

Serological Follow-up of *Trypanosoma cruzi* Infection from 1987 to 1994 in 32 Counties of the State of Jalisco, Mexico (Preliminary Report)

Seguimento Sorológico de Infecção por Trypanosoma cruzi entre 1987 e 1994 em 32 Municípios do Estado de Jalisco, México (Relatório Preliminar)

Francisco Trujillo-Contreras¹

Maria Angeles V. Yerenas¹

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In 1987 the University of Guadalajara performed a seroepidemiological survey on the prevalence of Chagas' disease in the 124 counties of the State of Jalisco, Mexico, arriving at a rate of 21.6 per 100 inhabitants. From December 1993 to June 1994, we studied 2238 individuals from 32 rural counties in this State. Of these, we found 276 positives (12.33%) and 1962 negatives (87.66%). Nevertheless, the series of serological differences found are very striking, since out of the 655 individuals that were seropositive in 1987, we noted that 276 individuals remained positive, while 50 individuals (7.63%) became negative. There were no flaws in the laboratory techniques. We believe that either the immune response of Mexicans is different or that the virulence of the Mexican strains of *Trypanosoma cruzi* may be not as great as that in the South America countries.

Key words: Chagas' Disease; Serology; *Trypanosoma cruzi* Infection

INTRODUCTION

Chagas disease is caused by a hemoflagellate protozoan producing varied clinical manifestations in humans. Many patients have an indeterminate, asymptomatic form of the disease. Some of these patients can develop lesions 10 or 20 years after the infection, 20% to 30% develop the cardiac form of the disease (Prata, 1985), and around 7% have megaesophagus (Castro et al., 1984). The prevalence of patients with megacolon is still unclear.

Mazzoti (1940), while attempting to diagnose malaria, made the discovery of the

first two human cases of Chagas' disease in Mexico in 1940. In the State of Jalisco, Cuartero et al. (1967) reported the first two cases of this disease in the county of Santa Maria de los Angeles. Later, in 1986, the most important outbreak of acute Chagas' disease of those reported thus far was detected in the county of Tuxcueca, Jalisco. Eleven individuals out of a family of 14 began to display the characteristic symptomatology (Hernandez et al., 1987).

In response to these events, the University of Guadalajara decided in 1987 to perform a seroepidemiological survey to measure the prevalence of Chagas' disease in the 124 counties of the State of Jalisco, arriving at a rate of 21.6 per 100 inhabitants (Trujillo-Contreras et al., 1987).

However, despite this very high rate of infection, from 1940 to 1994 only approximately 300 acute cases and 300

¹ Centro de Investigación de Enfermedades Tropicales, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara. Carlos Merida, No. 348, Colinas de la Normal, CP. 44270, Guadalajara, Jalisco, Mexico.

chronic cases have been reported in Mexico (Instituto Nacional de Diagnostico y Referencia, 1991). This is extremely interesting, since Mexico has the same environmental conditions (ecosystems), reservoirs, and parasites as those found in South America (Instituto Nacional de Diagnostico y Referencia, 1991), but we do not have the same high numbers of diseased persons as South American countries.

For this reason, the decision was made to perform a serological follow-up of *T. cruzi* infection in the same individuals studied in 1987 to see what changes, had occurred over a seven-year period, formulating a negative hypothesis where we did not expect to find changes in the individuals with positive and negative serology.

The purpose of this follow-up study was to perform the Indirect Hemagglutination test on all the individuals, that were involved in the research in 1987 in the 124 municipalities of the State of Jalisco.

MATERIAL AND METHODS

From December 1993 to June 1994, peripheral blood samples were taken from the inhabitants of various municipalities in the rural areas of the State of Jalisco, including

those mentioned in Table 1 of this preliminary report. Individuals born from 1987 on were excluded from the study, as were those who lived at the same address but were not previously included in the study. The characteristics of the dwelling were inspected to compare whether or not there had been any improvement in housing conditions. A search was made inside and outside the house for triatomids, and there was an attempt to identify signs and symptoms suggesting acute or chronic disease.

Samples were extracted by venipuncture and centrifuged at 2500 rpm for 15 minutes. The serum was transported under refrigeration to the Center for Research on Tropical Diseases at the University of Guadalajara. A sample was also taken on Wattman paper number 1.

For processing the samples, the Indirect Hemagglutination technique was used with a procedure similar to that used by the Mario Fatale Chaben Institute in Argentina (Ministerio de Salud y Accion Social, 1985), but following the technique defined by the commercial antigen, Diagno Chagas, of Wiener Laboratories (Wiener Laboratorios). The Tulawen strain was used again so there would be no antigen variation in the test.

TABLE 1. Serological Diferences Found among the Studies of 1987 and 1993. Guadalajara, Jalisco, Mexico

Year	Individuals Studied	Positive	Negative
1987	2956	655 (22.15%)	2301 (77.85%)
1994	2238	276 (12,33%)	1962 (87.67%)

- 256 Individual stayed positive.
- 50 Individual became negative.
- 20 Individuals positive, new cases.

RESULTS

A total of 2238 individuals were studied (75.71 % as compared to the 2956 tested in the 1987 study). Reasons for non-inclusion in the study were the following: migration – 362 (16.17%); refusal – 183 (8.17%); death – 91 (4.06%); and other causes – 80 (3.57%). Of the individuals studied, we found 276 positives

(12.33%) and 1962 negatives (87.66 %), differing from the seropositivity rate of the 1987 study, which was 22.15% for these 32 municipalities (Table 2). Nevertheless, the series of serological differences we found were very striking, since out of the 655 individuals that were seropositive in 1987, we noted that 50 (7.63 %) remained positive, while 20 (7.24 %) were discovered as new cases (Table 1).

TABLE 2. Municipalities Studied in Serological Follow-Up of *T. cruzi* Infection with Percentual Distribution From 1987 to 1994. Guadalajara, Jalisco, Mexico

Municipalities	Year 1987				Year 1994					
	Individuals Studied	Positives		Negatives		Individuals Studied	Positives		Negatives	
		N ^o	%	N ^o	%		N ^o	%	N ^o	%
Chapala	72	0	0	72	100.0	48	1	2.0	47	98.0
Zapotiltic	31	20	65.5	11	35.5	13	0	0	13	100.0
Guadalajara	51	1	1.9	50	98.1	24	0	0	24	100.0
Santa Maria de los Angeles	42	1	2.3	41	97.7	30	1	3.3	29	96.7
Tlajomulco de Zuñiga	172	11	6.3	161	93.7	129	7	5.4	122	94.6
Tequila	74	17	22.9	57	77.1	12	5	11.9	37	78.1
Techaluta	91	12	13.1	79	86.9	61	8	13.1	53	86.9
Jamay	42	23	54.7	19	45.3	30	0	0	30	100.0
San Juan de los Lagos	72	11	15.2	61	84.8	72	3	4.1	69	95.9
Zapopan	140	53	37.8	87	62.2	75	14	18.6	61	81.4
Atenguillo	67	23	34.3	44	65.7	54	17	11.4	37	68.6
Zapotiltic	50	18	36.0	32	64.0	22	0	0	22	100.0
Tuxcueca	196	18	24.4	148	75.6	103	19	18.4	84	81.6
San Martin Hidalgo	50	23	46.0	27	54.0	28	10	15.7	18	64.3
Valle de Juarez	92	11	11.9	81	88.1	58	11	18.9	47	81.1
Tonala	108	47	43.5	61	56.5	40	2	5.0	38	95.0
Villa Purificación	103	15	14.5	88	85.5	43	2	4.6	41	96.4
Extlahuacan de los Membrillos	73	1	1.3	72	98.7	54	3	5.5	51	94.5
Degollado	119	6	5.0	113	95.0	74	10	13.5	64	86.5
Arenal	103	20	19.4	83	80.6	58	5	8.6	53	91.4
Tapalpa	27	13	48.1	14	51.9	22	0	0	22	100.0
Tototlan	197	9	4.5	188	95.5	141	13	9.2	128	90.8
Zapotlanejo	95	4	4.2	91	95.8	40	0	0	40	100.0
Teuchitlan	70	30	42.8	40	57.2	46	15	32.6	31	77.4
Sayula	112	10	8.9	102	91.1	30	1	3.3	29	96.7
La Huerta	61	14	22.9	47	77.1	31	6	19.3	25	80.7
Ocotlan	170	14	8.2	156	91.8	72	9	12.5	63	97.5
Totatiche	52	2	3.8	50	96.2	14	12	85.7	2	14.3
Jocotopec	60	3	5.0	57	95.0	31	6	19.3	25	80.7
San Cristobal de la Barranca	148	0	0	146	100.0	82	3	3.6	79	96.4
Tolimán	78	6	7.6	72	92.4	34	6	17.6	28	82.4
Cuquío	138	24	17.3	114	82.7	49	4	8.1	45	91.9
Totals	2956	655	22.15	2301	77.85	2238	276	12.33	1962	87.67

When correlating the serological results with the dwelling's characteristics, we noted that there had been improvements in the following areas: 18.94% of the roofs had been improved; 16.12 % of the floors, and 9.7% of the walls.

DISCUSSION

When analyzing the above results, we found that the percentages of infection did not show a very extreme difference (from 22.15% to 12.33%), since these were obtained in relation to the number of study subjects; nevertheless, we found a high seroconversion, since 5.0 (7.6%) individuals had become negative in a period of 7 years. Although one might raise the possibility of were flaws in the laboratory technique, this can be dismissed because in all the tests, positive and negative control sera were used, in addition to the fact that a number of sera were provided to the Chagas' Disease Research Laboratory at the School of Medicine in Goiânia, Brazil, and processed by Dr. Alejandro Luquetti (Reference laboratory). The same Tulawen strain was used (Argentina), and techniques of Wattman filter paper number 1 (the same as in the 1987 study) and total sera (to form a serum reference group) were processed.

It is worth mentioning that in the dwellings where improvements in roofs, floors, and walls were reported, no triatomids were found, and the people reported not having been bitten by *barbeiros* since the previous study (1987). Interestingly, the 50 individuals that became negative corresponded to the inhabitants of these houses. Therefore, we believe that not living in infested houses and no longer being bitten produces negative seroconversion of antibody presence, due to a good initial immune response. When performing statistical analysis with the chisquare test, using the Mantel-Haenszel correction between the serology recorded in 1987 and that of 1994, we found a statistically significant difference with a p value of -0.05 (95% C.I.), and we found this same result by analyzing the presence of the vector with the serological results of the positive individuals in 1987 and 1994.

Luquetti (1987) studied 240 sera from megaesophagus patients in Goiânia, Brazil, analyzed by two serological techniques, and found that in 26 (10.8%), both serological tests performed gave clear-cut negative results. Twenty-four of these cases were suspected to be of chagasic etiology because of the epidemiological and clinical data. In 11 out of 24 of these individuals, xenodiagnosis was performed, and two were found to be positive.

In view of this situation, it is obvious that either the immune response of Mexican individuals is different or the virulence of the Mexican strains may be not as great as those in South American countries. This accounts partially for the presence of only 600 cases of the disease in its 54 years of existence.

However, this is only a preliminary report on the 32 municipalities studied, and further analysis is necessary in the remaining 92 municipalities.

Thus, there is reason to conduct further research on individual immune response or to compare the biochemical structure of our *T. cruzi* strains with those from South America.

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RESUMO

TRUJILLO-CONTRERAS, F. & YERENAS, M. A. V. **Seguimento Sorológico de Infecção por *Trypanosoma cruzi* entre 1987 e 1994 em 32 Municípios do Estado de Jalisco, México (Relatório Preliminar).** Cad. Saúde Públ., Rio de Janeiro, 11 (3): 501-505, jul/set, 1995.

Em 1987, a Universidade de Guadalajara realizou um inquérito sorológico sobre a prevalência da doença de Chagas em 124 municípios do Estado de Jalisco, México, chegando a uma taxa de 21.6 por 100 habitantes. Entre dezembro de 1993 a junho de 1994, os autores estudaram 2238 indivíduos de 32 municípios da área rural

desse mesmo estado. Encontraram 276 positivos (12.33%) a 1962 negativos (87.66%). Entretanto, as séries de diferenças sorológicas foram muito marcantes, uma vez que dos 655 indivíduos que testaram positivos em 1987, 276 permaneceram positivos, enquanto 50 (7.63%) tornaram-se soronegativos. Não houve falha de técnica laboratorial. Os autores acreditam que, ou a resposta imune dos mexicanos é diferente, ou então, que a virulência das cepas mexicanas de *Trypanosoma cruzi* não é tão intensa quanto aquela encontrada em países sul-americanos.

Palavras-Chave: Doença de Chagas; Sorologia; Infecção por *Trypanosoma cruzi*

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