

EXOTIC STOCK OF *TRYPANOSOMA CRUZI* (*SCHIZOTRYPANUM*) CAPABLE OF DEVELOPMENT IN AND TRANSMISSION BY *TRITOMA PROTRACTA PROTRACTA* FROM CALIFORNIA: PUBLIC HEALTH IMPLICATIONS

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Abstract. A stock of *Trypanosoma cruzi* was recovered from a *Triatoma dimidiata* from Tegucigalpa, Honduras. This stock was shown to be capable of development and transmission by native California *Triatoma protracta protracta*. Isozyme analysis indicated that this *T. cruzi* is closely related to the Tehuantepec strain and to a lesser extent the Miles' zymodeme 1 strain. The potential public health significance of development and transmission of exotic stocks of *T. cruzi* by native reduviids is discussed.

MATERIALS AND METHODS

On 16 May 1983, a single *Triatoma dimidiata* female was received from Tegucigalpa, Honduras. The bug had been captured in a modern house in that city 10 days before. Upon examination of feces from the hindgut, trypanosomes morphologically identical to *Trypanosoma cruzi* were seen.

An ICR inbred laboratory mouse was inoculated intraperitoneally with hindgut contents from the *T. dimidiata* on 19 May 1983 and maintained in arthropod-free quarters until 10 August, when it was killed and the heart aseptically removed and cultured in Nicolle's modified Novy and MacNeal medium (NNN).¹ The culture medium was examined microscopically on 2 September and found to contain epimastigote forms. Further serial passages were continued in Noguchi-Wenyon (N-W) media.¹

Eight wild-caught *Triatoma protracta protracta* from Pleasant Valley, near Winters, California, were examined by collecting fecal material from the rectum. All were negative for trypanosomes. These "clean" bugs were fed on inoculated mice on 10 May and 22 June 1984, and their feces were examined on 18 July and found positive for epimastigotes. Separately maintained cultures of material from the original *T. dimidiata*,

the isolations from the original *T. p. protracta*, and isolations from mice infected by feces from these *T. p. protracta* were studied in this investigation.

Isoenzyme analysis of the isolates

Epimastigotes from the three isolations were transferred to separate culture tubes of RPMI 1640 plus 20% fetal calf serum and serially passed until sufficient organisms had been collected for analysis. The methods used for growing, harvesting, lysing, and storing the organisms were those described by Tibayrenc and LeRay.²

Electrophoreses were carried out on cellulose acetate plates (Helena Laboratories, Beaumont, Texas). The following 14 enzyme systems were assayed: aconitase (aconitate hydratase, EC 4.2.1.3), adenylate kinase (EC 2.7.4.3), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose-6-phosphate isomerase (EC 5.3.1.9), glutamate dehydrogenase (EC 1.4.1.2), glutamate dehydrogenase (NADP⁺) (EC 1.4.1.4), isocitrate dehydrogenase (NADP⁺) (E 1.1.1.42) leucine aminopeptidase (cytosol aminopeptidase) (EC 3.4.11.1), malate dehydrogenase (EC 1.1.1.37), malate dehydrogenase (oxaloacetate decarboxylating (NADP⁺) or malic enzyme (E 1.1.1.40), peptidase 1 (ficin EC 3.4.22.3, formerly EC 3.4.4.12, substrate: leucyl-leucyl-leucine), peptidase 2 (bromelain, EC 3.4.22.4, formerly EC

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TABLE 1

Matrix of genetic distances between our Honduran stock, Tehuantepec, and Tulahuén reference strains, and Miles zymodeme 1

	Honduran	Tehuantepec	Tulahuén
Tehuantepec	0.17	—	
Tulahuén	1.13	1.15	—
Zymodeme 1	0.34	0.31	0.95

3.4.4.24, substrate: leucyl-L-alanine), phosphoglucomutase (EC 5.4.2.2, formerly E 2.7.5.1), and phosphogluconate dehydrogenase (E 1.1.1.44).

The assays for aconitase and adenylate kinase were performed according to Kreutzer et al.³ with slight modifications. Leucine aminopeptidase was performed according to Shaw and Prasad⁴ with modifications given by Tibayrenc and LeRay.² All other enzymes were assayed according to Lanham et al.⁵ with the modifications given by Tibayrenc and LeRay.² These 14 enzyme systems represent 15 enzymic loci, as malic enzyme yields two different loci.² Known *T. cruzi* reference strains (Tehuantepec and Tulahuén) were run simultaneously with each of the three sources of our *T. cruzi*.

Inoculation of ICR mice with *T. cruzi* isolate from *T. dimidiata*

Three male ICR mice 3 weeks of age were inoculated intraperitoneally with 50,000 organisms pooled from passages 4, 6, 7, and 8 of the *T. cruzi* organism isolated from *T. dimidiata* from Honduras. The cultures from which the inoculations were made were approximately 6, 5, 5, and 4 months old, respectively, at the time of use. The same mice were inoculated intraperitoneally a second time at 5 weeks of age with 100,000 organisms from passage 9 or 10 of the *T. dimidiata*-isolated *T. cruzi*. These cultures were 3 and 2 months old, respectively, at the time of use. These mice were used to feed the wild-caught *T. p. protracta*.

Feeding of wild-caught *T. p. protracta* on mice

Eight nymphal *T. p. protracta* were collected on 29 October 1983 in a wood rat nest from north Pleasant Valley, near the town of Winters, California. Examination of feces from these bugs did not reveal any trypanosome forms. These 8 bugs were fed on the inoculated ICR mice 1 and 2

months after the second inoculation. The bugs' feces were examined approximately 1 month after the second feeding. Thereafter, the bugs were fed at 1- and 2-month intervals for the next 12 months on uninoculated ICR mice and feces examined at each feeding.

Transmission of the Honduran *T. cruzi* by feces from infested *T. p. protracta*

Approximately 10 months after the *T. p. protracta* were found infested with the Honduran *T. cruzi*, all of the feces, passed from 2 bugs after feeding on an uninfected mouse, were collected. This fecal material (approximately 1×10^{-3} ml) was placed in the conjunctiva and buccal cavity of an anesthetized uninfected ICR male mouse. The mouse was maintained in a separate cage in arthropod-free quarters for 1 month before it was killed and its heart aseptically removed, ground in a sterile Tenbroeck tissue grinder, and the slurry cultured in N-W media.

RESULTS

Isoenzyme analysis

Three isolates (from the original *T. dimidiata*, the experimentally infected *T. p. protracta*, and the heart of the ICR mouse infected by feces from *T. p. protracta*) gave exactly the same patterns for all enzymes assayed. Although analysis of 121 known *T. cruzi* stocks from a wide geographic range has shown a large isozyme variability to exist⁷ two isozyme alleles (Adk1 and Mdh₂) were found to occur in all stocks, including all 3 of our isolates. In addition, another isozyme allele (Acon 1) was found in all but 1 of the 121 known *T. cruzi* stocks⁷ and all 3 of our isolates. We therefore consider our isolates to be *T. cruzi*. Evidence that all 3 of our isolates are indeed the same stock comes from the fact that all gave the exact same patterns for all enzymes assayed. These enzymes represent 15 different genetic loci and constitute a good characterization of a given stock. One pattern which is rare in *T. cruzi* but occurred in all 3 of our isolates is the slowest locus of malic enzyme. This locus exhibited a 3-banded heterozygous pattern. Since this pattern is rare among *T. cruzi* stocks it would be unlikely to find it in 3 separate isolates unless all of the isolates represented the same stock.⁶ Based upon our isoenzyme pattern we estimated the

genetic distances and hence the phylogenetic relationship of our Honduran stock.⁸ Genetic distance, estimated from isozyme data, gives the average number of codon differences per gene among populations, in this case of *T. cruzi* stocks. Table 1 gives such values⁷ between our Honduran stock, the two reference strains used in the present study, and Miles' zymodeme 1 (Miles et al.⁹). Our Honduran stock appears to be closely related to the Tehuantepec strain and to a lesser extent the Miles' zymodeme 1 strain.

Transmission of the Honduran T. cruzi by feces from infested T. p. protracta

Feces collected from 2 bugs following their feeding on an uninfected mouse on 28 May 1985 were placed into each conjunctival sac and the buccal cavity of an anesthetized uninfected mouse. The feces of both bugs were positive by wet mount examination for metacyclic forms. On 9 July 1985 2 out of 3 tubes of the N-W media, in which the mouse's ground-up heart had been placed 1 month after exposure were found to contain epimastigote forms of *T. cruzi*.

DISCUSSION

The identical results of the isoenzyme analyses of the isolates from the wild-caught Honduran *T. dimidiata*, the *T. p. protracta* infested by feeding on Honduran *T. cruzi*-inoculated mice and the heart of a mouse infested by feces from the *T. p. protracta* strongly suggests that all isolates are the same stock.

This demonstrates that some *T. p. protracta* from central California are sufficiently susceptible to Honduran *T. cruzi* that they can become infested when fed on mice with parasitemias low enough to be undetectable by blood smears during the first 2-3 weeks following injection. All 8 of the *T. p. protracta* became infected after feeding only twice on infected mice. Bugs remained infested for at least 12 months, and 2 of 8 remained infective to mice by conjunctival and buccal cavity contamination 10 months after initial infection.

Thirteen F₁ nymphs from the original 8 *T. protracta* were fed on a mouse infected by fecal contamination with Honduran strain of *T. cruzi* on 3 October 1985. On 7 January 1986, 4 of the 13 nymphs harbored epimastigotes and trypo-

mastigotes of *T. cruzi*; the other 9 nymphs were not examined.

Some strains of *T. cruzi* have shown greater infectivity to relatively local vectors than to more exotic ones. Little, Tay, and Biagi¹⁰ found the Mexican *T. barberi* more susceptible than Chilean *T. infestans* to 5 strains of Mexican *T. cruzi*. The degree of difference in susceptibility was not statistically significant, but the time from infective bloodmeal to first positive feces was shorter for *T. barberi*. All of their bugs were fed on mice with very high parasitemias (2.3×10^6 /cc); had mice with low level parasitemias been used, the difference in susceptibility might have been more apparent. Dias¹¹ also reported differences in susceptibility of Venezuelan vs. Brazilian *Rhodnius prolixus*. The Venezuelan bugs became infested with Brazilian *T. cruzi* only 55% of the time while those from Brazil became infested 90% of the time when fed on the same dog.

Ryckman¹² reported that *T. infestans* and *Panstrongylus megistus* infested with North American trypanosomes from rats consistently showed a low population density of metacyclic forms in their feces 30 days after feeding, and by the end of the third month metacyclic forms were virtually absent.

T. p. protracta is found over a wide area of the west and southwestern United States.¹³⁻¹⁶ *T. cruzi* has been reported to occur in several species of Reduviidae in the same areas,¹⁵⁻²¹ and in both wild and domestic animals throughout the United States.^{15, 18, 20-25} In addition, experimental infections of wild and domestic animals and humans have been achieved with strains of *T. cruzi* found in the United States.²⁶⁻²⁸

T. p. protracta in California is attracted to light, and adults will fly into houses or aggregate near street lights, particularly those that emit light in the ultraviolet wavelengths (i.e., mercury vapor street lamps and neon tube).^{29, 30} Of the 3 species of Triatominae found in California *T. p. protracta* is the most widespread, being found in both the Sierra Nevada foothills and coastal mountain ranges.³¹ Its major wildlife hosts are wood rats, particularly *Neotoma fuscipes* and *N. lepida*. The lifespan of *T. p. protracta* is approximately 1 year and involves an egg, 5 nymphal stages, and an adult stage.³¹ The major problem created by the bug in humans has been the reaction to its bite.³² The majority of the 110 cases of *T. p. protracta* bites reviewed by Walsh and Jones³¹ occurred between 12 midnight and 6 A.M.

Most people were bitten while in the home and found the bug in their bed.

T. cruzi is naturally transmitted in the United States to domestic animals and humans.^{22, 23, 33-36} The number of asymptomatic cases indicated by serologic surveys in asymptomatic populations of humans and animals indicates that transmission is occurring at a greater rate than is clinically suggested. Following the first naturally acquired case of Chagas' disease in California,³⁶ Navin et al.³⁷ found 6 of 10 dogs in the vicinity of the index case to have positive complement-fixing (CF) antibody to *T. cruzi*, 2.5% of 237 humans living nearby to be positive, as well as 0.7% of 1,706 humans positive in the community of the index case. Likewise 6 of 951 sera collected from people living in Georgia were positive for CF antibody to *T. cruzi*.³⁵ Two of these 6 were suffering from chronic, unexplained heart failure. Woody³⁸ also demonstrated 7 CF-positive children out of 500 sampled in Corpus Christi, Texas. He reported that the homes of all the seropositive cases were infested with triatomine bugs. Thus, while there have been only 3 human cases^{33, 34, 36} and 10 canine cases^{22, 23} of acute Chagas' disease reported as naturally acquired in the United States, actual transmission appears to be more frequent and widespread than clinical cases would indicate.

Pathogenic strains of *T. cruzi* do exist in the continental United States. The reason more cases from blood transfusion and natural transmission have not been detected is probably due to several factors: the limited distribution of pathogenic strains, lack of true domestic reduviid species in the United States as compared to Central and South America, and better home construction in most areas of the United States in comparison to Latin America.

The ability of native *T. p. protracta* to support exotic strains of *T. cruzi*, as shown by our work, is of public health importance. Wildlife reservoirs of *T. cruzi* such as wood rats, ground squirrels, opossums, coyotes, and skunks are widely distributed in the United States. Human contact with *T. p. protracta* occurs with some frequency, prompting natural transmission of native strains of *T. cruzi* in humans and domestic dogs. The introduction of pathogenic strains of *T. cruzi* from Central and South America could lead to established enzootic foci that would be very difficult to eliminate once in the wildlife or domestic animal reservoirs. Were such to occur, humans

could be exposed to potentially more pathogenic strains of *T. cruzi*.

According to the United States Immigration and Naturalization Service, 19,951 immigrants entered the U.S. from Honduras between 1975 and 1984, excluding 1982 for which data were not available. Considering the prevalence rate of *T. cruzi* infections in Hondurans reported by Ponce³⁹ there may be as many as 7,000 individuals in the U.S. with Honduran strains of *T. cruzi*. Cardiologists in the United States need to recognize the potential of Central and South American immigrants presenting with chronic heart disease due to *T. cruzi*.⁴⁰ Blood banking officials should also be aware of the potential for transmission of *T. cruzi* by blood transfusion and realize that at present no treatment of whole blood is carried out in the United States to kill *T. cruzi*. The lack of evidence of transfusion-induced Chagas' disease in the United States and the absence of clinical reports of chronic Chagas' disease in immigrants from endemic areas suggests that the number of pathological cases is low. However, cases of acute and chronic Chagas' disease in immigrant populations have been reported in Canada.⁴¹

Although the flow of immigrants from Chagas'-endemic Central American countries presently is low, due to political climate the number of refugees coming from these areas could increase virtually overnight. Many of these refugees could be infected, yet could appear healthy because of the long latent period before chronic heart failure develops. Since there is no current therapy to specifically treat chronic Chagas' disease, each infected refugee would constitute a potential reservoir. These individuals, should they donate blood, could also serve as a direct source of infection to recipients unless the blood was treated or they were screened out as donors by the blood bank. Local public health departments and blood banks are not prepared at present to provide such serologic screening.

Those refugees coming to communities in the United States declared to be sanctuaries would not be likely to even come to the attention of public health officials.

At present the prospects for the introduction of more virulent strains of *T. cruzi* from Central America to the United States may seem remote. Nevertheless the political, social, and biological conditions for such introductions appear to be in place.

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