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PREVALENCE OF PARASITEMIA AND SEROREACTIVITY TO *TRYPANOSOMA CRUZI* IN A RURAL POPULATION OF NORTHEAST BRAZIL*

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Abstract. Age-specific prevalence rates of parasitemia and seroreactivity to *Trypanosoma cruzi* were determined in a rural area endemic for Chagas' disease in Northeast Brazil. Parasitemia was detected by blood cultures and xenodiagnosis, and serum antibodies to the parasite were measured by the complement fixation (CF) and indirect immunofluorescence (IFA) tests. Of the 116 persons examined, 39 (33.7%) had antibodies and 23 (19.8%) had parasitemia. Ninety-six percent of parasitemic individuals were seropositive and 56% of seropositive individuals were parasitemic. The percentage of seropositive individuals with detectable parasitemia declined with age; all seropositive children in the 1- to 4-year age group and two-thirds of seropositive persons 5–19 years old had parasitemia while only one-third of seropositive adults above 19 years had parasitemia. CF and IFA tests were equally sensitive in detecting persons with parasitemia. Xenodiagnosis was more sensitive than culture for detecting parasitemia, but the two methods together were more sensitive than either method alone. Using the age-dependent relationship of parasitemia to seropositivity determined in this study, the prevalence rate of *T. cruzi* parasitemia was estimated in a much larger adjacent population in which seropositivity rates and the demographic structure were already known.

The prevalence of infection with *Trypanosoma cruzi* in man is usually determined serologically. Although serologic tests are usually reactive in individuals known to be infected,[1] the relationship of seroreactivity to parasitemia in populations in endemic areas has yet to be established. As antibodies to *T. cruzi* may persist indefinitely,[2,3] their presence may or may not indicate active infection.

In acute Chagas' disease, parasitemia usually can be detected by direct microscopic examination of peripheral blood, but often the primary infection is not clinically identifiable.[4] The age-distribution of seroreactivity to *T. cruzi* in endemic areas suggests that primary infection occurs at a relatively constant rate up to age 20 years,[5] but supportive parasitologic data are unavailable. In the chronic phase organisms may disappear from the circulation or may be present in such low numbers that they are not detectable by direct microscopy.[4] Parasitologic diagnosis then must rely on xenodiagnosis and culture, methods that support multiplication of the parasites. By these means, parasites have been detected in more than a third of individuals with chronic Chagas' cardiomyopathy.[6,7] However, data relating to the occurrence of parasitemia during the intermediate period between the acute and chronic stages are lacking.

We here report age-specific prevalence rates of parasitemia and seroreactivity to *T. cruzi* as determined in a population of a rural area endemic for Chagas' disease in Northeast Brazil. The study was designed to explore the relationship between seroreactivity and parasitemia in all age groups and to determine the relative sensitivity of xenodiagnosis and blood culture. Since all individuals within the study area were included,

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the age specific rates of parasitemia could be used to estimate the prevalence of parasitemia in a larger adjacent population for which the seroreactivity rates and demographic structure were already known.

POPULATION AND METHODS

Study area, population and methods of field study

The study was done in Fazenda Sape in the Municipio de Castro Alves, the State of Bahia, Brazil. This area is adjacent to the 10 fazendas where Mott et al.[5] studied the prevalence of seroreactivity to *T. cruzi* in 1974. In January of 1976 the Vector Control Service of the Brazilian Government (SUCAM) sprayed the interior of the houses with BHC insecticide to control infestation with *Panstrongylus megistus*. In March 1976 we mapped and censused the study area, using demographic methods previously described,[5] and identified 122 individuals living in 26 households. The population was examined in July and August 1976; 116 people participated. Four individuals refused or were not present on the day of examination, and two people had died in the interval since the census.

Two blood specimens were obtained from each individual by a single venipuncture using a multiple-draw Vacutainer® needle and holder. Blood for culture was collected in sterile 7-ml Vacutainer® tubes containing 25 IU of heparin and sufficient vacuum to obtain 5 ml. Blood for serum was collected in 10-ml Vacutainer® tubes.

For xenodiagnosis we used 10 *Triatoma infestans* 5th stage nymphs that had not been fed for 4 weeks. Five bugs were put into each of two 5 cm-diameter by 5 cm-high white plastic cups covered with nylon netting. The cups, secured with string, were placed for 30 min on the anterior thigh of young children or on the anterior forearm of older individuals.

Laboratory methods

The heparinized blood was processed for culture on the day of collection. Three milliliters were pipeted into a 15-ml capped centrifuge tube and lysed with 10 ml of 0.87% NH_4Cl for 15 min at room temperature.[8] The tubes were then centrifuged at 1,000 × *g* and the supernatant fluid was discarded. The sediment was washed once with 0.87% NH_4Cl, suspended in 2.0 ml of F29 medium and then overlaid on an NNN agar slant containing 7.5% rabbit blood.[9] The cultures were incubated in an air-conditioned room where the temperature ranged from 24° to 26°C. We examined preparations from the cultures under 400× phase contrast magnification every 3 weeks until parasites were found or until the cultures were discarded as negative after 15 weeks.

The bugs from each pair of xenodiagnosis cups were combined in a single larger cup and were kept at 24–26°C for 25 days. During the period 26–42 days after feeding, the rectal contents of individual bugs were dissected out, mixed with saline and examined under a coverslip for the presence of *T. cruzi* using 400× phase contrast magnification.

Blood for serologic studies was allowed to clot at ambient temperature and, after overnight storage at 4°C, the serum was separated and stored at −20°C. We performed the complement fixation (CF) test for antibodies to *T. cruzi* as previously described.[5] For the indirect immunofluorescence test (IFAT) we used the method of Miles[10] with mouse blood forms of the Y strain of *T. cruzi* as antigen and fluorescein conjugated anti-human IgG (Cappel Laboratories, Downington, PA, USA). With the IFAT, titers of 1:16 or greater were considered positive; control sera from 30 healthy individuals from non-endemic areas for Chagas' disease were not reactive at this dilution. Sera were also examined using the Venereal Disease Research Laboratory (VDRL) test.

RESULTS

The age distribution of patients with parasitemia and seroreactivity to *T. cruzi* is shown in Table 1. Of the 23 individuals with parasitemia, 22 (95.7%) were seropositive by CF or IFAT and one 58-year-old woman with a positive xenodiagnosis was negative by both serologic tests. When this woman was reexamined 2 months later, xenodiagnosis and the serologic tests were all negative. Fifty-six percent of persons who were seroreactive for *T. cruzi* were parasitemic. The percentage of seropositive individuals with parasitemia declined with age; all seropositive children in the 1- to 4-year age group and two-thirds of seropositive persons 5–19 years old had parasitemia while only one-third of seropositive adults

TABLE 1

Age-specific prevalence rate of T. cruzi *parasitemia and seropositivity in Fazenda Sape, Castro Alves*

Age in years	No. examined	Parasitemia*		Seropositivity†		Percent of seropositive individuals with parasitemia (1) ÷ (2) × 100
		No. pos. (1)	Percent	No. pos. (2)	Percent	
1	4	0	0	0	0	0.0
1–4	18	5	27.7	5	27.7	100.0
5–9	21	6	28.2	9	42.9	66.7
10–19	25	5	20.0	8	32.0	62.0
20–44	26	3	11.5	8	30.7	37.5
45+	22	4‡	18.1	9	40.9	33.3
Total	116	23	19.8	39	33.7	56.4

* Parasitemia detected by xenodiagnosis or culture.
† Seropositive by CF or IFA.
‡ One individual with parasitemia was seronegative.

above 19 years had parasitemia. Among seropositive individuals the median age was 15 years for those with parasitemia and 37 years for those without parasitemia. The ranked ages of seropositive individuals with parasitemia differed significantly from those without parasitemia by the Mann-Whitney Test ($P < 0.05$).[11] There appeared to be no difference in the rate of parasitemia between males and females. Among the 26 households, 14 had at least one member with parasitemia and 19 had at least one seropositive member.

Xenodiagnosis was more sensitive than culture for detecting parasitemia and the combined methods were more sensitive than either method alone; 8 individuals were positive only by xenodiagnosis and 3 were positive only by culture (Table 2). The parasites isolated were judged to be *T. cruzi* by their characteristic morphology and by their infectivity for laboratory mice. Of the 1,160 bugs used for xenodiagnosis, 53 (4.6%) were positive for *T. cruzi*. The number of positive bugs per individual ranged from 1–9 with a median and mode of 2 bugs. Cultures became positive in 3–15 weeks after inoculation with a median of 6 and mode of 3 weeks. During the 15-week observation period approximately one-fourth of the cultures became contaminated with bacteria but this was controlled by the addition of 300 μg of Gentamicin®. *T. cruzi* appeared in several cultures contaminated with bacteria and continued to grow in those already positive.

The CF and IFAT results for individuals with and without parasitemia are compared in Table 3. The geometric mean titers (GMT) by CF and IFAT in those with parasitemia did not differ significantly from those in seropositive individuals without parasitemia. Two individuals, 64 and 69 years old, one of whom was seroreactive for *T. cruzi*, had positive VDRL reactions.

DISCUSSION

Unlike other epidemiologic studies of parasitemia with *T. cruzi*,[7,12] an entire population including young children was examined. We were able to show that the prevalence rate of parasitemia (parasite rate) in individuals with serologic evidence of exposure to infection declined with age. The high rates we observed in seropositive children probably reflected recent primary infection during which parasitemia is easily detectable.[13] Dubois et al.,[14] in a preliminary report, also found that seropositive children have higher parasite rates than seropositive adults.

Despite the age-related decline in the parasite rate, *T. cruzi* was detectable in over one-third of the seropositive adults. Other investigators, employing different xenodiagnostic and cultural methods, have found even higher parasite rates

TABLE 2

Comparison of culture and xenodiagnosis for detection of T. cruzi *parasitemia*

Culture	Xenodiagnosis		Total
	Positive	Negative	
Positive	12	3	15
Negative	8	93	101
Total	20	96	116

TABLE 3

Comparison of complement fixation (CF) and indirect fluorescent antibody (IFA) results in individuals with and without T. cruzi *parasitemia*

Age group	Individuals with parasitemia				Individuals negative for parasitemia			
	No. examined	CF*		IFA† pos.	No. examined	CF*		IFA† pos.
		Pos.	AC‡			Pos.	AC‡	
<1	0	0	0	0	4	0	0	0
1–4	5	4	1	5	13	0	0	0
5–9	6	6	0	6	15	2	0	3
10–19	5	5	0	5	20	3	1	3
20–44	3	3	0	3	23	5	0	5
45+	4	3	0	3	18	5	1	6
Total	23	21	1	22	93	15	2	17

* Significant titer for CF considered to be 1:8.
† Significant titer for IFA considered to be 1:16.
‡ AC = anticomplementary serum.

in selected seropositive adult populations. In Venezuela, Pifano detected parasitemia in 57% of such individuals,[12] and Petana et al.[15] found *T. cruzi* in 70% of seropositive adults living in a triatomine-controlled area near Rio de Janeiro. The latter authors used serial xenodiagnoses with 40 *T. infestans* 3rd or 4th stage nymphs for 3 consecutive months; the cumulative parasite rate was 51% with one xenodiagnosis, 62% after the second and 70% after the third xenodiagnosis. Thus, with repeated attempts, it is probable that a higher parasite rate would have been found in the seropositive adults of our study. Together these studies suggest that parasitemia can persist, perhaps for life, in the majority of individuals who have antibodies to the parasite.

On the basis of the age specific rates of parasitemia among seropositive individuals in Fazenda Sape, we estimate that over 20% of the rural population[5] of Castro Alves probably has parasitemia, illustrating the magnitude of the human reservoir. Since methods used to detect *T. cruzi* parasitemia are relatively insensitive, particularly in the chronic phase of the disease, the true prevalence of parasitemia may be even higher than our estimate.

The relationship between persistent parasitemia and the development of the chronic manifestation of Chagas' disease is not known. Our unpublished studies in Fazenda Sape, and data from the population studied by Moleiro et al.,[16] in Venezuela, indicate that electrocardiographic (ECG) abnormalities occur equally in seropositive individuals with or without parasitemia. Maekelt found that ECG abnormalities were 6.3 times more prevalent in seropositive than in seronegative individuals.[7] Further population-based studies are needed to determine whether parasitemia per se has a role in the pathogenesis of the disease.

The CF and IFA tests were equally sensitive in detecting 22 of 23 (95.7%) individuals with parasitemia. The degree of sensitivity of the CF and IFA tests agrees with results reported for selected chronically infected individuals in whom xenodiagnosis was positive.[1] However, in two recently reported epidemiologic studies, the CF test was less sensitive in detecting infected individuals. Maekelt,[7] in Venezuela, reported negative CF tests in 18 of 96 (18.8%) xenodiagnosis-positive individuals in a hospital-based study, and Zeledon et al.[17] in Costa Rica reported negative CF tests in 4 of 14 (28.6%) parasitemic individuals in a rural population-based study. In the one serologic "false negative" individual in our study, the xenodiagnosis was positive but the blood culture and a second xenodiagnosis were negative suggesting the possibility of a technical error. Unfortunately, this individual has not been available for further study.

Under field conditions we found xenodiagnosis to be more sensitive than culture for detecting parasitemia, although the combined methods were more sensitive than either method alone. This confirms other reports[18, 19] that neither xenodiagnosis nor culture alone are superior for detecting *T. cruzi* in the chronic phase. The use of both tests increases sensitivity. In both methods, sensitivity is dependent on the amount of blood used

and can be increased by using more bugs for xenodiagnosis or inoculating a larger volume of blood for culture.[18] In comparing the two methods, we examined approximately equal amounts of blood; 3.0 ml was concentrated for culture. Ten *T. infestans* 5th stage nymphs suck an average of 3.1 ml (range 2.8–3.9 ml).[20]

There are advantages and disadvantages to xenodiagnosis and culture for detecting *T. cruzi* parasitemia in field studies. Some of our cultures became contaminated with bacteria. Although we, and others,[18, 19] have observed similar sensitivity, some investigators,[21, 22] for unexplained causes, have found culture highly insensitive compared to xenodiagnosis. For xenodiagnosis some investigators have suggested employing 40 bugs or more for maximum sensitivity in detecting chronic infections.[21] However, in our experience, the use of such a large number of bugs for xenodiagnosis would be unacceptable to many participants. Moreover, the task of examining the extra bugs would limit the application of xenodiagnosis in field studies. Another serious problem with xenodiagnosis has been hypersensitive skin reactions which were frequent and sometimes severe in Fazenda Sape.[23] Although xenodiagnosis remains the method of choice for detecting *T. cruzi* infection, it may be necessary to use cultural techniques in order to avoid problems of hypersensitivity.

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