

CLINICAL AND EPIDEMIOLOGIC STUDIES OF CHAGAS' DISEASE IN RURAL COMMUNITIES IN OAXACA STATE, MEXICO, AND A SEVEN-YEAR FOLLOW-UP: I. CERRO DEL AIRE^{1, 2}

R. S. Goldsmith,³ R. J. Zárate,⁴ L. G. Zárate,⁴ I. Kagan,⁵ and L. B. Jacobson⁶

In 1971, serologic studies in the Mexican state of Oaxaca revealed unusually high chagasic infection in certain communities. This article reports the results of 1973 and 1980 follow-up work performed in one of those communities, Cerro del Aire, which shows that the infection caused significant electrocardiographic abnormalities in seropositive persons (as compared to seronegative persons), without there having been evident progression of those abnormalities as of the end of the follow-up period in 1980.

Introduction

The frequency and severity of Chagas' disease in the Americas varies by geographic region (1). In Mexico before 1970 the infection was not thought to occur commonly or to cause significant cardiac pathology. Prevalence rates reported from several communities ranged from 7% to 13% (2-5). However, seroepidemiologic surveys conducted in 1971 in 60 rural communities on the Pacific side of Mexico's Oaxaca State showed unusually high rates of *Trypanosoma cruzi* infection (6-9). In particular, antibody prevalence rates for adults living in the three communities of Cerro del Aire, Chila, and Tataltepec

were 76%, 58%, and 51%, respectively. These rates were similar to some of the highest rates reported in South America.

Although many community-based cross-sectional studies of Chagas' disease have been reported (10-21), relatively few community-based longitudinal studies (22-28) have been described. Such longitudinal studies are needed to provide information about the natural history of the disease—which is not fully understood (22, 29).

This and companion reports present recent findings of both cross-sectional and longitudinal studies in several Oaxaca communities. These studies sought (1) to determine the pathologic

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³Professor of Tropical Medicine, Department of Epidemiology and International Health, University of California, San Francisco, California 94143, United States.

⁴Researcher, Ecological Research Center of the Southeast (*Centro de Investigaciones Ecológicas del Sureste*), San Cristóbal de las Casas, Chiapas, Mexico.

⁵Director, Parasitology Division, Centers for Disease Control, Atlanta, Georgia, United States.

⁶Attending Cardiologist, Department of Medicine, Pacific Medical Center, San Francisco; and Associate Clinical Professor, Department of Medicine, University of California, San Francisco.

Figure 1. A map of Mexico's Oaxaca State showing the location of Cerro del Aire.

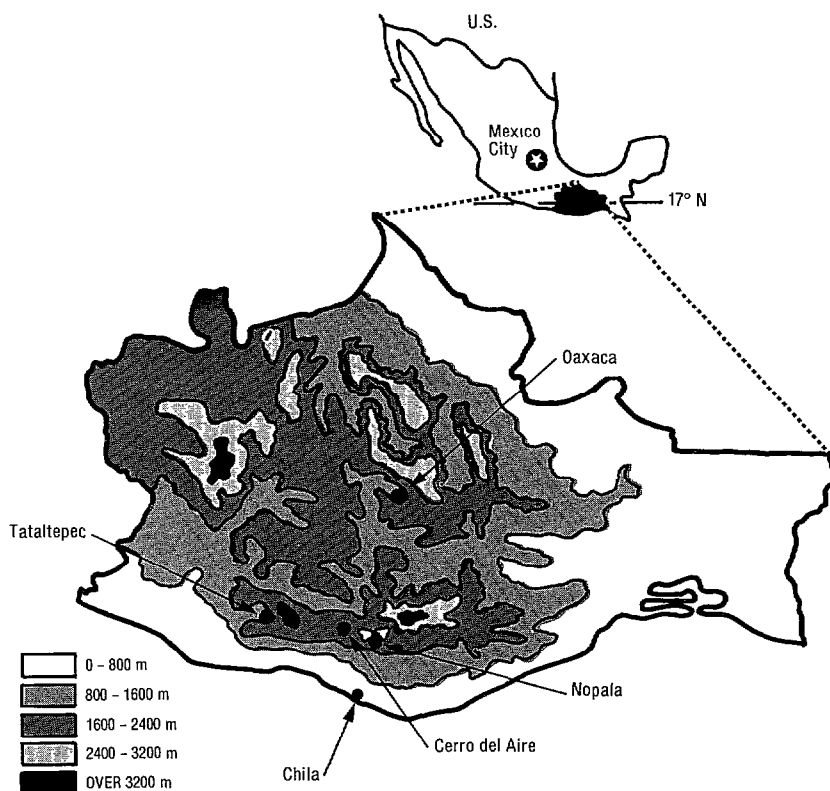


Table 1. Age and sex distribution of 248 subjects tested in Cerro del Aire in 1971 and age-specific indirect hemagglutination (IHA) test results. A titer ≥ 128 was considered positive. This study population included 89% of Cerro del Aire's total 1971 population of 280 residents.

Age group (in years)	% of study population in each age group	% males in each age group	IHA test results					
			Males		Females		Total	
			No./No. pos./tested	% positive	No./No. pos./tested	% positive	No./No. pos./tested	% positive
0-3	2	83	0/5	0	0/1	0	0/6	0
4-6	10	68	1/17	6	0/8	0	1/25	4
7-9	9	68	0/15	0	0/7	0	0/22	0
10-12	10	54	2/13	15	2/11	18	4/24	17
13-15	8	53	4/10	40	3/9	33	7/19	37
16-19	10	38	8/10	80	7/16	44	15/26	58
20-29	16	41	7/16	44	14/23	61	21/39	54
30-39	16	40	14/16	88	17/24	71	31/40	78
40-49	10	50	12/12	100	12/12	100	24/24	100
50-59	5	46	5/6	83	5/7	71	10/13	77
≥ 60	4	20	2/2	100	8/8	100	10/10	100
Total	100	49	55/122	45	68/126	54	123/248	50

impact of *T. cruzi* infection in humans, (2) to investigate the natural history of the disease, and (3) to confirm that serologically positive persons were parasitologically positive. This report summarizes the clinical, electrocardiographic, serologic, and parasitologic findings of cross-sectional and longitudinal studies conducted in the community of Cerro del Aire. The results of similar studies in three other communities—Chila, Tataltepec, and Nopala—will be reported later.

Additional aims of the Cerro del Aire work were (1) to compare the sensitivity of xenodiagnosis and two hemoculture methods—one using LIT medium (30)⁷ and the other employing MK₂ tissue culture cells—for isolation of *T. cruzi* from serologically positive persons, and (2) to monitor resumption of Chagas' disease transmission that could be occurring as a result of a reduction in the frequency of residual insecticide spraying by the malaria control program in and near Cerro del Aire.

In the 1971 survey (6, 7), only 1.4% of 1,289 children under 10 years of age tested in the 60 communities were seropositive. This low level of infection in young children, reflecting near-cessation of transmission of infection to humans in the region, was associated with concurrent disappearance of the vector and was attributed to the malaria control program that began in 1962. From 1976 onward, however, spraying was discontinued in some communities, and the frequency of spraying was reduced in others. Thus, one objective of the Cerro del Aire study was to monitor possible seroconversion in children and adults for an indication that the vectors were returning and transmission had resumed.

Methods

The Study Area

Oaxaca, one of Mexico's southernmost states, is at about latitude 17° North and longitude 96° West (Figure 1). The community of Cerro del

Aire (also known as La Cañada de Atotonilco) is located on the Pacific side of the state at an altitude of about 800 meters. The annual rainfall is about 525 mm, with most of the precipitation occurring between May and October. The forest cover is deciduous.

Until 1980, Cerro del Aire was isolated and was about 10 kilometers from the nearest unimproved road. Except for a few men, most of the inhabitants had not lived or traveled outside the local area. During 1971-1973, some 280 persons lived in the town; our 1971 sample of 248 persons (Table 1) represented 89% of the population (6, 7). By 1980 the population had grown to 1,200, with most of the new residents having come from the nearby community of Teotepec located about eight kilometers to the north and 200 m higher. Houses in Cerro del Aire usually have dirt floors, walls made of wood slats and adobe, and ceilings made of palm fronds or straw.

Field Trips to Cerro del Aire

In 1971, during the first field trip to Cerro del Aire, 248 persons were included in the serologic survey. The results of this survey, summarized in Table 1, have been reported previously (6-9).

In May 1973, during a second trip, the cardiac status of 130 persons seen in 1971 was evaluated by clinical and electrocardiographic examinations, and new blood specimens were tested by indirect hemagglutination (IHA) serology.

In July 1980, during a third field trip, a seven-year longitudinal study was begun and the number of persons in the cross-sectional study was increased. (Of the 248 persons seen in 1971, 19 had died by 1980, generally of unknown causes.) In 1980, as Table 2 indicates, blood specimens were obtained from 237 persons; 55 had been tested in 1971 and 1973, while the remaining 182 were tested for the first time. All 237 specimens were tested by IHA and direct agglutination (DA) methods; because of limited volumes of blood collected in capillary tubes, only 86 specimens could be tested by complement-fixation (CF). Electrocardiograms (ECGs) were obtained from 83 persons; 37 had been

⁷LIT medium = liquid liver-infusion-tryptose medium.

Table 2. Age and sex distribution of 237 subjects tested in Cerro del Aire in 1980 and age-specific serologic test results. All 237 subjects were tested by indirect hemagglutination (IHA) and direct agglutination (DA), and 86 subjects were tested by complement fixation (CF). A subject responding positively to any one test (with an IHA titer ≥ 128 , a DA titer ≥ 256 , or a CF titer ≥ 8) was considered seropositive. This study population included 20% of Cerro del Aire's total 1980 population of 1,200 residents.

Age group (in years)	% of study population in each age group	% males in each age group	Serologic test results					
			Males		Females		Total	
			No./No. pos./tested	% positive	No./No. pos./tested	% positive	No./No. pos./tested	% positive
0-3	16	45	1/17	6	2/21	10	3/38	8
4-6	14	61	2/20	10	0/13	0	2/33	6
7-9	10	61	0/14	0	0/9	0	0/23	0
10-12	8	53	1/10	10	0/9	0	1/19	5
13-15	5	45	1/5	20	1/6	17	2/11	18
16-19	3	33	1/2	50	1/4	25	2/6	33
20-29	12	46	6/13	46	6/15	40	12/28	43
30-39	8	35	5/7	71	9/13	69	14/20	70
40-49	15	39	11/14	79	18/22	82	29/36	81
50-59	3	50	4/4	100	4/4	100	8/8	100
≥ 60	6	53	6/8	75	4/7	57	10/15	67
Total	100	48	38/114	33	45/123	37	83/237	35

tested in 1973, while 46 were tested for the first time.

Overall, during all of the cross-sectional studies, ECGs were obtained from 176 subjects, medical histories from 130 (52 in 1973 and 78 in 1980), and physical examinations from 101 (40 in 1973 and 61 in 1980).

Blood Collection, Storage, and Testing

Blood specimens were collected in heparinized venipuncture tubes in 1971 and in heparinized capillary tubes thereafter. Specimens were centrifuged at about 700 RPM for 10 minutes and processed aseptically; the resulting plasma was stored on "wet" ice (H_2O) in the field and at $-20^\circ C$ in the laboratory. The *T. cruzi* antigen used was a saline extract of lyophilized epimastigotes, the latter having been delipidized with benzene before extraction (4, 5). The serologic methods employed in this study for the IHA, CF, and DA tests have been described elsewhere (4, 5, 31). Positive titers in these tests were ≥ 128 for IHA, ≥ 256 for DA, and ≥ 8 for CF.

T. cruzi Isolation by Xenodiagnosis and Hemoculture

Xenodiagnosis. Forty fourth-instar nymphs of the triatomid vector *Rhodnius prolixus* were used per patient. The bugs had not been fed for four weeks. Patient exposure time was 30 minutes. After four to seven weeks, the bugs fed on the patient were individually examined for *T. cruzi* infection by thoroughly and entirely triturating each bug in 1 ml of normal saline. The parasites that were isolated were identified as *T. cruzi* by their morphology and their infectivity for laboratory mice.

Hemocultures. The first hemoculture method employed closed test-tube tissue cultures of MK₂ cells. The source of the MK₂ cell line, the composition of the maintenance medium, and the mode of inoculation have been described previously (6, 7). Two tissue-culture tubes were each inoculated with 2 ml of plasma.

The second hemoculture method used LIT medium (30, 32, 33). Two tubes were each inoculated with one-half the volume of packed

cells recovered after centrifuging 10 ml of heparinized blood at 2,000 rpm for 15 minutes; before use, the packed cells were washed once with LIT medium and recentrifuged.

Both sets of cultures were kept at ambient field temperatures for one week. Thereafter, in the laboratory, the LIT medium cultures were kept at 24°C and the MK₂ cell cultures were kept at 37°C. The LIT medium cultures were examined over a fifteen-week period for the presence of flagellates by fresh-film microscopy. The MK₂ tissue-cultures were examined at weekly intervals for approximately one month.

Electrocardiograms

ECGs were recorded with either Cambridge or Burdick portable ECG machines powered by a portable generator. The ECGs contained the six standard limb leads and 3-6 standard precordial leads. A cardiologist (LBJ) recorded the rhythm, rate, PQRSTU contours, PR interval, and the QRS axis, duration, and configuration of each ECG in accordance with standard criteria but without knowledge of the patient's serologic status.

Results

Serologic Tests, 1971

In 1971, 248 blood specimens were collected and tested by IHA for antibody to *T. cruzi* (6, 7). The results of these tests, by age and sex of the subjects, are shown in Table 1. Only one of the 53 children tested who were under the age of 10 years was seropositive. For each of the age groups between 10 and 49 years the percentage of seropositives increased progressively—from 17% to 100%. A total of 54% of the females and 45% of the males were seropositive, but this difference between the sexes was not statistically significant. Overall, 50% of the 248 persons tested were seropositive, but a much higher pro-

portion (76%) of the 126 subjects 20 years of age or older were seropositive. These overall 1971 prevalences are very likely to have been within two percentage points of the town's true prevalences because the sample tested included 89% of the population of Cerro del Aire.

Serologic Tests, 1980

In 1980, blood specimens were collected from 237 residents of Cerro del Aire (Table 2) and tested by IHA and DA; 86 specimens were also tested by CF. As indicated in the table, a specimen found positive by one or more of the tests was considered seropositive.

Of 182 persons tested for the first time in 1980, 42 (23%) were found seropositive by one or more of the three tests. Of the 59 persons 20 years of age or older, 34 (58%) were found to be seropositive. No significant differences between results for males and females were observed when the data were analyzed by sex.

Of 38 children under three years of age, three (8%) were found to be seropositive; and of 33 children four to six years old, two (6%) were found to be seropositive; all of the children tested were born in Cerro del Aire. The test results for the five serologically positive children under age seven and the serologic status of their mothers are presented in Table 3. One of the mothers was seropositive and three were seronegative; no blood sample was available from the fifth mother.

Regarding seroconversion, of 55 persons tested in 1971 or 1973 and retested in 1980, none of the 13 people found seronegative initially had converted to seropositive as of 1980. Of 42 persons considered seropositive in 1971 and retested in 1980, all remained seropositive except for three persons. These three had individual IHA titers of 128, 256, and 512 in 1973; but in 1980 they all had IHA titers of 64, DA titers of 64, and CF titers of zero. The second of the three subjects was xenodiagnosis-positive in 1980 (see below).

Table 3. Serologic test results for the five 1980 study subjects under seven years old who were found to be seropositive, and corresponding serologic test results for their mothers.

Subjects tested	Age (in years)	Sex of child	Serologic test results (titers)			Serologic status
			IHA	DA	CF	
Child	3	M	32	64	16	+
Mother	30		1,024	512	128	+
Child	4	M	<32	32	16	+
Mother	30		<32	16	AC ^a	0
Child	1	F	<32	<16	16	+
Mother	40		<32	16	<16	0
Child	1	F	<64	<16	32	+
Mother	37		<32	16	NT ^b	0
Child	6	M	512	256	AC	+
Mother	unknown		NT	NT	NT	NT

^aAC = anticomplementary.^bNT = not tested.*Comparative Sensitivity of the IHA, DA, and CF Tests*

Eighty-three of the 237 blood specimens collected in 1980 were positive by one or more of the three tests; 13 (5%) were positive only by the IHA test and 10 (4%) only by the CF test. None were positive by the DA test alone.

Of the 237 specimens examined by IHA and DA tests (Table 4), 29% were positive by the IHA test and 21% were positive by the DA test. Overall, 218 of the specimens were either positive by both tests or negative by both tests, resulting in 92% agreement between the two methods.

Of the 86 sera examined by IHA and CF tests (Table 5), 43% were positive by the IHA test and 45% were positive by the CF test. Overall, 62 of the specimens were either positive by both tests or negative by both tests, resulting in 72% agreement between the two methods.

Of the 86 sera examined by CF and DA tests (Table 6), 31% were positive by the DA test and 44% were positive by the CF test. Overall, 63 of the specimens were either positive by both tests or negative by both tests, resulting in 73% agreement between the two methods.

Cross-sectional Clinical and Electrocardiographic Studies

Comparison of clinical findings for seropositive and seronegative subjects. Histories of symptoms were provided by 130 subjects, 94 of whom were seropositive and 36 of whom were seronegative (Table 7). Differences in the reported frequency of certain symptoms were statistically significant in the following instances: (1) chagoma or edema of the face or body was recalled by 13 (13.8%) of the seropositive subjects but by none of the seronegative subjects ($p < 0.02$); (2) palpitations were reported by 59 (62.8%) of the seropositive subjects but only by 14 (38.8%) of the seronegative subjects ($p < 0.02$); and (3) precordial pain was reported by 10 (10.6%) of the seropositive subjects but by none of the seronegative subjects ($p < 0.05$).

Physical examinations were performed on 101 subjects, of whom 74 were seropositive and 27 seronegative (Table 8). There were no statistically significant differences between the findings for the two groups, although seven (9.5%) of the seropositives and none of the seronegatives had cardiomegaly.

Table 4. Comparison of indirect hemagglutination (IHA) and direct agglutination (DA) test results obtained with the 237 Cerro del Aire sera collected in 1980. IHA titers ≥ 128 and DA titers ≥ 256 were considered positive.

		DA test					
						Total	
		+		-			
		No.	(%)	No.	(%)	No	(%)
IHA test	+	49	(21)	19	(8)	68	(29)
	-	0	(0)	169	(71)	169	(71)
Total		49	(21)	188	(79)	237	(100)

Table 5. Comparison of indirect hemagglutination (IHA) and complement-fixation (CF) test results obtained with 86 Cerro del Aire sera collected in 1980. IHA titers ≥ 128 and CF titers ≥ 8 were considered positive.

		CF test				Total	
		+		-			
		No.	(%)	No.	(%)	No.	(%)
IHA test	+	26	(30)	11	(13)	37	(43)
	-	13	(15)	36	(42)	49	(57)
Total		39	(45)	47	(55)	86	(100)

Table 6. Comparison of complement-fixation (CF) and direct agglutination (DA) test results obtained with 86 Cerro del Aire sera collected in 1980. DA titers ≥ 256 and CF titers ≥ 8 were considered positive.

		CF test				Total	
		+		-			
		No.	(%)	No.	(%)	No.	(%)
DA test	+	21	(24)	6	(7)	27	(31)
	-	17	(20)	42	(49)	59	(69)
Total		38	(44)	48	(56)	86	(100)

Table 7. A comparison of findings derived from medical histories of seropositive and seronegative subjects.

Findings from histories	Abnormalities found among:				Statistical significance by Chi square (test; NS = not significant)
	94 seropositive subjects		36 seronegative subjects		
	No.	%	No.	%	
<hr/>					
<i>History of acute illness:</i>					
Chagoma or edema of the face or body	13	14	0	0	p<0.02
<i>History of chronic illness:</i>					
Fatigue	1	1	0	0	NS
Weight loss	14	15	4	11	NS
Palpitations	59	63	14	39	p<0.02
Dyspnea on exertion	34	36	9	25	NS
Orthopnea	5	5	0	0	NS
Precordial pain	10	11	0	0	p<0.05
Loss of consciousness	11	12	1	3	NS
Convulsions	3	3	0	0	NS
Peripheral edema	3	3	2	6	NS
Dysphagia (mild)	17	18	10	28	NS
Dysphagia (severe)	0	0	0	0	—
Regurgitation	23	24	10	28	NS
Constipation	1	1	1	3	NS

Table 8. A comparison of clinical findings derived from physical examination of seropositive and seronegative subjects.

Clinical findings	Abnormalities found among:				Statistical significance (by Chi square test; NS = not significant)
	74 seropositive subjects		27 seronegative subjects		
	No.	%	No.	%	
Arrhythmia	1	1	1	4	NS
Cardiac murmurs	29	39	11	41	NS
Gallops	9	12	3	11	NS
Cardiomegaly	7	9	0	0	NS
Cardiac insufficiency	1	1	0	0	NS
Large bowel fecal mass	0	0	0	0	—

Comparison of electrocardiographic findings for seropositive and seronegative subjects. ECGs were obtained from 176 subjects, of whom 93 were tested only in 1973, 37 were tested in both 1973 and 1980, and 46 were tested only in 1980 (Table 9). Of the 176, 111 were seropositive by one or more of the three serologic tests and 65 were seronegative by all three. The age

distribution of these subjects, grouped according to their serologic status, is shown in Table 10.

As Table 9 indicates, one or more electrocardiographic abnormalities were found in 51 (46%) of the seropositive subjects and in 14 (22%) of the seronegative subjects. This difference is statistically significant ($p<0.005$). Intraventricular conduction delays (IVCDs) were present in 18

Table 9. Electrocardiographic abnormalities found among 111 seropositive and 65 seronegative subjects. These findings were derived from ECGs recorded in 1973 (in 93 subjects), in 1980 (in 46 subjects), and in both years (in 37 subjects).

Specific electrocardiographic abnormality	Abnormalities found among:				Statistical significance (by Chi square test; NS = not significant)
	111 seropositive subjects		65 seronegative subjects		
	No.	%	No.	%	
Intraventricular conduction delays (IVCDs)	18	16	3	5	p<0.05
Right bundle branch block (total)	13	12	1	2	p<0.02
RBBB alone	8	7	1	2	NS
RBBB with left anterior fascicular block	4	4	0	0	NS
RBBB with superior, rightward axis	1	1	0	0	NS
Left anterior fascicular block alone	3	3	2	3	NS
Left bundle branch block	1	1	0	0	NS
rSr _i in V ₁ , QRS = 0.10 sec	1	1	0	0	NS
1° atrioventricular block	2	2	0	0	NS
Right axis deviation	1	1	1	2	NS
R>S in lead V ₁	5	5	1	2	NS
Left atrial abnormality	0	0	1	2	NS
Myocardial infarction, probable	0	0	1	2	NS
Myocardial infarction, possible	3	3	0	0	NS
Primary ST-T-U abnormalities ^a	20	18	6	9	NS
Premature ventricular contractions	7	6	0	0	p<0.05
Premature atrial contractions	7	6	3	5	NS
Short PR interval (<0.12 sec.)	3	3	1	2	NS
Left ventricular hypertrophy	1	1	0	0	NS
Subjects with one or more abnormalities	51	46	14	22	p<0.005

^aST-T-U abnormalities occurring in the presence of normal QRS complexes.

Table 10. Right bundle branch block by age group and serologic status for 176 persons on whom ECGs were conducted in 1973 and/or 1980. If ECGs were conducted in both years, the subject's age group was determined according to his or her age at the time of the 1973 test.

Age group (in years)	Seropositive subjects		Seronegative subjects		Statistical significance (by Chi square test; NS = not significant)
	No./with RBBB/ No./tested	% with RBBB	No./with RBBB/ No./tested	% with RBBB	
8-19	0/15	0	0/21	0	—
20-29	1/20	5	1/24	4	NS
30-39	7/27	26	0/9	0	NS
40-49	4/26	15	0/5	0	NS
50-59	1/12	8	0/3	0	NS
≥60	0/11	0	0/3	0	—
Total	13/111	12	1/65	2	p<0.02



Two typical houses in the study area display (above) wood pole walls with a tile roof and (below) mud walls with a palm thatch roof.



(16%) of the seropositives as compared to three (5%) of the seronegatives ($p < 0.05$). Right bundle branch block (RBBB) alone or with other findings occurred in 13 (12%) of the seropositives as compared to one (2%) of the seronegatives ($p < 0.02$). In the latter seronegative subject, RBBB occurred without other IVCDs. Among those with IVCDs, RBBB accounted for 13 of 18 IVCD abnormalities in the seropositives and for one of three IVCD abnormalities in the seronegatives. Premature ventricular contractions (PVCs) occurred in seven (6%) of the seropositives as compared to none of the seronegatives ($p < 0.05$). All of the subjects with PVCs were found to have at least one other ECG abnormality.

Other ECG abnormalities were found to occur at different frequencies in the seropositive and seronegative subjects, but these differences were not statistically significant. RBBB alone occurred in eight (7%) of the seropositive and one (2%) of the seronegative subjects. RBBB with left anterior fascicular block (LAFB) occurred in four (4%) of the seropositives and none of the seronegatives. $R > S$ in lead V_1 occurred in five (5%) of the seropositives and in one (2%) of the seronegatives. Primary ST-T-U abnormalities occurred in 20 (18%) of the seropositives and in six (9%) of the seronegatives.

Findings for subjects with RBBB. Of the 13 seropositive subjects with RBBB, one was between 20 and 29 years of age, seven were between 30 and 39, four were between 40 and 49, and one was between 50 and 59 (see Table 10). Seven of the subjects were men and six women. The 13 were all positive by IHA, with IHA titers ranging from 128 to 4,096. Of the 11 tested by DA, all were positive with DA titers ranging from 256 to 4,096. Finally, of eight tested by CF, five were positive with titers ranging from 16 to 128. Parasitologic isolation from eight of these subjects was attempted by three methods, but was successful in only one case (by xenodiagnosis); these eight subjects were part of the group of 33 from whom parasite isolation was attempted.

The one seronegative person with RBBB was

a man 27 years of age who had a negative history for Chagas' disease; physical examination of this subject revealed no abnormalities.

Longitudinal Study: Progression of Electrocardiographic Abnormalities

Comparison of the ECGs obtained from the same 37 subjects in 1973 and 1980 revealed no significant differences between the earlier and later ECGs. Rather, there were merely minor nonspecific changes in T wave contours and in the appearance or disappearance of atrial and ventricular premature beats.

*Isolation of *T. cruzi**

Isolation of *T. cruzi* was attempted by three methods. This was done in order to confirm that serologically positive subjects were parasitologically positive and to compare the sensitivity of the isolation methods used. Thirty-three persons who were seropositive in 1971 and 1973 were seen again in 1980 and tested for *T. cruzi* infection by xenodiagnosis, inoculation of LIT medium with plasma, and inoculation of MK₂ tissue cultures with serum. *T. cruzi* was isolated from 15 (45%) of the 33 persons tested (Table 11). Nine of the 15 isolates were obtained by xenodiagnosis alone, two were obtained by xenodiagnosis and the tissue-culture method, three were obtained by both culture systems, and one was obtained by LIT medium alone. Overall, 11 (73%) of the 15 isolates were obtained by xenodiagnosis, five (33%) by the MK₂ tissue-culture system, and four (27%) by the LIT medium.

When the 15 parasitologically positive patients were retested serologically in 1980, 10 of the 15 were found positive by IHA, nine of the 15 were found positive by DA, and eight of the 15 were found positive by CF (one of the subjects was not tested by CF). As Table 11 shows, one of the subjects (number two) yielded negative serologic results in 1980 by all three methods.

Table 11. Methods used to isolate *T. cruzi* from 15 of 33 seropositive subjects, showing the age and sex of each subject yielding an isolate, the results obtained with each isolation method, and the subject's serologic status.

No. of isolates	Subject's age in 1980 (in years)	Sex of subject	Results with the indicated <i>T. cruzi</i> isolation methods			Serology titers			
			Xeno-diagnosis	LIT medium	MK ₂ tissue culture	IHA		DA	CF
						1973	1980		
1	21	F	+	0	0	1,024	256	64 ^b	0 ^b
2	70	M	+	0	0	128	64 ^b	64 ^b	0 ^b
3	45	M	+	0	0	1,024	128	128 ^b	16
4	42	M	+	0	0	1,024	256	128 ^b	0 ^b
5	46	M	+	0	0	1,024	128	1,024	0 ^b
6	46	F	+	0	0	256	128	512	64
7	30	F	+	0	0	128	128	128 ^b	0 ^b
8	45	F	+	0	0	NT ^a	64 ^b	2,048	NT ^a
9	29	F	+	0	0	256	128	256	32
10	30	F	+	0	+	512	64 ^b	1,024	0 ^b
11	32	M	+	0	+	1,024	256	256	16
12	59	M	0	+	+	2,048	512	512	32
13	56	M	0	+	+	2,048	256	256	16
14	39	M	0	+	+	1,024	256	256	64
15	33	M	0	+	0	512	<32 ^b	128 ^b	8
Total			11	4	5	14+	10+	9+	8+

^aNT = not tested.

^bResults negative.

Discussion and Conclusions

Prevalence of *T. cruzi*

Between 1962 and 1971 the transmission of *T. cruzi* to humans nearly ceased in Cerro del Aire, as evidenced by the very few seropositive reactors found among children less than 10 years old in the 1971 survey (6, 7). However, before 1962 and the fortuitous onset of transmission control, chagasic infection was holoendemic (6, 7). Indeed, age-specific infection rates in the 1971 survey indicated that, in the absence of control, more than 50% of the population became infected by their early teens, and also that with advancing age and continuing exposure to infected triatomines, most of the remaining uninfected persons eventually became infected.

The near-absence of antibody in young children correlates with both the commencement of

residual insecticide spraying by a malaria control program and the disappearance of the triatomine vector or vectors from Cerro del Aire and other communities in the Pacific Coast study region of Oaxaca (6, 7). Review of malaria campaign records through 1980 showed that DDT had been used almost exclusively in spraying in the region since 1962 (34).

This is especially interesting because DDT is not generally considered an effective agent for control of triatomines (35). Initially, the insecticide was applied every four months, but beginning in 1976 the schedule was changed to twice yearly in Cerro del Aire and other communities, and in some areas spraying was discontinued entirely.

In 1973 we searched for the triatomine vectors by day and night (with black lights at night) and sprayed houses with pyrethrin to activate the insects. Despite these efforts, we recovered only

one specimen of *Rhodnius prolixus* in Cerro del Aire and one specimen of *Triatoma dimidiata* in the nearby town of Nopala. On showing villagers bug specimens in 1973, they reported that the bugs had not been seen for some seven to 10 years. As previously discussed, we presume that the vectors present in the region before the onset of spraying were *Triatoma phyllosoma* spp., *T. dimidiata*, and *R. prolixus* (6, 7). No systematic search for vectors was made in 1980.

In the 1980 serologic survey, 94 children under 10 years old who were born in Cerro del Aire were tested serologically for *T. cruzi* antibodies. Five of these children, ranging from one to six years of age, were seropositive. Four mothers of these five seropositive children were also tested; three were seronegative, indicating that the infections in their children were not congenitally transmitted but had been acquired postpartum as a result of vector transmission. Thus, during the period 1971-1980 vector transmission of Chagas' disease in Cerro del Aire appears to have continued at a low level.

Comparative Sensitivity of the Serologic Tests

The IHA and DA tests probably measure different antibodies. The IHA detects IgG immunoglobulins more efficiently than IgM immunoglobulins, while the DA measures IgM more efficiently than IgG. For this reason, in a population with only chronic infections, such as that in Cerro del Aire, IHA tests should be and were more sensitive than DA tests (see Table 4). CF is the test of choice for detecting chronic Chagas' disease; like IHA, CF efficiently measures IgG but not IgM. In this study, the CF test was also more sensitive than the DA test and was as sensitive as the IHA test (see Tables 5 and 6).

In determining the sensitivity and specificity of serologic tests, a cutoff level must be selected to differentiate between positive and negative titers. The cutoff levels used in this study were taken from routine diagnostic serology at the U.S. Centers for Disease Control (4, 5, 31). The fact that reinfection had not occurred in this

population for about 18 years (1962-1980) may have contributed to the antibody decay seen over this period for IHA titers (see Table 11). Decay of DA antibody presumably also occurred, as exemplified by the cases of six subjects listed in Table 11 who had negative DA titers (64 or 128) but after isolation tests were shown to be parasite-positive. The concordance between DA and IHA tests was 92%; the concordance between CF and IHA, and CF and DA tests were 72% and 73%, respectively. A higher concordance for the latter pairs might have been obtained had we arbitrarily lowered the cutoff level of the IHA and DA tests to compensate for IHA and DA antibody decay.

Clinical Manifestations of T. cruzi Infection in Cerro del Aire

The clinical manifestations of Chagas' disease vary in different regions of its geographic distribution (1). In cases of chronic infection, the heart is the most commonly afflicted organ. Severe chronic myocarditis can progress to cardiomegaly and heart failure. In another form of the infection, the conduction system is affected; this can lead to a variety of arrhythmias that can result in sudden death before the onset of clinically apparent hemodynamic abnormalities. In this study of chronically infected subjects, the physical examinations yielded findings with regard to cardiomegaly, congestive heart failure, arrhythmias, cardiac murmurs, and gallops that were not significantly different for seropositive and seronegative subjects. The subjects' histories, however, indicated that palpitations and precordial pain were significantly more frequent among seropositive persons.

Acute Chagas' disease probably occurs in Cerro del Aire but is not recognized because of the complete absence of trained medical observers in the community. However, in response to our inquiries about possible findings at the onset of infection, chagoma- or Romaña-like lesions were reported more frequently by seropositive than by seronegative persons. Sudden and unexpected

death of young adults, a feature of the disease in some areas of South America, does not appear to occur in Cerro del Aire. In some regions of South America, particularly central Brazil, infection may also result in motility disturbances of the esophagus and distal colon or in "mega" syndromes of these organs. However, the findings we obtained from histories and physical examinations in Cerro del Aire do not suggest that the infection causes pathologic changes in the intestinal tract.

Electrocardiographic Findings

Intraventricular conduction delays and arrhythmias are cardinal ECG features of chronic chagasic cardiomyopathy. Complete right bundle branch block (RBBB) is the most frequent finding; left anterior fascicular block is reported to coexist at about the same frequency. Other common ECG findings include atrioventricular (AV) block, premature ventricular contractions (PVCs), and ST-T-U abnormalities (11, 36-38).

In Cerro del Aire, ECG abnormalities statistically more frequent among seropositives than seronegatives were RBBB (with or without other abnormalities), IVCDs in total, and PVCs. For RBBB in particular, 12% of the seropositives but only 2% of the seronegatives had the finding; this difference is statistically significant ($p < 0.02$).

Pinto-Dias (36) has commented that a region endemic for Chagas' disease could be recognized electrocardiographically by one of two findings: (1) a prevalence of RBBB higher than 2% and (2) a marked disproportion between right and left bundle branch block. In his work, the ratio of RBBB to LBBB was 28:1. In our study, among seropositives, it was 13:1.

In addition to being caused by Chagas' disease, RBBB can occur as an expression of atherosclerotic heart disease (generally in people over age 60), in people with hypertension, as a rare manifestation of rheumatic heart disease, and as an isolated finding in the absence of other cardiac abnormalities. In Cerro del Aire, the relatively young age of most people tested by

ECG and the near-absence in the population of hypertension or murmurs suggestive of rheumatic heart disease rules against these other potential causes as providing an explanation for the high frequency of RBBB.

Since 46% of the seropositive persons tested by ECG had one or more abnormalities, compared to 22% of the seronegatives ($p < 0.005$), it can be concluded that a relatively large proportion of the persons infected with *T. cruzi* in Cerro del Aire exhibited cardiac pathology. Nevertheless, limitations of the sampling methods do not permit extrapolation from these findings for the purpose of determining a prevalence rate for chagasic cardiopathy.

Progression of the Disease Over a Seven-Year Period

In our longitudinal study, the paucity of major or minor ECG changes among 37 seropositive persons followed for a seven-year period is unexpected and surprising. In contrast to our findings, community-based longitudinal studies of Chagas' disease conducted by others have shown progression of abnormalities in seropositive persons. Pifano (24), working in Venezuela's Carabobo State, reported a 17% to 39% increase in electrocardiographic abnormalities among 218 seropositive persons followed for seven years. Puigbo and colleagues (23), working in Belén, Venezuela, described a 14% increase among 429 persons followed for four years. Prata (22), working in São Felipe, Brazil, reported that less than 5% of a group of patients showed evolution of their cardiac status during each year of the study; but Maguire and colleagues (25), working in the Brazilian state of Bahia, reported progression or new conduction defects in 9% of the seropositive subjects studied, as compared to 2% of the seronegative subjects studied, over a three-year period. Finally, Apt and colleagues (28) reported a 9% increase in IVCDs among 28 seropositive subjects from Chile's Elqui Valley who were followed for four years, and a 10% increase among 43

seropositive subjects from the Limiri Valley (also in Chile) who were followed for four years.

It is possible that the Cerro del Aire strain of *T. cruzi* is less pathogenic, and that as a result cardiac pathology proceeds at a much slower rate. A second hypothesis takes into consideration a difference between the Cerro del Aire study area and the areas of the other longitudinal studies. In Cerro del Aire, transmission of the infection was nearly nonexistent throughout the study period, whereas in the other studies transmission apparently continued. So, although *T. cruzi* infection persists in humans indefinitely, repeated reinfection (39-42) could possibly be an important factor in the mechanism responsible for progression of cardiac disease. With regard to the time when cardiac damage occurs, our epidemiologic findings also support a view on pathogenesis that most of the damage occurs during the acute phase of the disease (43) and possibly also at times of new infections, but not as a result of persistent infection in the chronic stage.

We conclude from the cross-sectional and longitudinal ECG studies and from the *T. cruzi* isolations from seropositive patients in Cerro del Aire that infection in this region of Oaxaca can induce a substantial degree of cardiac electrical abnormality. Whether the damage responsible for these abnormalities results in significant morbidity or in early death is not yet established. Our knowledge is also incomplete with regard to the frequency and severity of both acute and congenital *T. cruzi* infections.

Given the pathogenic potential of the infection in Cerro del Aire, the serendipitous but effective control of *T. cruzi* transmission through the malaria control program, and the relatively recent administrative decision to decrease insecticide spraying in the region, it is essential that sylvatic searching for the vector be continued, and also that monitoring continue for repopulation of houses by the bugs. In addition, serologic surveillance, particularly through serial surveys in children, should be continued so that the resumption of *T. cruzi* transmission to humans can be detected.

Isolation of *T. cruzi*

Although most investigators accept positivity in serodiagnostic tests as evidence of infection with *T. cruzi* (1), confirmation was sought by isolation of the organism from seropositive persons. Well-documented cross-reactions with *T. cruzi* antigen have been observed only with *Leishmania*; however, leishmaniasis does not occur in our study region of Oaxaca. In 1974 (8, 9) we reported finding no cross-reactions with *T. rangeli* in Oaxaca sera; to date this latter parasite has not been recognized in Oaxaca residents or in *Rhodnius prolixus* specimens from Oaxaca.

Of the 33 serologically positive persons from whom *T. cruzi* isolation was attempted, 45% yielded *T. cruzi* isolates by one of the three methods employed. Xenodiagnosis, in which each bug was examined individually for *T. cruzi* infection, was the most sensitive method, being nearly twice as effective as the two hemoculture methods combined (see Table 10). An evaluation of the sensitivity obtained by examining the gut contents of each individual bug for *T. cruzi*, as compared to the sensitivity obtained by the usual method of pooling gut contents (25, 29), will be reported separately. Generally, for chronically infected patients, the isolation of *T. cruzi* by xenodiagnosis is seldom successful in over 60% of the cases, as compared to a success rate of about 30% for hemoculture. In the hands of some workers, however, these ratios have been reversed (1, 30, 44, 45). Thus, our findings correspond to the usual experience that xenodiagnosis is the more sensitive procedure, while indicating that xenodiagnosis plus hemoculture is more sensitive than either method alone.

Regarding hemoculture with LIT medium, it has been reported (30) that inoculation of LIT medium with packed red blood cells obtained from 30 cc of blood provided by serologically positive subjects has resulted in the recovery of *T. cruzi* from 55% of the subjects tested. Our failure to achieve this high recovery rate with LIT medium could have been due in part to the smaller volume of inoculum employed. (We used only 10 cc of blood as the source of packed

cells, this being the maximum volume of whole blood we could conveniently obtain by venipuncture under outpatient conditions.) Nevertheless, the LIT medium method (as well as the MK₂ cell culture method) permitted isolation of three

strains of *T. cruzi* from subjects not yielding isolates by xenodiagnosis, and LIT medium alone also succeeded in isolating the parasite from one subject negative by both of the other isolation methods.

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SUMMARY

In 1971, serologic surveys conducted in the Mexican state of Oaxaca revealed unusually high levels of antibody to the Chagas' disease agent, *Trypanosoma cruzi*, in certain communities. The survey in one of these communities, Cerro del Aire, showed that of 248 persons tested who were over 20 years of age, 76% were seropositive. However, only 2% of those under 10 years of age were seropositive, and the absence of the vector indicated that transmission of the infection had not occurred in the community for about 10 years. Further studies were undertaken in 1973 and 1980 to (1) determine the pathologic impact of these infections in humans, (2) investigate the natural history of the disease, (3) confirm that people yielding positive serologic responses were parasitologically positive, and (4) compare *T. cruzi* isolation methods.

Of 237 residents (124 under age 16) in Cerro del Aire tested by indirect hemagglutination, direct agglutination, and complement fixation tests in 1980, 35% were seropositive. Clinical and electrocardio-

graphic (ECG) examinations showed significant differences between 111 seropositive and 65 seronegative persons. Seropositive persons who provided medical histories reported chagoma-like or Romaña-like lesions more frequently than did seronegative persons ($p < 0.02$). Differences were also significant for precordial pain ($p < 0.05$) and palpitations ($p < 0.02$). ECG abnormalities of one or more types occurred in 46% of the seropositive persons but only in 22% of the seronegative persons ($p < 0.005$). Right bundle branch block occurred in 12% of the seropositives and 2% of the seronegatives ($p < 0.02$), and premature ventricular contractions occurred in 6% of the seropositives but in none of the seronegatives ($p < 0.05$). Histories of sudden, unexpected deaths of young adults and of intestinal tract abnormalities were not found.

In the longitudinal study, paired ECGs from 37 persons seen in 1973 and again in 1980 showed little change, suggesting minimal progression of cardiac pathology during the seven-year interval. This lack of

progression could relate to the lack of reinfection with *T. cruzi* during this interval as a result of antimalarial DDT spraying (since 1966) that greatly reduced local populations of triatomid vectors and resulted in a near-cessation of transmission of the infection.

T. cruzi was isolated from 45% of 33 seropositive persons. Xenodiagnosis carried out by dissection of individual bugs was more than twice as sensitive as the combined use of LIT medium and MK₂ tissue culture for these isolations.

Further information is needed from Cerro del Aire as to whether the *T. cruzi* infections there cause significant morbidity, premature deaths, or congenital malformations. In view of the administrative decision to decrease insecticide spraying in the community in 1976, monitoring for repopulation of houses by the vector and serologic surveillance—particularly of children—should continue so that resumption of *T. cruzi* transmission to humans can be detected.

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POLIOMYELITIS IN THE AMERICAS, 1983

The countries of the Americas reported 776 poliomyelitis cases to the World Health Organization in 1983, as compared to 840 in 1981 and 787 in 1982. Nineteen countries, mostly smaller ones, reported no cases in 1983. Others (including Brazil, the Dominican Republic, Ecuador, and Paraguay) reported a decline in the incidence of cases. On the other hand, some countries (including Colombia, El Salvador, Guatemala, Haiti, and Mexico) reported relatively high or increasing incidences. It is difficult to determine whether these latter reflect the actual epidemiologic situation or results obtained by improved surveillance and reporting efforts.

On the whole, the poliomyelitis situation in the Americas seems to be relatively stable, with a clear downward trend appearing in those countries with relatively high vaccination coverage of the population.

Source: World Health Organization, *Weekly Epidemiological Record* 60(23):175, 1985.