

Seroprevalence of *Trypanosoma cruzi* Infection in Students at the Seven-Fourteen Age Range, Londrina, PR, Brazil, in 1995

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Seropositivity for Chagas disease was evaluated in 834 children aged between 7 and 14 from the Municipal Teaching System in the district of Londrina, State of Paraná. A seroprevalence rate of 0.1% was found through the use of an indirect immunofluorescent test and an enzyme-linked immunosorbent assay. This low rate of seroprevalence provides evidence that the vectorial transmission of Chagas disease has been eliminated in Londrina. The main reason for the elimination of vectorial transmission of Trypanosoma cruzi infection, as evaluated by serological tests, may be a remarkable change in the economic structure of the northern region of Paraná in the 1960's. At that time coffee production was almost completely replaced by soy beans, wheat and grazing in the rural areas. This change deeply affected the rural ecology and caused an exodus of the population from rural to urban areas as well as a decrease in the total number of the population of that region. The measures introduced for controlling the disease through the Program of Chagas Disease Control established by the Fundação Nacional de Saúde of the Brazilian Ministry of Health, certainly, had a positive impact on the reduction of American trypanosomiasis prevalence in the area under study. However, it does not seem that this was the most relevant factor responsible for the elimination of vectorial transmission of Chagas disease in Londrina.

Key words: Chagas disease - epidemiology - control program - Brazil

Trypanosoma cruzi is part of an exclusively American ecosystem and is found in large extensions of this continent stretching from southern United States, where sporadic cases of Chagas disease have been reported, to southern Argentina and Chile. Although data concerning the prevalence and morbidity of Chagas disease increased in the 1980's, it is still difficult to accurately establish its geographical distribution as well as its prevalence in Latin America (Rassi et al. 1989, Rey 1991, WHO 1991, Wanderley & Corrêa 1995).

In the American continent the prevalence of human infection by *T. cruzi* is estimated at 16 to 18 million cases, although an additional 90 million, i.e., 25% of the population of the continent is

exposed to the risk of being infected. Among the 211 million inhabitants of the American Continent in the South Cone, some 11 million are infected and around 54 million are under the risk of being infected, representing 31% of the population. Based upon a number of studies carried out in Brazil, it is estimated that about 30% of the infected population present symptoms and signs of the disease. Thus, it is assumed that approximately 4.8 to 5.4 million people present clinical manifestations attributed to Chagas disease. According to the World Health Organization (WHO) more than 50,000 people a year die of Chagas disease (Situación de la enfermedad de Chagas en las Americas 1984, WHO 1986, 1991, Chagas disease 1990, Rey 1991, Chuit 1993, Kirshhoff 1993, 1996, Moncayo 1993, Elimination of transmission of Chagas disease in southernmost Latin America 1994, Guariento 1994, Wanderley 1994, Corrêa et al. 1996, Ferreira & Ávila 1996, Meyer et al. 1996).

In order to obtain information concerning the distribution of *T. cruzi* infection in the country, a wide serological survey on the prevalence of Chagas disease was carried out in Brazil from 1975

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to 1981, under the coordination of Conselho Nacional de Pesquisa (CNPq) and with the collaboration of Superintendência de Campanhas (Sucam) of the Brazilian Ministry of Health. The survey was restricted to the rural population and the estimated prevalence of positive reactions was recorded as 4.2% in Brazil as a whole. The states with the highest rates of positive reaction were Rio Grande do Sul (8.8%), Minas Gerais, (8.8%) and Goiás (7.4%). In the State of Paraná and in the district of Londrina the rates of seropositivity were 4% and 3.7%, respectively (Brasil, Ministério da Saúde 1979, Camargo et al. 1984).

The 4% prevalence in Paraná corresponds to an estimated 166,511 people infected by *T. cruzi* in a population of 4,164,943 inhabitants from the rural area. These figures rank Paraná as the fourth state in terms of the estimated number of infected patients in Brazil, being surpassed only by Minas Gerais, Bahia, and Rio Grande do Sul (Toledo et al. 1997).

Recent data published by the Brazilian Program for the Control of Chagas Disease (PCDCh) in Brazil – a signatory of the “South Cone Initiative” for the reduction of the transmission by means of spraying insecticides in residences and through the maintenance of serological tests in blood banks in six countries of South America - indicate that the transmission eradication in Brazil is imminent (Chagas success in Brazil and Colombia 1996). The virtual elimination of *T. infestans* in Brazil is demonstrated not only by the entomological indicators adopted but also by the results of the serological surveys carried out with groups of young people, which confirm the impact of the preventive actions on the transmission. Data relating to morbidity and mortality also strongly, indicate that the vectorial control has led to a distinct reduction in the number of cases of Chagas disease and of deaths caused by that disease (Dias 1991, Silveira & Rezende 1994, Dias & Gontijo 1995).

This study evaluates the prevalence of the infection by *T. cruzi* - using two serological tests - enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IFI) - in children aged between 7 and 14 (students of elementary school from the municipal educational system) living in both urban and rural areas of Londrina.

MATERIALS AND METHODS

The target population comprised students of the basic cycle (elementary school) of the Municipal School System of Londrina.

The students sample was simple casual stratified (urban and rural areas).

For a population of 26,573 children aged between 7 and 14, living in Londrina, with an esti-

mated prevalence of positivity for infection by *T. cruzi* reaching 0.035 (with an error margin of 0.0125), the calculated size of the sample was 805 students, with a confidence interval of 95%. In order to decrease the error margin, 834 students were evaluated. Data on age, sex, address and place of birth were collected and recorded.

The decision to study a number of rural area children twice as large as those of the urban area was based on the assumption that the prevalence of seropositivity for *T. cruzi* infection in those children would be higher than that observed in children from the urban area of the district.

Due to the ease with which the tests could be carried out, the easy transportation of the material and in order to reduce of costs, a digital collection of blood was made, (upon the permission of the children's parents or guardians) with the use of a paper filter as support, according to the standardized technique developed by Camargo et al. (1984). The reliability and quality of this method has been demonstrated by a number of studies previously performed in our country. The paper filter utilized was the 3 MM CHR (Whatman Laboratory Division Maidstone, England).

The immunological diagnosis of the infection by *T. cruzi* was performed by researchers of the Adolfo Lutz Institute, São Paulo, who employed the IFI and ELISA techniques. The ELISA for detecting class IgG antibodies was carried out according to the method described by Voller (1975). The aliquots were tested at four dilutions (1/64 to 1/512) and a reactive dilution ³ 1/128 was considered as meaningful. The cut-off point of the test was 0.250.

The IFI was carried out according to the method of Fuchs et al. (1980), and the results presenting a value of ³ 1/32 were considered meaningful. The sensitivity and specificity of both reactions (ELISA and IFI) are 98.5% and 95.2%, and 95.2% and 96.3%, respectively.

The students who presented two positive reactions after confirmation were considered seroreactive.

In order to confirm the results obtained from the serological tests of the students, venous blood from the only seropositive child was collected and re-analyzed using the same techniques (IFI and ELISA). In addition, blood samples of all other members of that child's family were collected through venous puncture and processed by IFI and ELISA.

A descriptive statistic was used in order to calculate the averages and standard deviations of the students' age, confidence intervals of the proportions and variables distribution in charts.

RESULTS

The average age was 9.1 ± 1.6 years. Out of 834 students, 424 (50.8%) were male.

With regards to their address, 589 (70.6%) students were from the rural area and 245 (29.3%) from the urban area of Londrina. As for their place of birth, most of them, i. e., 652 (78.2%) students, were born in the district and 182 (21.8%) were migrant children.

Out of 834 blood samples examined, only one was seropositive both for IFI and ELISA (Table I). That child was born on 4 November 1984 and had been living in the country area of Londrina since birth. The mother was born in an endemic area of Chagas disease in Minas Gerais and lived there for 18 years prior to moving to the rural area of Londrina. The mother's health records, obtained from the Hospital Universitário Regional do Norte do Paraná in Londrina, revealed that she was seropositive for *T. cruzi* infection (indirect immunofluorescent and complement fixation techniques) when the child was born. These tests were also positive with a sample of cord blood.

The confirmatory serologic tests of the child revealed titers of 1:2048 for ELISA and 1:512 for IFI. The confirmatory tests in the mother presented the same titers. The father and the two brothers of the child were non-reagent by the same two techniques.

TABLE I

Distribution of the students according to the test results of indirect immunofluorescence (IFI) and enzyme-linked immunosorbent assay (ELISA) for the diagnosis of *Trypanosoma cruzi* infection

IFI and ELISA	No.	%	Reliable interval (95%)
Reagents	1	0.1	0 - 0.7
Non-reagents	833	99.9	99.3 - 100
Total	834	100	

In Table II it can be observed that the serological inquiry (IFI) for the diagnosis of infection by *T. cruzi*, carried out between 1976 to 1979 in Paraná examining a sample of 1,318 residents in the rural area of Londrina, revealed seropositivity for 46 persons. Although the total number of persons in terms of age range is not available, the study revealed that only two children in the 7 - 14 age range were serum reactive (Brasil, Ministério da Saúde 1979).

DISCUSSION

Entomological evaluations have proved that vectorial transmission of the *T. cruzi* infection has effectively been interrupted in Londrina (Brasil, Ministério da Saúde 1996). However, we have not found any report on serological studies carried out with children which objectively shows the impact of PCDC and other factors on that occurrence. Thus we decided to perform a serological survey of students with ages ranging from 7 to 14, living in both rural and urban areas of Londrina.

Out of 834 children submitted to serological examination for the diagnosis of *T. cruzi* infection (employing the ELISA and IFI techniques), only one child was positive, corresponding to a positivity rate of only 0.1% (0% - 0.7% with 95% reliability). If we consider just the portion of the children residing in the rural area of the district, the rate was 0.2% (0% - 0.9%) also with 95% reliability.

We could not ignore the possibility that the child had been infected by congenital transmission. There was some indirect evidence that favored this possibility, including the fact that the mother of that child was infected when the child was born; the positivity of serologic tests for *T. cruzi* infection in blood samples obtained from umbilical cord by ELISA and IFI techniques; and the presence of advanced cardiomyopathy in the infected child. Triatomas density in the area where the child had lived since birth had always been very low (since 1983) (Brasil, Ministério da Saúde 1977, 1996),

TABLE II

Distribution of seropositive patients for *Trypanosoma cruzi* infection in serological tests of indirect immunofluorescence carried out in the rural zone of the district of Londrina, Paraná, according to the age range (1976-1979)

Serological test results	Age range (Years)					Total		Reliable interval (95%)
	1 - 6	7 - 14	15 - 29	30 - 44	≥ 45	No.	%	
	No.	No.	No.	No.	No.	No.	%	
Reagents	0	2	15	16	13	46	3.5	2.6 - 4.6
Non-reagents	a	a	a	a	a	1,272	96.5	95.4 - 97.4
Total	a	a	a	a	a	1,318	100	

a: not available. Source: Health Ministry: Public Health Campaign, Regional Board of Paraná, 1979.

and no evidence of the presence of triatomas had been detected by technicians of the Fundação Nacional de Saúde (FNS).

When our results were compared with those obtained by the National Serological Inquiry carried out from 1976 to 1979 in the general rural population, it was shown that out of 589 children from the rural area, only one was seropositive for *T. cruzi* infection, while in the previous inquiry, out of the total 1,318 persons of all ages, 46 were seropositive. It is important to notice that out of this number only two belonged to the 7 to 14 age range group (Brasil, Ministério da Saúde 1979). As the total data from that inquiry as to age group are not available, it is not possible to perform a comparative statistical analysis. However, the results indicate that, even at that time (before starting PCDCh) the rate of seropositivity for *T. cruzi* infection in children aged between 7 and 14 was already low, in common with what was demonstrated in our study.

The results of our research were similar to those obtained in studies carried out in different regions of Brazil and in the country as a whole. In the whole country the serum prevalence of *T. cruzi* infection in children aged between 7 and 14 was recorded as 2.4% in 1980. However, by 1994, this prevalence had reduced to only 0.1%, corresponding to a 95% reduction of American trypanosomiasis incidence in that age group (Silveira & Rezende 1984, Chagas success in Brazil and Colombia 1996).

According to Carneiro and Antunes (1994b), some studies carried out in Brazil, in areas under PCDCh intervention, demonstrated that there was a decrease in the level of *T. cruzi* infection in the human population within the area under evaluation. Some workers consider that this reduction was directly connected with the control actions developed; while some other researches contend that the observed decrease was due mainly to the social, economic and cultural changes that occurred during the intervention period.

Carneiro and Antunes (1994a, b) evaluated the efficiency of PCDCh in a study carried out in Minas Gerais. The PCDCh efficiency was estimated by comparison with the results obtained by the National Serological Inquiry (1975-1980) performed before the program started. According to these authors the results showed an association between the PCDCh actions and the decrease in *T. cruzi* infection which was evaluated through serological tests.

The FNS Minas Gerais Program has been performing serological tests for the diagnosis of American trypanosomiasis in students of the rural area aged between 7 and 14 with the purpose of

analyzing the results obtained by the PCDCh, preferably in the districts already under epidemiological surveillance. Preliminary results from this study suggest effectiveness of PCDCh in Minas Gerais (Azeredo et al. 1997). According to Moreno et al. (1996) comparison of the results from the inquiry carried out with students with the results obtained by the National Serological Inquiry reveals differences in the relative values, demonstrating a decrease of more than 90%. That decrease confirms, therefore, the success of the PCDCh program in reducing vectorial transmission in the group of children born after the program implantation.

According to Silva (1981, 1990), the analysis of the causes of the disappearance of endemic Chagas disease in São Paulo indicated that the rural population decrease, and the consequent reduction in the number of residences was a relevant factor to the disappearance of Chagas disease in that state. Moreover, in Silva's opinion, some resistance to the elimination of endemic disease was observed in the areas of São Paulo where there was no decrease of rural population. Wanderley (1993) stated that the rural exodus was followed by the destruction of houses at that time, suggesting that the destruction was selective, that is, it happened in a sequence from the worst houses to those of a better standard, affecting primarily those houses inhabited by triatomas. Many authors consider that this factor, along with the direct action for the control of the vector, which were intensified in this period were extremely relevant to the process of interrupting the transmission of Chagas disease in São Paulo.

Silva (1996) claims that as has happened to many large urban centers of the country since the 1960's, Londrina has also gone through an accelerated urbanization process. The population in the urban area increased from 47% in 1950 to nearly 95% in 1995 (Table III). At present the population is concentrated mainly in the city with the remainder distributed throughout the various towns nearby following the same pattern as other small towns found in Paraná.

Table III shows that in 1970, although the rural population of the district of Londrina had already reached the highest point (64,573 persons) in absolute figures, the proportion of rural inhabitants had already decreased in relation to the number of inhabitants in the urban area. Furthermore, in 1980, there was a relevant reduction of the rural population not only as an absolute figure but also proportionally. At that time the annual growth rate of the district had already decreased in relation to the 70's and, especially, in relation to the 60's. These figures indicate that the coffee plantation declined, the consequent rural exodus and population defla-

TABLE III

Evolution of population living in the district of Londrina in the years 1950, 1960, 1970, 1980, 1991 and 1995

Year	Urban	%	Rural	%	Total	Growth rate/year
1950	34,230	47.9	37,182	52.1	71,412	-
1960	77,382	57.4	57,439	42.6	134,821	6.6
1970	163,528	71.7	64,573	28.3	228,101	5.4
1980	266,940	88.5	34,771	11.5	301,711	2.8
1991	366,676	94	23,424	6	390,100	2.3
1995	431,578	94.9	23,233	5.1	454,811	-

Source: population - Census 1950 IBGE/Preliminary Synopsis of Census Demofigura; 1960/IBGE/Census Demofigura - 1970, 1980 and 1991; IBGE/IPPUL - estimate - 1995.

tion of the district that occurred at that time may have been the main reasons for the eradication *T. cruzi* vectorial transmission in Londrina. As PCDC control measures were introduced after all those changes, our study suggests that their impact was less significant.

As stated by Silva (1981, 1990), it would be difficult or even impossible to assess the relative effect of the different factors involved in the extinction of endemic disease in São Paulo. That author pointed out that the specific combat activities were determined by politico-economical and technical-scientific injunctions, and the development that took place in the rural area of São Paulo was a result of those determinants. A campaign against a given disease as well as the rural exodus are both derived from the evolution of a society. When we analyze the disconnection of the relational system that allows the existence and the dissemination of the disease we must include the control actions along with other so called "social-economical" factors.

Through these considerations, one can conclude that the social, economical and ecological transformations that took place in São Paulo were similar to those that occurred in Paraná some decades later. In the period between 1940 and 1960, when the colonization and the coffee plantation were at their peak in Paraná, the coffee plantation in São Paulo was already in decline and the rural-urban exodus had already started. These transformations in Paraná had their start in the 1960's and reached their peak in the 1970's.

Although the methodology of our work does not allow us to have a full evaluation of the impact of the multiple factors involved in the elimination of vectorial transmission of infection by *T. cruzi* in Londrina, several facts suggest that the main causes for this elimination were the rural-urban exodus that took place in the 60's and 70's and the decrease in the population of the district. The lat-

ter resulted from changes in the rural economy which turned from a predominant coffee production to the production of other crops such as soy beans and wheat that require a comparatively smaller labor force. This change led to an exodus from the rural areas and caused important ecological and social modifications in the rural setting. The resulting situation seems to have been the determining factor in the eradication of vectorial transmission of the Chagas disease in the district of Londrina.

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