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EPIDEMIOLOGIC STUDIES OF CHAGAS' DISEASE IN OAXACA, MEXICO[1]

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A survey in southern Mexico for antibodies to Trypanosoma cruzi, *the agent of Chagas' disease, indicated higher prevalences of such antibodies than had previously been found in Mexico. The data were derived from tests of blood samples collected on filter paper strips from 4,023 people residing in 60 communities. Prevalences were particularly marked among subjects 20 years of age and over, 35 per cent of whom were found to be seropositive, and among residents of one village in which 76 per cent of the adult test subjects yielded positive results.*

Introduction

Chagas' disease, the most important cause of heart disease in some areas of South America (*1*), has not been considered an important infection in Mexico. Nevertheless, a survey conducted among persons living in a Pacific coastal region of the State of Oaxaca, Mexico, has shown an unexpectedly high prevalence of antibodies to the disease agent, *Trypanosoma cruzi*. The survey was carried out in 60 communities, after evaluating three serologic tests used for detecting Chagas' disease antibodies and determining the effectiveness of filter paper strips as a means of collecting and storing blood to be used in the indirect hemagglutination (IHA) test (*2*). The percentage of positive reactors ranged from 0 in six communities to 50 per cent in the sample from the community of Cerro del Aire, where the 248 persons tested represented most of the population.

Information on the distribution, prevalence, and severity of Chagas' disease in Mexico is limited. Four previous serologic surveys conducted in Mexican towns (*3, 4, 5, 6*) obtained seropositive rates of 4 to 13 per cent with the complement fixation (CF) test. Clinically manifest Chagas' disease has not been diagnosed frequently. According to past reviews of data on Chagas' disease in Mexico (*7, 8*), only 72 cases of the disease (including 5 from the State of Oaxaca) were confirmed parasitologically. In 1972 we reported the results of a survey for antibody to seven parasites in the present study area of Oaxaca (*9*). These results showed that 47 of 161 persons tested (29 per cent) had antibody that reacted with *T. cruzi* antigen. This finding confirmed the prediction by Tay, et al. (*10*) that Pacific coastal areas of Oaxaca might have endemic zones of Chagas' disease.

The objectives of the study reported here were: (a) to extend our knowledge of the distribution and prevalence of antibodies to *T. cruzi* in Oaxaca; (b) to isolate the parasite from man, thereby confirming that the detected antibodies resulted from

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T. cruzi infections; and (c) to find an area of active transmission that would permit subsequent detailed epidemiologic studies of the domestic and sylvatic life cycles of *T. cruzi*.

Study Area and Methods

Oaxaca, one of Mexico's southernmost states (see Figure 1), is located at about latitude 17° North and longitude 96° West. The study region, in the southwest part of the state, is approximately 260 km by 90 km and encompasses the five political districts of Jamiltepec, Juquila, Pochutla, Putla, and Sola de Vega. Lying between the Pacific Ocean to the south and the Sierra Madre del Sur to the north, the region has an altitude ranging from sea level to 1,800 meters. Topographically (moving from south to north) it begins as a narrow, flat, lowland coastal zone that is succeeded by rapidly rising foothills.

Five different ethnic groups, each with its own dialect, live in this region. Because only three roads cross the area, most communities within it are isolated. Houses usually have dirt floors, walls made with wood slats and adobe, and ceilings of palm fronds, straw, or tile. The study region has a population of approximately 330,000, its local communities ranging in size from *rancherías* with only a few inhabitants to one town with a population of 10,500.

Survey Areas

For sampling purposes, the study region was divided into 16 zones of about equal size. The initial plan was to obtain a sample from four to six communities in each zone. Overall, 60 communities of varying size were studied; in most zones four or more communities were included, but in six zones only one or two communities were sampled.

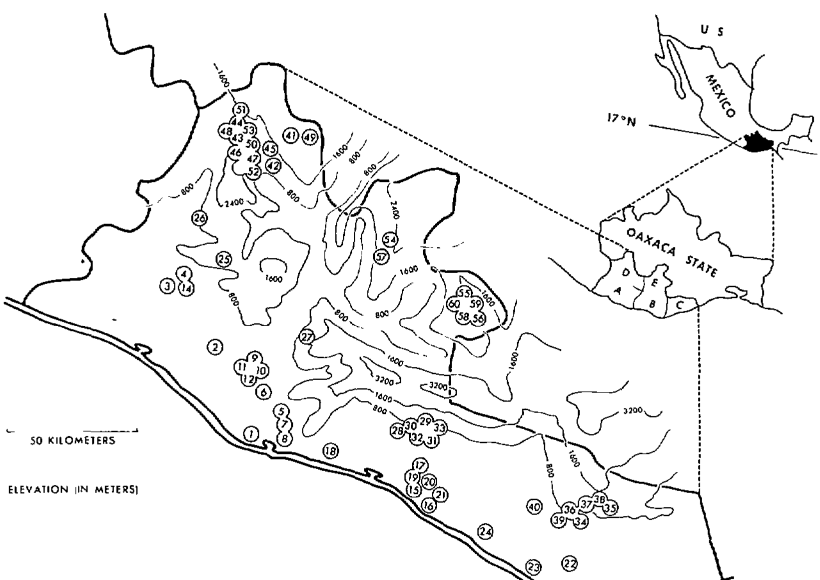


Figure 1. A region along the Pacific coast of Oaxaca State, Mexico, showing the locations and code numbers of the 60 communities studied. The political subdivisions of the region are: (A) Jamiltepec, (B) Juquila, (C) Pochutla, (D) Putla, and (E) Sola de Vega.

Within each community, the subjects tested were persons living in a cluster of adjacent houses. All residents of the selected houses who were at home when our team visited provided blood specimens and demographic information. However, the specific community and the cluster of houses selected within the community were determined by where malaria campaign personnel were spraying insecticides at the time our technicians arrived to work in a particular zone. Our acceptance by the local people and their willingness to provide blood specimens was facilitated by having our technicians accompany the malaria workers (who were well-known locally) as they proceeded sequentially from house to house.

The name, number of inhabitants (in 1971), approximate altitude, and sample size of each community are presented in Table 1. This table combines data for communities where blood specimens were obtained from fewer than 30 persons. Figure 1 uses a code number for each community (cited in Table 1) to show its geographic location and topographic setting. Figure 2 provides the geographic location of the eight "areas" into which the 60 communities were grouped (see Table 1). Although arbitrarily designated, the bound-

Figure 2. Grouping of communities into study areas based on longitude, altitude, and topography, and showing the percentage of positive reactors among those tested in each community and each area. (Each circle represents one community; the size of the circle reflects the percentage of test subjects yielding a positive response. Areas I to III are at altitudes of 0-400 meters; areas IV to VIII are at 600-1,800 meters.)

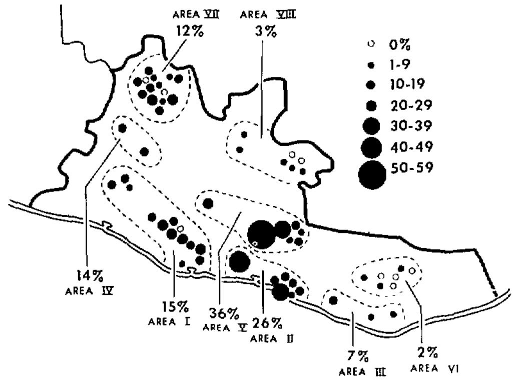


Table 1. Results of indirect hemagglutination testing of blood specimens from 4,023 subjects with *Trypanosoma cruzi* antigen (number positive over number tested). Data are broken down by subjects' age group (0-9 years, 10-19 years, total sample) and place of residence.

Area (Altitude in meters)	Selected Communities*			Number and % of positive reactors in total sample		Number of positive reactors in specific age groups (No. positive/No. tested)	
	Code Number	Name	Population	No. positive / No. tested	% Positive	Ages 0-9 years	Ages 10-19 years
Area I (0-400 m)	1	Charco Redondo	268	1/44	2	0/21	0/10
	2	Jamiltepec	10,516	7/41	17	0/3	0/14
	3	Tlacamama	1,213	5/46	11	0/21	0/8
	4	San Pedro Atoyac	2,700	6/40	15	0/13	0/5
	5	Tututepec	1,641	11/78	14	0/40	0/20
	6	El Ciruelo	61	14/49	29	0/15	0/16
	7	La Luz	1,239	11/52	21	0/21	1/13
	Σ	8, 9, 10, 11, 12, 13, 14	2,233	22/178	12	1/55	4/40
		Subtotal	19,871	77/528	14.6	1/189	5/126
Area II (0-400 m)	15	Chila (Bajos de)	2,578	102/289	35	2/69	11/66
	16	La Barra de Colotepec	639	6/47	13	0/33	0/16
	17	San Pedro Mixtepec	1,413	29/181	16	0/58	0/48
	18	Río Grande	4,683	17/39	44	1/14	3/6
	Σ	19, 20, 21	74	7/75	9	0/21	0/6
		Subtotal	9,387	161/631	25.5	3/195	16/142

Table 1. (cont.)

Area (Altitude) in meters)	Selected Communities* Code Number	Name	Population	Number and % of positive reactors in total sample: No. positive / No. tested	% Positive	Number of positive reactors in specific age groups (No. positive/No tested): Ages 0-9 years	Ages 10-19 years
Area III	22	San Pedro Pochutla	4,395	4/70	6	0/26	0/14
(0-400 m)	23	Santa María Tonameca	1,081	2/120	2	0/30	0/59
	24	Cozoaltepec	809	17/84	20	0/29	3/19
	40	Santo Domingo del Estado	481	3/88	3	1/28	0/26
		Subtotal	6,766	26/362	7.2	1/113	3/118
Area IV	25	San Juan Cacahuatepec	2,033	9/49	18	0/15	0/7
(800-1,300 m)	26	Santa María Zacatepec	1,766	11/98	11	0/41	0/15
		Subtotal	3,799	20/147	13.6	0/56	0/22
Area V	27	Tataltepec de Valdéz	1,312	93/303	31	5/71	12/83
(600-1,800 m)	28	Cerro del Aire	280	123/248	50	1/53	26/69
	29	El Corozal	119	9/31	29	0/8	1/11
	30	San Juan, Lachao Nuevo	1,288	15/39	38	0/11	1/5
	31	Santa Rosa, Lachao Nuevo	290	8/47	17	0/19	0/9
	Σ	32, 33	30	2/23	9	0/7	0/5
		Subtotal	3,319	250/691	36.2	6/169	40/182
Area VI	34	Candelaria, Loxicha	879	2/161	1	1/20	1/141
(600-1,800 m)	35	Pluma Hidalgo	878	0/37	0	0/15	0/5
	36	Buenavista, Loxicha	1,100	0/42	0	0/9	0/14
	37	San Isidro (del Camino)	325	0/34	0	0/6	0/24
	38	La Galera Santiago	1,264	1/27	4	0/9	0/3
	39	San Francisco, Loxicha	343	1/36	3	0/11	0/10
		Subtotal	4,789	4/337	1.2	1/70	1/197
Area VII	41	Zimatlán	506	5/200	3	0/77	1/77
(800-1,000)	42	San Miguel Reyes	481	22/87	25	0/22	6/36
	43	San Juan Lagunas	715	30/239	13	5/96	6/84
	44	Malpica	307	0/52	0	0/33	0/11
	45	Cuadrilla	250	0/43	0	0/23	0/20
	46	Sesteadero	238	13/51	25	0/20	4/17
	47	San Isidro Palizada	177	5/60	8	0/23	0/15
	Σ	48, 49, 50, 51, 52, 53	1,387	29/245	12	1/91	4/81
		Subtotal	4,061	104/977	10.6	6/385	21/341
Area VIII	54	Textitlán	385	3/95	3	0/25	0/13
(1,400-1,800 m)	55	Santa María Sola	548	0/45	0	0/14	0/5
	56	Sola de Vega	2,304	5/73	7	0/32	0/8
	57	Santa María Zaniza	742	3/52	6	0/14	1/8
	58	San Ildefonso	171	1/41	2	0/14	0/8
	Σ	59, 60	678	1/44	2	0/13	0/9
		Subtotal	4,828	13/350	3.7	0/112	1/51
		Total	56,820	655/4,023	16.3	18/1,289 (1.4%)	87/1,179 (7.4%)

*The communities selected are those in which the sample included 30 or more persons. Data for the remaining communities, which are listed by code number only, are combined under the summation symbol (Σ).

aries of these eight areas are based principally on parameters of longitude, altitude, and topography. Areas I, II, and III are located along the coast; they have altitudes ranging from 0 to 400 meters, tropical deciduous forest vegetation, a mean annual temperature of 28°C, and a mean annual rainfall of 1,016 mm. Areas IV through

VIII have altitudes of 600 to 1,800 meters, forest cover ranging from deciduous vegetation to pine-oak zones in the higher altitudes, mean temperatures up to 20°C, and an annual rainfall of up to 525 mm in some places. Areas VII and VIII are separated from each other by the Rio Verde basin and from the other six areas by highlands with elevations up to 3,000 meters. Most of the precipitation received by all the areas occurs from May to October, and a dry period is characteristic from November to April. Although nights may be cool in the higher villages, freezing does not occur.

Between August and October 1971, blood specimens from 4,478 persons were collected on filter paper strips. However, 455 specimens from subjects who had travelled outside the study region—for as little as a few days—were subsequently excluded, leaving 4,023 specimens for analysis. The sample size and total population in each of the five political districts were as follows:

District	*Population*	*Sample size*
Jamiltepec	103,000	300
Juquila	55,800	1,599
Pochutla	79,500	699
Putla	53,600	1,075
Sola de Vega	40,900	350

The sex and age distributions of the 4,023 subjects included in the study are presented in Table 2. Because men were often working in the fields at the time a household was visited, there were fewer male than female subjects in the age groups above 16 years of age.

Procedures for Blood Collection, Storage, and Testing

Blood Collection. After pricking the subject's finger with a lancet, fingertip blood was obtained without squeezing and allowed to saturate both sides of the marked circle on a filter paper strip. Further details on the use of the filter paper strips are presented in a companion paper (*2*). Performance of the indirect hemagglutination test (IHA), using a *T. cruzi* extract as antigen, was the main serologic procedure. The antigen and serologic methods used in this study have been described previously (*9*).

Xenodiagnosis. Xenodiagnosis was carried out using fourth and fifth instar nymphs and adults of *Triatoma barberi* and *Rhodnius prolixus*.[7] After the bugs had

[7] *T. barberi* was obtained from Dr. R. E. Ryckman, whose colony was started from bugs collected at Sola de Vega, Oaxaca; *R. prolixus* was obtained from the Hooper Foundation colony, which was initiated with bugs collected in Venezuela.

Table 2. Results of indirect hemagglutination testing of blood specimens from 4,023 subjects, by age and sex.

Age group (years)	% males in each age group	Males			Females			Total		
		No. tested	No. positive	% positive	No. tested	No. positive	% positive	No. tested	No. positive	% positive
0-3	60	147	3	2.0	98	3	3.1	245	6	2.4
4-6	52	265	4	1.5	248	1	0.4	513	5	1.0
7-9	49	262	1	0.4	269	6	2.2	531	7	1.3
10-12	53	381	12	3.1	342	8	2.3	723	20	2.8
13-15	54	128	12	9.4	108	14	13.0	236	26	11.0
16-19	37	82	20	24.4	138	21	15.2	220	41	18.6
20-29	30	161	48	29.8	369	75	20.3	530	123	23.2
30-39	30	128	60	46.9	304	99	32.6	432	159	36.8
40-49	33	84	46	54.8	167	65	38.9	251	111	44.2
50-59	44	71	44	62.0	90	43	47.8	161	87	54.0
60 +	35	63	23	36.5	118	47	39.8	181	70	38.7
Total	44	1,772	273	15.4	2,251	382	17.0	4,023	655	16.3

been starved for three weeks, they were fed on human subjects (approximately 16 *R. prolixus* and 6 *T. barberi* per subject) for 30 minutes. Only engorged bugs were kept for study, and these were kept at a temperature of 28°C and 50 per cent relative humidity. Subsequently, at intervals of 3, 7, and 11 weeks, expressed feces were examined for flagellates. At 11 weeks the bugs were separated into lots of six; for each lot, mid and hind guts were removed, ground together, suspended in saline, and inoculated into three weanling white mice. At 15 and 45 days after inoculation blood was removed from the tail of each mouse and the white blood cell layer examined for trypanosomes by the heparinized capillary tube method (*11*).

Isolation methods. To isolate *T. cruzi*, blood samples were inoculated into Offutt's medium (*12*) and closed test tube tissue cultures of MK2 cells, a cell line obtained from Microbiological Associates, USA. The tissue cultures, used within 10-14 days after seeding, were maintained with minimum essential medium with 10 per cent fetal bovine serum, supplemental nonessential amino acids, vitamins, and antibiotics; the pH of the medium was maintained between 7.1 and 7.6. Inocula were added to tissue cultures (drained of medium) and then allowed to absorb for 2 hours before fresh medium was added to the cultures.

The procedure followed in 1971 was to add 2-3 drops of fresh whole blood (without anticoagulants) to each of two test tubes of Offutt's medium and each of two tubes of tissue culture. In addition, thick and thin blood smears were stained with Giemsa stain and searched for trypanosomes. In 1972 and 1973, however, fresh blood was not used; instead, 2-3 test tubes of both culture systems were inoculated with 0.25-0.5 ml of a plasma concentrate obtained as follows (*13*): 1 mg of Bacto-Phytohemagglutinin (Difco, Code 0528), freshly rehydrated in 0.1 ml of normal saline, was added to 10 ml of heparinized whole blood. The suspension was shaken gently, allowed to stand for 5 minutes at room temperature as agglutination proceeded, and then centrifuged at approximately 700 rpm for 5 minutes. The supernatant plus the buffy coat were then removed and recentrifuged at 1,500 rpm for 10 minutes. The resulting pellet, diluted with 2 ml of the plasma, was used as the inoculum. The Offutt's medium cultures were kept at ambient field temperatures for 2 weeks, and thereafter at 25°C in the laboratory—where they were examined weekly over an 8-week period for the presence of flagellates. The tissue cultures were held at 37°C in the laboratory; 3 weeks after inoculation, the supernatant was centrifuged, and the sediment examined for trypanosomes.

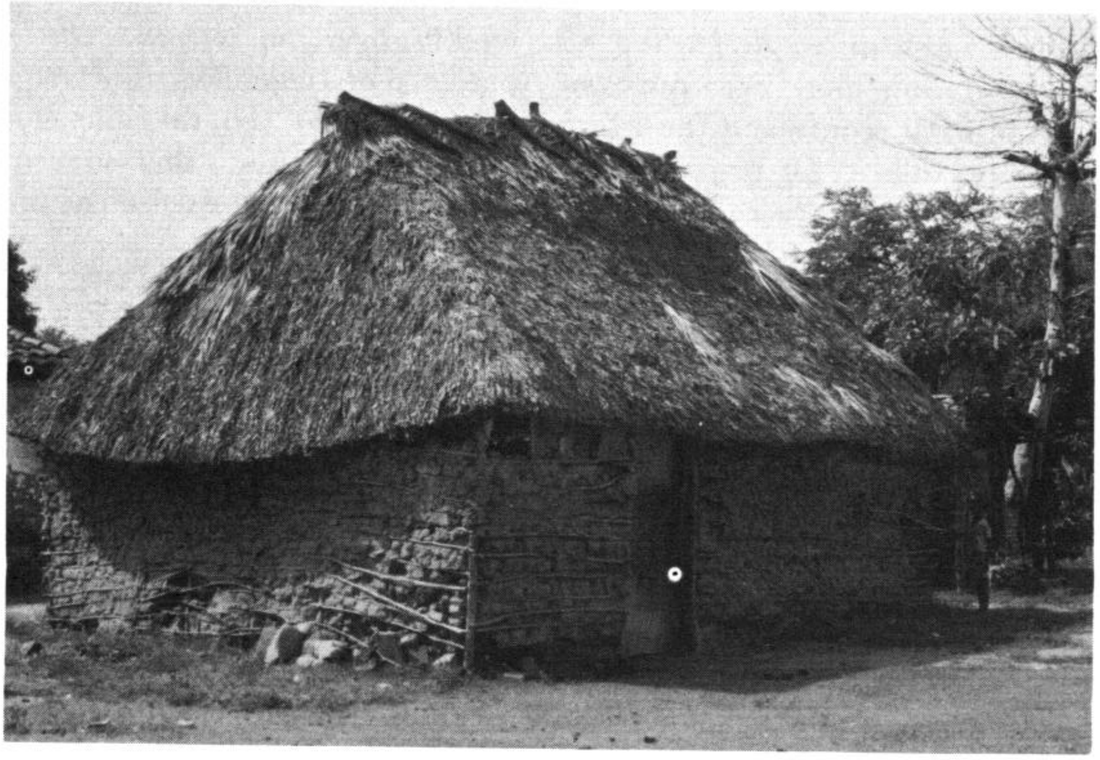
Results

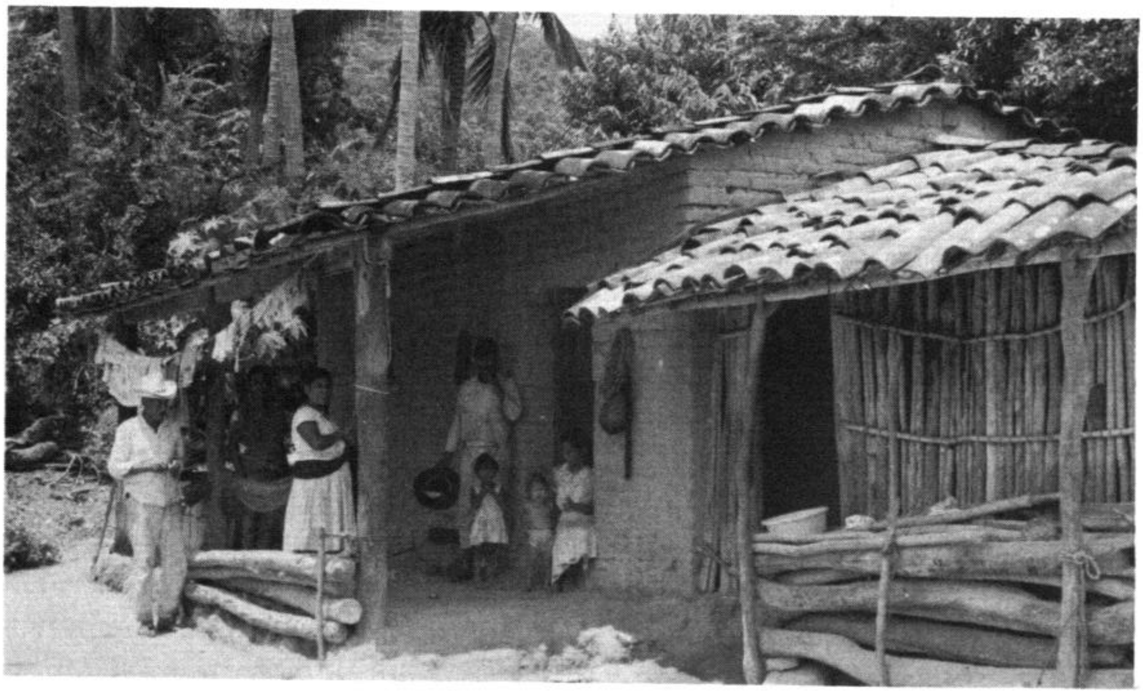
Frequency Distribution of IHA Titers

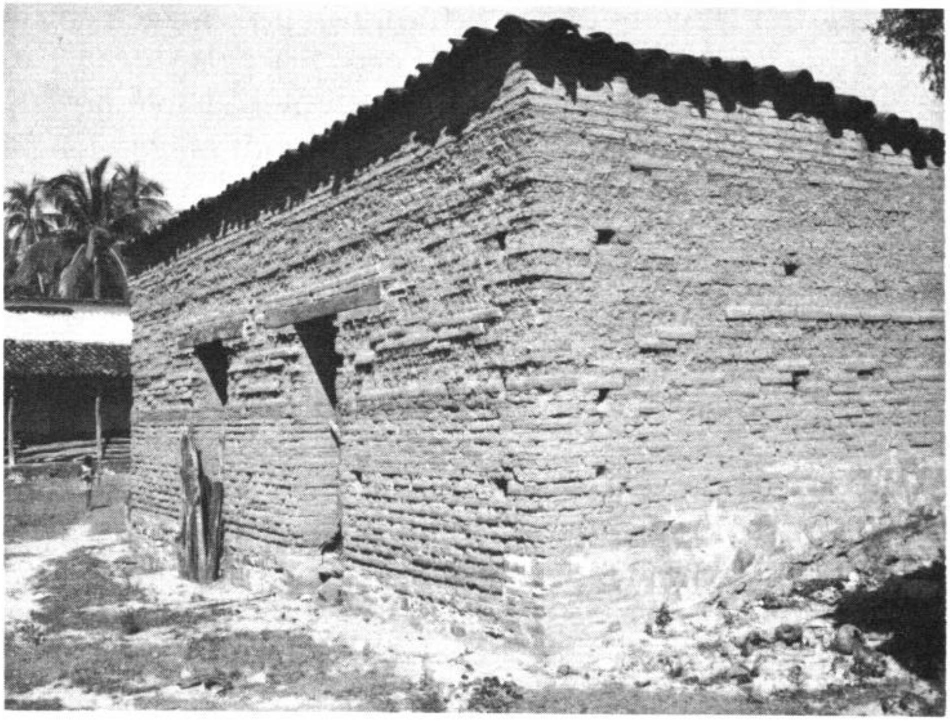
A total of 4,023 IHA antibody titers to *T. cruzi* were obtained by processing blood specimens collected on filter paper strips from subjects who had never travelled outside the study region of Oaxaca. The frequency distribution of these IHA titers (Figure 3) resulted in a bimodal curve with a peak titer at 512. The first mode represented nonspecific reactions and the second mode specific reactions. It was thus appropriate to select some point on the ascending slope of the second mode as the level of a positive titer. The nature of the curve obtained, plus previous experience with the IHA test for *T. cruzi* (*9*), led us to consider titers of 128 and higher as positive.

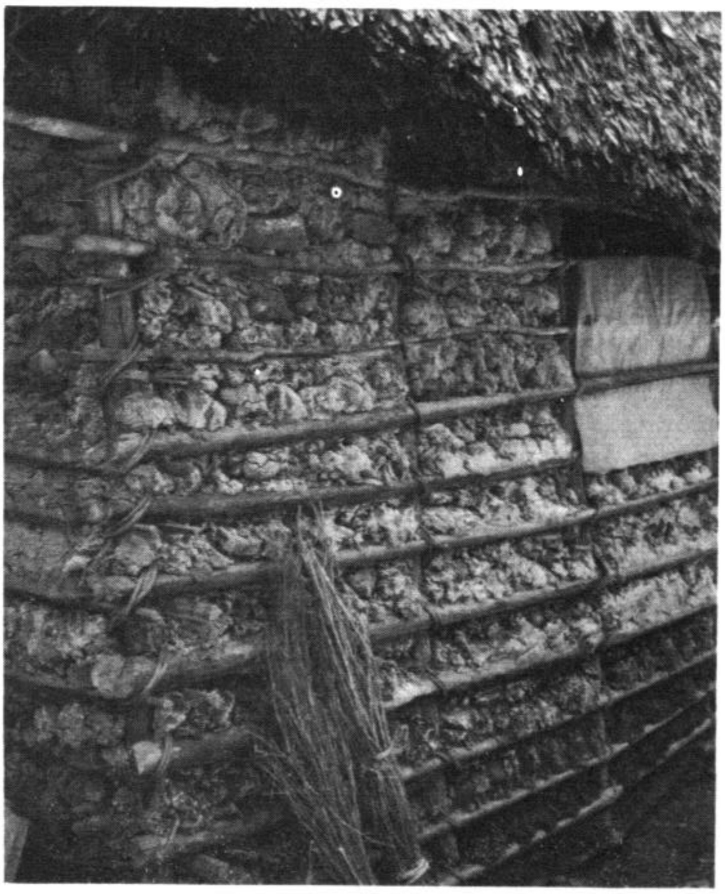
Geographic Distribution of Positive Reactors

The 4,023 persons providing blood specimens lived in 60 communities whose locations are shown in Figure 1. For each community with a sample including over 30 test subjects, Table 1 lists the total number of







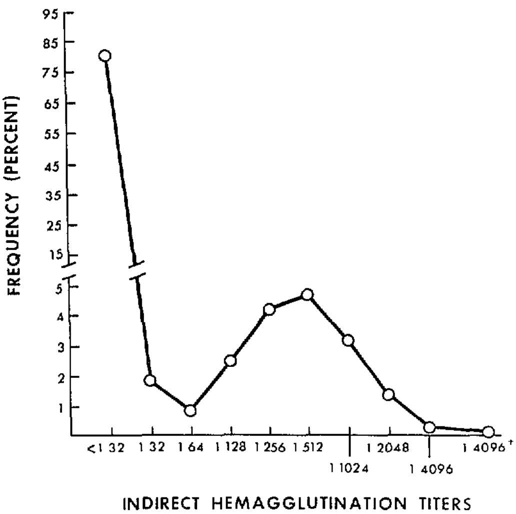


Photos, top left, reading clockwise:

(1) Research team member collects a blood sample on filter paper.

Some typical houses in the study area display: (2) wood pole walls, (3) mud walls and a palm-thatched roof, (4) adobe walls and a tile roof, (5) adobe and wood pole walls and a tile roof, and (6) mud walls (a closer and more detailed view).

Figure 3. Frequency distribution of 4,023 IHA titers obtained with eluates of blood stored on filter papers strips and an antigen prepared from *T. cruzi.*



inhabitants, the number of persons tested, and the percentage of positive reactors in the sample. Where community samples included fewer persons the data have been combined; these combined data are presented in Table 1 under the summation symbol Σ. Antibody prevalence rates for the samples from the 60 communities were 0 (no positive reactors) in 6 communities, 1 to 14 per cent positive in 38, 15 to 45 per cent positive in 15, and 50 per cent positive in 1.

In the town of Cerro del Aire, where 50 per cent of the 248 persons tested were seropositive and the sample included 89 per cent of the whole population (see Table 3), the sample prevalence rate was statistically within two percentage points of the town's true prevalence rate because of the large size of the sample. Sample prevalence rates for other communities were less precise approximations of their true rates—even though the samples included 10 per cent or more of the inhabitants in 36 of the 60 communities—because the households included in the sample were not randomly chosen. Table 3 presents age-specific seropositive rates for subjects in three other towns where specimens were obtained from large numbers of people. In Tataltepec 31 per cent of 303 subjects tested were positive; in Chila 35 per cent of 289 were positive; and in San Juan Lagunas 13 per cent of 239 were positive.

To help find areas with relatively high or

Table 3. Seropositive persons, by age group, among test subjects from four communities.

Age group (years)	Cerro del Aire (Code no. 28)		Tataltepec (Code no. 27)		Chila (Code no. 15)		San Juan Lagunas (Code no. 43)	
	No. positive / No. tested	% Positive	No positive / No. tested	% Positive	No. positive / No. tested	% Positive	No. positive / No. tested	% Positive
0-3	0/6	0	2/11	18	0/22	0	3/13	23
4-6	1/25	4	0/29	0	0/27	0	2/34	6
7-9	0/22	0	3/31	10	2/20	10	0/49	0
10-12	4/24	17	3/38	8	2/27	7	2/68	3
13-15	7/19	37	3/19	16	1/18	6	1/7	14
16-19	15/26	58	6/26	23	8/21	38	3/9	33
20-29	21/39	54	23/63	37	23/44	52	2/19	11
30-39	31/40	78	17/38	45	22/32	69	6/16	38
40-49	24/24	100	16/23	70	18/34	53	3/10	30
50-59	10/13	77	14/17	82	18/24	75	2/2	100
60 +	10/10	100	6/8	75	8/20	40	6/12	50
Total	123/248	50	93/303	31	102/289	35	30/239	13
Total population	(280)		(1,312)		(2,578)		(715)	

low transmission levels of the agent responsible for the *T. cruzi* antibodies, we clustered the communities studied into eight areas as previously described. To provide a graphic representation of the prevalences observed, we then mapped the results by indicating the percentage of positive reactors among subjects from different communities with black circles of varying size (see Figure 2). The highest prevalence rate for any one area (36 per cent) was registered by area V, located in the center of the study region. Area II, just to the south, had the next highest rate (26 per cent). Surrounding areas had lower rates, the lowest (1.2 per cent) being found in the easternmost area (area VI).

Prevalence rates for the political districts of the state (see Figure 1) were as follows: Jamiltepec (A), 14 per cent; Juquila (B), 28.3 per cent; Pochutla (C), 4.2 per cent; Putla (D), 10.7 per cent; and Sola de Vega (E), 3.7 per cent.

Prevalence rates also varied with altitude: 21.3 per cent of the subjects living at 100-800 meters were seropositive (518 of 2,432 tested); but only 8.6 per cent of those living at 800-1,800 meters were seropositive (137 of 1,591 tested).

Crude Antibody Prevalence Rates and Age- and Sex-Specific Rates

Of the 4,023 persons tested for antibodies to *T. cruzi* (see Table 2), 655 (16.3 per cent) were positive. But the percentages of seropositive reactors were very low in the age groups under 12 years of age, ranging between 1.0 and 2.8 per cent. Thereafter the percentage of positive reactors increased progressively up to age 59 for each age group studied, the highest rate (54 per cent positive) being found in the 50-59 group.

In the total sample of 1,555 test subjects who were at least 20 years of age, 35.4 per cent were positive. In the four communities with large samples (Table 3), the percentage of seropositive reactors among those 20 or over was 76.2 per cent in Cerro del Aire, 51.0 per cent in Tataltepec, 57.8 per cent in Chila, and 32.2 per cent in San Juan Lagunas.

Prevalence rates for males and females of all ages were about equal, 15.4 per cent of the males and 17.0 per cent of the females being positive. However, age-specific prevalence rates for the two sexes showed that males had higher rates than females in all age groups between 16 and 59 years, with men aged 50-59 years of age having the highest positive rate (62 per cent).

Additional data indicating a near-absence of *T. cruzi* antibody in young children are shown in Tables 1 and 3. Of 1,289 children under 10 years of age, only 18 (1.4 per cent) had positive reactions (see Table 1). Of these, 5 were from Tataltepec, 5 were from San Juan Lagunas, 2 were from Chila (Table 3), and the remaining 6 were from 6 different towns. Thus, in 51 of the 60 towns surveyed, 919 children under 10 were tested and none was found to be seropositive; but in these same 51 communities the seropositive rate for 995 adults (age 20 or over) was 25 per cent.

Search for the Vector

Persistent efforts were made to find triatomines in four towns. Initially an entomologist worked for one week each in Chila (August 1971), San Pedro Mixtepec (August 1971), and Tataltepec (August 1972) conducting daytime and nighttime searches in and about houses. No triatomines were found. Next, several hundred children were motivated to search for bugs, again with no success. In August 1973, searches were conducted for one-week periods; black (fluorescent) lights were set up in Chila and Cerro del Aire from dusk to midnight, these being placed within houses and in a variety of ecologic habitats in and on the periphery of the towns; but no triatomines were found. In February 1975, houses in the towns of Chila, San Juan Lagunas, and

Cerro del Aire were sprayed with a 1 per cent pyrethrin solution in kerosene in an attempt to enhance collection of bugs. This also was unsuccessful. Furthermore, villagers queried in these and other communities consistently confirmed the absence of the vector; when triatomine specimens were displayed, people repeatedly said that although the bugs had once been common in houses, they had not been seen for about seven years or more.

Previous Oaxaca State studies by others had indicated the presence of four triatomine species: *Triatoma dimidiata*, *T. phyllosoma*, *T. barberi*, and *Rhodnius prolixus*. In June 1973 we did find one adult specimen of *Triatoma dimidiata maculipenis* in a house at Nopala, a town near Cerro del Aire. In addition, in October 1973 one adult specimen of *Rhodnius prolixus* was found at a home in Cerro del Aire.

Isolation of T. cruzi *from a Seropositive Subject*

During the month of August in 1971, 1972, and 1973 four methods were used to detect *T. cruzi* or isolate the organism from seropositive individuals in the towns of Chila, San Pedro Mixtepec, Tataltepec, and Cerro del Aire. This work (see Table 4) proceeded as follows: (a) examinations of thick and thin Giemsa-stained blood smears from 80 persons were conducted in 1971 and found negative for trypanosomes; (b) xenodiagnosis was carried out on 27 subjects with negative results; (c) whole blood from 80 persons, including those tested by xenodiagnosis, was inoculated into Offutt's medium and found negative; and (d) plasma concentrates from 89 subjects were inoculated concurrently into 2-3 test tubes of Offutt's medium and 2-3 test tubes of the MK2 cell line. This latter work yielded negative results in the tissue culture system, but one isolation of *T. cruzi* was made in Offutt's medium. The isolate was from a 30-year-old woman who had lived in Chila all her life. Her antibody titer was 1,024 by IHA and 128 by CF, she was free of symptoms, and her physical examination and electrocardiogram were normal. The isolation was confirmed by inoculating the infected medium into mice and later recovering from their blood trypanosomes with typical *T. cruzi* morphology: C-shape with central nucleus and large terminal kinetoplast. The mice died about two weeks after

Table 4. Methods used in different years to isolate *T. cruzi* from serologically positive residents of Chila, Tataltepec, Cerro del Aire, and San Juan Lagunas.

	1971		1972 and 1973	
	Xenodiagnosis	Whole blood inoculated into 2 tubes of Offutt's medium	Plasma concentrate inoculated into	
			2-3 tubes of Offutt's medium	2-3 tubes of MK2 tissue culture cells
Number of persons tested	27[1]	80[2]	89	89
Number of *T. cruzi* isolations	0	0	1	0

[1]Whole blood specimens from the 27 persons tested by xenodiagnosis and 53 others were inoculated into Offutt's medium.

[2]Examination of thick and thin blood smears from 80 persons were negative for trypanosomes.

inoculation. Pseudocysts containing amastigotes were found on histologic examination of the heart and other tissues.

Discussion

The IHA test has previously been used for seroepidemiologic studies of Chagas' disease in Brazil (*14*), Colombia (Kagan, unpublished), and Oaxaca (*9*). In this study filter papers were used to collect blood specimens because of their convenience and the demonstrated accuracy of previous tests conducted with specimens collected in this way (*2*).

The frequency distribution of IHA titers obtained with 4,023 blood specimens collected on filter paper from persons residing in 60 study region communities (Figure 3) is quite similar to the frequency distribution of IHA titers we previously obtained with 161 Oaxaca sera collected in and near 3 communities of the region (*9*). (The new frequency distribution curve shows a peak titer of 512, as compared to a peak titer of 256 in the first study.) Thus the results of the present enlarged study confirm our initial impression that large numbers of people had probably been exposed to *T. cruzi*.

The data from the present study indicate higher *T. cruzi* antibody prevalence rates than had previously been reported from Mexico—rates equal in some instances to the high rates reported from parts of South America. In the town of Cerro del Aire, for example, 50 per cent of all persons tested were found positive, as were 76 per cent of the test subjects 20 years of age and over. Another unusually high rate of positive reactors (62 per cent) was found among the 71 male test subjects in the 50-59 age group (see Table 2).

The mean antibody prevalence rate for all 4,023 subjects tested was 16.3 per cent, but for those 20 and over the rate was 35 per cent. Both rates exceeded mean prevalence rates of 4 to 13 per cent reported by four previous serologic surveys conducted in Mexico that had employed the complement-fixation test. The results of these earlier surveys were as follows: In Apatzingán, Michoacán (1947), 4 per cent of 48 persons tested were found seropositive (*4*); in Tetitlán, Guerrero (1964), 8.5 per cent of 216 subjects were found seropositive (*3*); in Tuxpan, Michoacán (1966), 7.2 per cent of 148 were found seropositive (*5*); and in Zacatecas (1968), 13 per cent of 75 were found seropositive (*6*). In addition, in Motul, Yucatan (1975), 1.8 per cent of 223 subjects tested by IHA yielded positive results (*15*).

Although antibody to *T. cruzi* was found in residents of all eight areas covered by the present study, particularly high rates (26 per cent and 36 per cent, respectively) were found among residents of study areas II and V (see Figure 2). The factors responsible for these high rates remain unknown.

Our inability to obtain more than one isolation of *T. cruzi* despite concerted efforts (see p. 246) was disappointing. Possible reasons could be that some seropositive reactions were due to other infections or that the methods used were not sensitive enough to isolate the organism. It is regrettable that xenodiagnosis—with large numbers of bugs belonging to local species—could not be more extensively used because of its unacceptability to the population.

The question of the specificity of the IHA antibody detected in the Oaxaca sera with *T. cruzi* antigen cannot be completely answered at present. Nevertheless, we believe that the greater part of this antibody was due to *T. cruzi* infections. In our companion paper (*2*), which reports on laboratory tests conducted in advance of the field studies, we show that some of the antibody could be due to previous infections with *Leishmania mexicana* or *Trypanosoma rangeli*. However, evidence that most of the antibody did actually result from *T. cruzi* infections is provided by the following: (a) a high degree of agreement between seropositive and seronegative reactions in our preliminary study (*2*) when paired sera were simultaneously tested by CF and IHA tests; (b) the isolation of *T. cruzi* from a seropositive person; (c) a relatively large number of abnormal electrocardiograms obtained from young seropositive persons (to be reported); and (d) the low antibody prevalance rates observed in young children—an observation associated in time with the reduction in populations of house-dwelling triatomine bugs.

A significant finding derived from the data is that transmission of the agent responsible for the antibody had been interrupted in the previous decade. Antibody was nearly absent in children under 12 years old, and age-specific prevalence rates for the four youngest age groups—between 0 and 12 years (Table 2)—did not show the expected progressive increase with age.

There is no reason to believe that *T. cruzi* infections are acquired primarily in adult life in Oaxaca and that children are spared. Still, only 1.9 per cent of 2,012 children 0-12 were positive, while the high seropositive rate (35.4 per cent) for adults over 20 indicates that transmission to man was once a very frequent event. Further study is needed to determine what proportion of the few infections seen in young children may have been due to congenital transmission.

To sum up, Chagas' disease appears to have been controlled in the study region. However, the parasite is probably still transmitted in sylvatic foci. Should triatomines become resistant to insecticides, or should the malaria control program be discontinued or become less effective, then the bugs would return to human habitations and transmission of the parasite to man would resume. Therefore, additional triatomines should be found in order to test and confirm that they are indeed sensitive to DDT, a finding that would contrast with their commonly reported resistance to the insecticide elsewhere in Latin America (*16*). Studies are currently being planned in order to search for the vector in sylvatic foci, to determine reservoir hosts for *T. cruzi*, and to elucidate other aspects of the epidemiology of Chagas' disease in Oaxaca State.

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SUMMARY

This article describes the distribution and prevalence of antibody to *Trypanosoma cruzi*, the agent of Chagas' disease, among residents of the Mexican state of Oaxaca. It is based on a study in which 4,023 blood specimens were collected from persons living in 60 Pacific coastal communities and tested by indirect hemagglutination with *T. cruzi* antigen. These tests indicated that 16.3 of the subjects were seropositive, and that a considerably higher proportion—35.4 per cent—of those age 20 and over were seropositive.

Within individual communities the prevalence rates ranged from no positive reactors in 6 communities to 1-14 per cent in 38, 15-45 per cent in 15, and 50 per cent in 1. In the last community, the percentage of seropositive reactors among those 20 years of age and older was 76 per cent. In general, the observed rates were higher than those found by previous surveys in Mexico, the latter rates ranging from 2 to 13 per cent.

Transmission of the agent to man is now infrequent and under control in the study area, probably as a result of DDT spraying connected with the area's malaria control program. Initiation of that program, 10-12 years before this study was carried out, correlates with the reported disappearance of the vector from houses and with a near-absence of *T. cruzi* antibody in young children. Of 2,012 children tested in the 0-12 age group only 1.9 per cent were seropositive, as compared with 14.7 per cent of 456 children 13 to 19 years old and 35.4 per cent of 1,555 subjects who had reached age 20.

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The Concept of Development

The purpose of development is to enable individuals to realize to the full their capacity to improve themselves continuously and to contribute to the overall development of their society and thus to the maximum well-being of its members. It is a process of change from one form of society to another in which all citizens enjoy a better quality of life.

IV Special Meeting of Ministers of Health of the Americas—Final Report and Background Document PAHO Official Document No. 155, Pan American Health Organization, Washington, D.C., 1978, p. 20.