Agent of Chagas' disease from Honduran vector capable of developing in California insects: Implications for cardiologists

An exotic strain of *Trypanosoma cruzi* recovered from Triatoma dimidiata from Tegucigalpa, Honduras, was shown by isoenzyme studies to be closely related to the Miles' zymodeme 1 and laboratory reference strain Tehuantepec. It was injected into Swiss random-bred ICR mice. Clean *Triatoma protracta* nymphs and adults, which had been captured in Winters, California, fed on inoculated mice and were then examined over a 15-month period. Their feces contained multiplying epimastigote and infective trypomastigote forms of *T. cruzi*. This shows that exotic strains of *T. cruzi* can develop and survive for long periods in local California vectors. The increasing number of immigrants from Central America who enter California and other states may have public health implications in regard to the introduction of pathogenic strains that are capable of producing cardiomyopathy. Cardiologists who examine patients with cardiomyopathy from Central and South America should rule out Chagas' disease as a cause, since pathogenic *T. cruzi* strains are present in most Central and South American countries. (AM HEART J 110:605, 1985.)

Jerold Howard Theis, D.V.M., Michel Tibayrenc, M.D., Steven K. Ault, M.Sc., and Dean T. Mason, M.D. Davis and San Francisco, Calif.; and La Paz, Bolivia

*Trypanosoma cruzi* is a protozoan that is responsible for the cardiac and visceral lesions known as Chagas' disease, named after Carlos Chagas who first discovered the organism. *Trypanosoma cruzi* is transmitted naturally via an insect vector. However, it is not by the bite of the vector but rather by the feces of the insect that the infective forms reach the vertebrate host. In Central and South America the important vectors of *T. cruzi* have a habit of defecating right after a blood meal, hence fecal material that may contain infective forms is deposited on the vertebrate host as soon as the bug finishes feeding. The important vectors to humans in Latin America live and reproduce in the houses of the rural and urban poor. Once the bugs become infected, usually by feeding on a person who is in the chronic stage of the disease, they remain infected for their entire lifetime, which may be 2 or more years. In the endemic areas human infections are usually acquired during childhood. The majority of individuals survive the acute stages of the disease and it may be 15 to 20 years before the chronic stage manifests itself clinically.

Nonpathogenic strains of *Trypanosoma cruzi* are
found throughout large areas of the United States and potential vectors are even more widely distributed. Thus, like the mosquito vectors of malaria and yellow fever which also occur in parts of the United States, the potential exists for the introduction of exotic strains of T. cruzi and their establishment in local vectors. Although up to the present time this has been considered only a theoretical possibility with Chagas' disease, the continued immigration of people from endemic areas of Latin America to the United States increases the possibility of such introductions. Historically this has already occurred with malaria and yellow fever. The investigation reported herein suggests that there may be strains of T. cruzi in Latin America that will adapt to vectors in the United States and indicates that pathogenic strains of T. cruzi may be capable of establishing themselves in the United States.

METHODS

A Triatoma dimidiata female arrived from Tegucigalpa, Honduras, on May 16, 1983. The bug had been captured alive in a modern house on May 6, 1983. The bug's feces were examined on May 18, 1983, and live trypanosomes, morphologically compatible with that of T. cruzi, were seen. The bug was dissected on May 19, 1983, and fecal material from the hindgut was injected intraperitoneally into an adult female Swiss random-bred ICR mouse.

The mouse was killed on August 10, 1983, the heart was removed aseptically, ground in a Tenbrook tissue grinder, and the slurry was placed into a tube of NNN media. Subpassages of the original culture were made in NNN media and Noguchi-Wenyon (N-W) media through April 4, 1984. On that date eight, 3-week old Swiss random-bred ICR mice were inoculated intraperitoneally with 0.1 cc of pooled media from N-W cultures put up in October, November, and December, 1983. The concentration of organisms in the pooled inoculum, determined by counting in a hemocytometer chamber, was 5 x 10⁷/cc. The trypanosomine organisms were characterized by the isoenzyme technique.

Ten uninfected Triatoma protracta protracta collected near Winters, California, were allowed to feed on the anesthetized, previously inoculated mice 1 and 2 months after initial inoculation. Fecal material from the fed T. protracta protracta were examined 2 months after the initial feeding. The 10 T. p. protracta were fed on uninfected mice and the bugs' feces were examined following each feeding during a 15-month period.

RESULTS

The heart culture of the original mouse inoculated with feces from the Triatoma dimidiata was found to be positive for trypanosomes on September 2, 1983, approximately 1 month after the heart was removed and cultured. The cultured organisms were successfully passed through 13 serial passages, using N-W and NNN media. The isoenzyme characterization of the strain of T. cruzi isolated showed this strain to be closely related to the Miles' zymodeme 1 and to the laboratory reference strain Tehuantepec. Our T. cruzi is heterozygous for one locus of malic enzyme; this feature has been found in only two other stocks isolated from French Guiana.

The feces from uninfected Triatoma p. protracta fed on the mice inoculated with cultured organisms were found positive for T. cruzi 2 months after the initial feeding. Periodic examinations of feces from 10 infected T. p. protracta bugs fed on uninfected mice have shown infective forms of the Honduran T. cruzi to be maintained in the California insect for over 15 months.

DISCUSSION

Chagas' disease is a well recognized and serious problem in many areas of Central and South America. The wildlife reservoirs and domestic animal hosts, together with the semidomesticated and domesticated reduvid vectors, provide an opportunity for the establishment of rural foci which are a constant threat to the health of people living in these areas. Urban foci of infected bugs in Central and South America have also been reported.

Within the United States, T. cruzi infected reduvid bugs have been found throughout much of the southwestern U.S. including California. The geographic range of human virulent strains of T. cruzi has generally been reported to extend from 25 degrees N latitude (northern Mexico) to 38 degrees S latitude (central Argentina). Indeed, there have only been three reported naturally acquired cases in the United States. Nevertheless, the results of serologic tests on humans sampled from Lake Don Pedro, the site of the index California case, showed 6 of 241 humans (2.5%) to have positive complement fixing titers to T. cruzi. This was considerably higher than the percent of positive serums collected from people in a nonendemic area (San Francisco, California), where only 1 of 654 samples were complement fixation positive (0.2%).

These data indicate that people in the Lake Don Pedro area in Tuolumne County are being exposed to the local strains of T. cruzi in spite of the fact that the major vector in California, Triatoma p. protracta, does not readily defecate on the host following feeding as do the major South and Central American vectors. Contaminative transmission via infective feces from the insect vector is the major means of transmission to the vertebrate host. Since T. p. protracta does not usually defecate following a blood meal until it leaves the host, humans would most likely have to crush the bugs on their skin or
otherwise contaminate the bite site with feces in order to effect transmission.

The virulence to humans of the California strains appears in general to be low, since none of the followed-up seropositive individuals at Lake Don Pedro had evidence of either acute or chronic Chagas’ disease. The index case, however, did present with fever, fatigue, and anorexia. On examination by a physician the patient was noted to have bilateral conjunctivitis and a macular, erythematous rash on her trunk and limbs. She showed no lymphadenopathy or hepatosplenomegaly, and her ECG was normal. Organisms were discovered in a blood smear made from the patient and cultures of her blood on selective media grew T. cruzi. The patient also developed a complement fixation titer of 1:512.

In the endemic areas of pathogenic T. cruzi infections there are two phases to the disease. The acute phase begins 1 to 2 weeks after exposure. Fever, fatigue, and headache are seen initially. The fever rarely goes above 40°C and may be continuous or recurrent in nature. The fever may last 4 to 5 weeks before gradually subsiding. During the second week of the acute phase, there is edema of the face and, less frequently, of the body. Enlargement of the lymph nodes and hepatosplenomegaly is common. In addition, patients may have episodes of vomiting and diarrhea and may develop a cutaneous rash and meningencephalitis. The cardiovascular system appears to be most frequently involved in the acute stage, as evidenced by a tachycardia irrespective of the intensity of the fever. Heart enlargement, hypotension, and heart failure may occur during the acute phase. The ECG shows sinus tachycardia, low voltage, prolonged PR intervals, and primary T wave alterations. However, up to 50% of the patients in the acute phase will show no ECG abnormalities. The cerebrospinal fluid (CSF) may show abnormalities, even in patients without central nervous system involvement. Recovery of T. cruzi by culture of cerebrospinal fluid in appropriate media has been reported. Elevation of albumin in the cerebrospinal fluid was seen in some patients, and when the level of albumin was higher than 8 mg/dl, T. cruzi was always culturable from the CSF.

The acute phase of Chagas’ disease usually lasts 5 to 6 weeks. Mortality varies between 2% and 10%. The clinical signs and symptoms often disappear at this time, leaving only about 15% of the individuals with an abnormal ECG. The infection, however, is not terminated and instead enters its chronic stage. In this phase heart disease and enteromegaly may develop over a 10- to 20-year period. The individual may remain asymptomatic for much of this period. However, as time progresses, increasing numbers of the chronically infected individuals will begin to show abnormal ECG patterns. It is during the third and fourth decade of life that the majority of chronically infected individuals begin to evidence heart disease.

The chronic disease is due to the pathophysiological effects of (1) the destruction of parasympathetic and sympathetic ganglia that supply the smooth muscle of the gastrointestinal tract and the myocardium, and (2) the inflammatory reaction in heart muscle stimulated by the immunologic reaction against endocardium, vascular structures, and interstitium of muscle due to EVI antibody. Antibodies produced against T. cruzi will cross react with antigens found in endocardium, vascular structures, and interstitium of the human host’s tissues, hence its designation EVI antibody. The ECG in these patients is characterized by conduction disturbances, right bundle branch block, left anterior hemiblock, inverted T waves, and alterations indicating apical myocardial necrosis. In addition, the chronic disease is also characterized by arterial hypotension. In some patients with chronic chagasic cardiomyopathy experiencing heart failure there is a sinus bradycardia.

In the chronic phase of the disease the parasitemia is very low. Blood smears will usually fail to demonstrate organisms. Blood cultures may reveal organisms but serologic tests are usually resorted to for the diagnosis of chronic Chagas’ disease. The complement fixation test (CF) and indirect fluorescent antibody test are quite reliable and positive in a high percentage of chronic infections.

Data available from the United States Immigration Service indicates that since 1975 approximately 19,951 Honduran immigrants have settled in the United States. At least 1593 of these have settled in California. Contrary to statements that human infections with T. cruzi have not been reported from Honduras, the infection in Hondurans appears quite prevalent. Ponce and Zeledon report that they found 95 of 210 T. dimidiata captured in the city of Tegucigalpa positive for T. cruzi. Eight patients, six of whom had the hemifacial edema and lymphadenopathy referred to as the Romaña sign, were culture positive for T. cruzi. Serologic tests were done on 304 individuals living in rural areas of Honduras and 36.8% were found to have a positive CF titer for T. cruzi. One hundred eight patients with chronic heart disease and an ECG compatible with Chagas’ disease were tested by the CF test and 59.2% were found positive serologically. Fifty blood donors were also tested by the CF test and 28% were found serologically positive. Ponce and Zeledon...
concluded that Chagas' disease in Honduras appeared to present a serious public health problem, particularly with regard to heart disease.

Considering the apparent prevalence rate in Honduras, the period of asymptomatic disease, and the number of Hondurans that have immigrated to the United States, there may be as many as 7000 individuals infected with Honduran strains of *T. cruzi* in the United States at the present time. Even though these individuals are asymptomatic for the disease they have organisms in their circulation in sufficient quantities to infect insect vectors that may feed on them; in addition, they may pass the infection on to persons that may receive their blood in transfusions. Both facts are important to keep in mind from a public health point of view in this country.

We have shown, from our feeding experiments, that the strain of *T. cruzi* isolated from a *T. dimidiatia* from Honduras can survive and produce infective forms in the hindgut of a local reduvid, *T. p. protracta*, for at least 15 months. This may mean that immigrants with chronic *T. cruzi* infection could serve to introduce potentially pathogenic strains of *T. cruzi* to North American reduvid vectors should these people be fed upon by the bugs. *Triatoma p. protracta* is a very common reduvid in California, is found in houses, and will bite people in order to obtain a blood meal.12,23,24

Transmission of *T. cruzi* via whole blood transfusions is a potential problem in Central and South America,17 and has led to the routine treatment of whole blood with a 1:4000 (final dilution) of crystal or gentian violet for 24 hours before use.25 This kills the trypomastigote form of *T. cruzi*. No such treatment of whole blood is carried out in the United States and the low risk at present for transmission of *T. cruzi* via blood transfusions does not warrant such treatment. However, blood donors should be questioned about their country of origin and the possibilities of chronic asymptomatic Chagas' disease should be ruled out by serologic tests if a blood donor has a history of having lived in an endemic zone.

The authors would like to thank Dr. Lynn Wiley, Division of Reproductive Biology and Medicine, School of Medicine, University of California, Davis, California, who provided the Swiss random-bred ICR mice for this work; and the Western Heart Institute, San Francisco, California, for partial funding of the project.

REFERENCES


