogenate of heart tissue. *T. cruzi* was cultured from two of three mice that had been held for 24 hours at 25 C while three out of three mice were positive after 24 hours at 5 C. The present study was conducted to more accurately assess the relationship between holding temperature and parasite survival in dead mice that were chronically infected and to compare the efficiency of in vitro cultivation with animal inoculation for detecting *T. cruzi* in tissue homogenates of dead animals.

The Calluromys LNX strain of *T. cruzi* was used in our current study since it typically produces a less intense acute phase parasitemia followed by a chronic infection in which mice have an extended survival time. Chronic infections were achieved by injecting mice subcutaneously with approximately 6 x 10^4 trypomastigotes (determined by the method of Herbert and Lumsden, 1976) obtained from mice with acute infections. ASU Lilac-Wild cross mice were used for this purpose since these animals are hardy and tend to develop chronic infections consistently. Mice were considered to be in the chronic phase when microscopic examination of tail blood from previously patent animals revealed very low or negative parasitemias. In most mice this was around 50 days postinoculation.

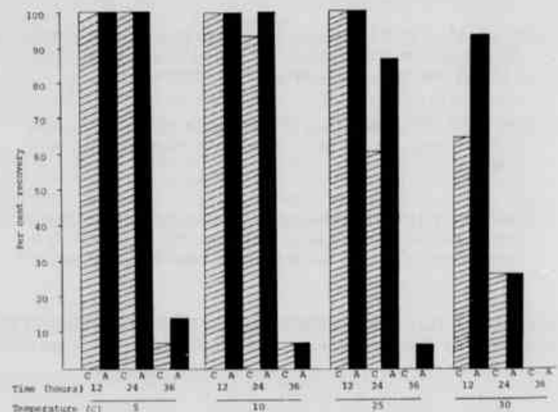
Chronic phase mice were sacrificed and held at temperatures ranging from 5 C to 35 C. Holding periods ranged from zero to 48 hours for lower temperatures while 36 hours was maximum time for higher temperatures. The heart and liver were aseptically removed from each animal and homogenized in a Ten-Broeck tissue grinder with 2.0 ml sterile Locke's solution containing 18.5 µg/ml gentamicin to prevent bacterial growth. From each homogenate, two mice (ASU Piebald) were injected subcutaneously with 0.5 ml portions and two tubes of modified Tobie's diphasic medium (with human blood) containing gentamicin were inoculated with 0.2 ml each. ASU Piebald mice were used for this phase of the study because of their susceptibility to infection. Mice were checked for parasitemias periodically for a period of 30 days after which time they were declared negative. In vitro culture tubes were incubated at 25 C for 10 days before discarding.

Results of the study indicate that survival time of *T. cruzi* in chronically infected mice is highly dependent on the holding temperature (Figure). At 5 C *T. cruzi* was detected in 100% of the mice held for as long as 24 hours when tested by in vitro cultivation or by animal inoculation. With increasing temperature, the recovery rate of *T. cruzi* from dead animals decreased. At 10 C the recovery rate for 24 hours was still 100% by animal inoculation and 93% by in vitro cultivation. The 24-hour positive fell to 60% and 87% for in vitro cultivation and animal inoculation respective at 25 C while at 30 C the rate dropped to 27% for both methods. The Table reveals that no parasites were detected in animals held at 35 C for 24 hours.

Table. Recovery of *Trypanosoma cruzi* from chronically infected mice by in vitro cultivation and mouse inoculation.

Holding time (hours)	Holding temperature (°C)	Recoveries by in vitro cultivation[a]	Recoveries by mouse inoculation[b]
0	5	15/15	15/15
6	"	15/15	15/15
12	"	15/15	15/15
24	"	15/15	15/15
36	"	1/15	2/15
48	"	0/15	0/15
0	10	15/15	15/15
6	"	15/15	15/15
12	"	14/14	14/14
24	"	14/15	15/15
36	"	1/15	1/15
48	"	0/14	0/14
0	25	15/15	15/15
6	"	15/15	15/15
12	"	15/15	15/15
24	"	9/15	13/15
36	"	0/18	1/15
48	"	0/15	0/15
0	30	15/15	14/15
6	"	14/14	14/14
12	"	10/15	14/15
24	"	4/15	4/15
36	"	0/15	0/15
0	35	15/15	15/15
6	"	14/15	15/15
12	"	2/15	3/15
24	"	0/15	0/15
36	"	0/15	0/15

[a]A modified Tobie's diphasic medium was inoculated with heart-liver homogenate and incubated at 25 C.
[b]Mice were injected subcutaneously with heart-liver homogenate.



C = Per cent recovery by inoculation of a modified Tobie's diphasic medium with heart-liver homogenate.
A = Per cent recovery by subcutaneous injection of mice with heart-liver homogenate.

Figure. Relationship of holding time and holding temperature to recovery rate of *Trypanosoma cruzi* from chronically infected mice.

The results also suggest that animal inoculation is a somewhat more sensitive method than in vitro cultivation for detecting *T. cruzi* in the tissue of dead animals. It was clear, however, from trial runs with several strains of mice that a highly susceptible line of mice is important for this purpose.

In the epidemiology of *T. cruzi* it appears likely that dead chronically infected animals might serve as a source of infection for scavenging animals for a period of at least 24 hours at moderate temperatures and possibly as long as 36 hours at cool temperatures. The results also point to in vitro or in vivo cultivation from heart-liver homogenates as an acceptable method for assessing the distribution and incidence of *T. cruzi* in wildlife. These animals could be road kills or those taken by fur trappers.

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LAWRENCE C. HINCK and JAMES L. STEVENS, Department of Biological Sciences, Arkansas State University, State University, AR 72467; and ROGER D. MEURER, Merck Institute for Therapeutic Research, Rahway, N.J.

EFFECT OF THE PAYMENT-IN-KIND (PIK) PROGRAM ON THE *PSOROPHORA COLUMBIAE* MOSQUITO POPULATION OF A NORTHEAST ARKANSAS RICEFIELD COMMUNITY

Since 1904, when rice was initially cultivated as a commercial crop within the state, Arkansas has developed into one of the five major rice-producing states in the U.S. with an annual production of ca. 6,000,000 ha (Meisch, et al., 1982). Although some rice is grown in central and southwestern counties, the majority of the crop is planted in the eastern half of the state, particularly in the "Grand Prairie" area of east-central Arkansas which includes Arkansas, Lonoke, Monroe, and Prairie counties.

It has been well established by a number of investigators in Arkansas, and in other rice-growing states, that the rice agroecosystem provides suitable breeding sites for several mosquito species including the dark ricefield mosquito, *Psorophora columbiae* (Dyar and Knab). In Arkansas, Schwardt (1939), Horsfall (1942), Whitehead (1951), Meisch and Coombes (1975), and Olson and Huggins (1983), among others, all have reported *P. columbiae* to be the dominant mosquito species wherever rice is cultivated. Whitehead (1951), in particular, noted that ricefield mosquito numbers have increased in direct proportion to the state's increased rice acreage.

In Craighead County in NE Arkansas, land devoted to rice production in 1981 and 1982 was 33,590 and 33,376 ha, respectively (Olson and Huggins, 1983). Light trap studies conducted in Jonesboro by these investigators indicated that, of 34,041 adult mosquitoes captured between 30 May and 2 October of 1981, 21,085 (61.9%) were *P. columbiae*. During the same period in 1982, *P. columbiae* numbered 24,675 (72.3%) of the 34,114 mosquitoes trapped.

In 1983, a federally funded payment-in-kind (PIK) program was implemented in an attempt to reduce national agricultural surpluses and to improve market prices by paying growers to keep land out of commercial crop production. As a result, American farmers idled some 31,000,000 ha including significant rice acreage in NE Arkansas. Under the PIK program, the amount of Craighead-County rice land was lowered by 39.0% to 20,350 ha (Fagala, Craighead Co. Extension Service, pers. comm.).

The primary aim of this study was to evaluate the effect which this acreage reduction had upon the relative abundance of *P. columbiae* in Jonesboro. To accomplish this objective, a standard New Jersey light trap was placed at each of four locations within the city limits. Sampling dates and trap locations (two peripheral and two central) were identical to those described by Olson and Huggins (1983). Light trap catches were collected daily and adult mosquitoes sorted and identified. Daily trap totals were summed for each week of the 18-week study period and compared with data obtained in 1981 and 1982.

It should be noted that, as in 1981 and 1982, all traps were in areas subjected to periodic applications of a mosquito adulticide by ground-operated ULV cold-aerosol generators. This undoubtedly lowered the total number of mosquitoes collected, particularly in the central-city area which was further from rice fields which served as the main source of *P. columbiae* reinfestation.

A total of 18,232 adult mosquitoes was captured between 30 May and 2 October of 1983, with the PIK program in effect. This represented an overall decrease in the general mosquito population of 46.5% in comparison with 1981 and 1982 pre-PIK totals reported by Olson and Huggins (1983). As anticipated, *P. columbiae*, with 13,394 trapped individuals, was the dominant species and it comprised 73.5% of the season's catch. Other genera contributing to the remainder of the total were *Anopheles* (16.0%), *Aedes* (6.5%), *Culex* (4.0%), and *Culiseta* (less than 0.1%). It was observed that the 39.0% decrease in Craighead-County rice acreage in 1983 was accompanied by a 43.4% reduction in the *P. columbiae* population trapped within the Jonesboro city limits. This was believed to have been a direct result of the lessening of available breeding sites normally