

## CHAGAS DISEASE IN A COMMUNITY IN SOUTHEAST BRAZIL. I. A SEROLOGIC FOLLOW-UP STUDY ON A VECTOR CONTROLLED AREA

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### SUMMARY

The prevalence of *Trypanosoma cruzi* infection was evaluated in Berilo, Minas Gerais (MG), Brazil, from January to July 1997. A serological survey using the indirect immunofluorescence test (IFT) in dried blood collected on filter-paper was performed in a sample of 2,261 individuals. The overall prevalence rate of *T. cruzi* infection was 18%, and reached 50% in individuals older than 30 years from rural areas. The percentage of seropositivity was 0.17% among individuals younger than 10 years old, suggesting that vectorial transmission is controlled in the area. A decrease in prevalence rates among people born after 1960 and 1970 was observed and this appears to be correlated with the beginning of control programs. A reduction in *T. cruzi* infection rates was observed when comparing our results with the rates estimated in a serologic study carried out in Berilo in 1983<sup>11</sup>.

**KEYWORDS:** *T. cruzi* infection; Chagas' disease prevalence; Epidemiology; Vectorial control; Chagas disease transmission.

### INTRODUCTION

It has been estimated that more than 20 million people in Brazil, are at risk of infection by *Trypanosoma cruzi*<sup>10</sup>. In 1975, the Chagas Disease Control Program (CDCP) was initiated in this Country, the priority being control of *Triatoma infestans* using residual action insecticides. There was a reduction in the percentage of counties with *T. infestans*, from 30.4 in 1983 to 7.6 in 1993<sup>21</sup>. Serologic studies in children show the impact of control activities in reduction of transmission. A survey carried out by National Health Foundation (NHF), on 15,314 schoolchildren from various regions of Minas Gerais State, found a prevalence rate of 0.09%<sup>18</sup>. More than 80% of the counties of endemic areas have infestation rates less than 5%<sup>8</sup> and there is a virtual absence of acute Chagas disease<sup>9</sup>.

Even though vectorial transmission of Chagas infection has diminished, the chronic form of Chagas disease continues to represent a serious public health problem. A national survey carried out between 1979 and 1981 estimated a total of 5 million seropositive inhabitants in Brazil. Approximately 20% of infected people present the cardiac form that may be the cause of death in 10 thousand individuals each year<sup>7</sup>.

A serologic survey was carried out in the county of Berilo, Minas Gerais State (MG) Brazil in 1983<sup>11</sup> when the estimated *T. cruzi* infection prevalence was 35%. In 1987, AGUILAR<sup>1</sup> studied epidemiological, clinical and social aspects of Chagas disease in the county of Berilo and estimated the prevalence to have fallen to 12.9%; there were still children younger than five years infected and *P. megistus* was the most important vector. At this time, the author examined 100 seropositive patients and

found electrocardiographic alterations in 52 and megaesophagus in 8 individuals. During the last 10 years, the National Health Foundation has been carrying out control actions in Berilo, based principally on spraying those dwellings which were found to be positive with insecticides.

The objective of this work was to study the prevalence of Chagas infection in Berilo and based on previous studies and information from the Chagas disease control program (CDCP) perform a retrospective analysis of the transmission dynamics.

### MATERIALS AND METHODS

**Studied area:** Berilo is situated in Minas Gerais state, southeast Brazil, about 660 Km northeast of the state capital Belo Horizonte (Fig.1). It covers approximately 581.5 Km<sup>2</sup> and the average elevation is circa 500 - 800 m. Its vegetation is generally "cerrado" and the annual rainfall of the zone is usually less than environmental water necessities. Berilo has a population of about 13,102 people, of which 80% live in rural and 20% in urban areas.

**Studied population:** Prevalence of *T. cruzi* antibodies was evaluated in a sample of the population in the Berilo region, from January to July, 1997. The study sample was composed of 2,261 individuals, of which 80% (1,811) resided in rural areas and the remainder (20%) in urban zones. Thus the distribution according to rural or urban origin of the sample population was proportional to the distribution of the Berilo population as a whole. The sampling unit was the household. In urban

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Fig. 1 - Location of Berilo, in Minas Gerais State, Brazil.

areas, a systematic selection was made of one from every six inhabited houses. In the rural area, the population was divided into nine sectors and the sample was proportionally distributed between these sectors. Based on ease of access, we selected two or three communities from each sector, and eventually studied 23 communities from different regions of the municipality. Whenever access was feasible all the houses in these communities were included in the study. All inhabitants aged over six months living in the selected houses were examined; those children that were at school during the home visit, were examined at a later opportunity.

**Data collection and laboratory tests:** Personal information was registered using a questionnaire developed specifically for this study. In each family, an adult was also interviewed regarding characteristics of the household and knowledge about Chagas disease. A blood sample was collected on filter-paper from all participants for serological diagnosis of *T. cruzi* infection. Blood samples were eluted and processed by indirect immunofluorescence antibody test (IFAT) at the Department of Tropical Medicine, Oswaldo Cruz Institute. Venous blood (10 ml) obtained by venipuncture using vacuum tubes were collected from seropositive individuals. Blood samples were centrifuged and the sera was stored at minus 20 °C until examined by Indirect Immunofluorescence Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA) to confirm the diagnosis of *T. cruzi* infection. IFAT was performed using human anti gammaglobulin type IgG (Biolab®) and formalinized epimastigote forms of Y strain of *T. cruzi* maintained in culture. To perform ELISA test a commercial kit produced by Biomanguinhos (FIOCRUZ) was used. This test is based on the reactivity of serum with soluble and purified antigens from *T. cruzi* culture.

Information about vectorial aspects and control program activities was obtained from Chagas Disease Control Program files (Fundação Nacional de Saúde, Distrito Sanitário de Curvelo, MG).

**Data processing and analysis:** Epi - Info software was used to analyze the information. To compare seropositive rates obtained in 1987 and 1997 surveys, we considered only the communities that were studied at both times. In Berilo City, 506 individuals were studied in 1987 and

322 in 1997. In rural areas, we compared the seropositivity found in residents from seven communities, 526 in 1987 and 574 in 1997. A cohort analysis for the age groups was performed.

The *T. cruzi* infection prevalence rates among residents of rural area were standardized in order to compare the results with the prevalence rates obtained in a previous study carried out in Berilo in 1983<sup>11</sup>.

**Ethical procedure:** Informed written consent was obtained from all adult participants of this study and from the parents or legal guardians of minors. This work complied with resolution number 196/1996 from the National Health Council for research involving humans.

## RESULTS

Age distribution of the sample was similar to the age distribution of Berilo population recorded in 1991 IBGE (Brazilian Institute of Geography and Statistics) census. Of the study sample 53% were female. The majority of individuals studied (86.1%) were natives of Berilo. Of the individuals from others states or counties, 23.0 and 14.7% respectively were from the contiguous municipalities of Virgen da Lapa and Chapada do Norte, 19.3% from São Paulo state and 23% from other municipalities of Jequitinhonha Valley region (Araçuaí, Francisco Badaró and Medina).

Of 311 adult men studied in rural area, 91% were involved with agriculture and cattle ranching. In relation to type of dwelling, it was observed that 21.9% of the study population, was living in houses with brick walls, 77.5% in houses with adobe walls and 0.6% (13 individuals) resided in dwellings with mud walls and dirt floor, known locally as “*de pau a pique*”.

**Serology of *T. cruzi* infection:** The analysis of the seroprevalence of *T. cruzi* infection, was made considering the results of the IFAT on filter-paper corrected by the results of the IFAT and ELISA on sera. Therefore, false positive individuals according to IFAT on filter-paper were considered to be negative<sup>1</sup>.

Table 1 shows the age-specific seroprevalence rates. Of 2,261 people, 418 (18.4%) were positive. While out of 566 children younger than ten years of age included in the sample, only one (0.17%) was seropositive.

Regarding the urban area residents, (Berilo and Leliveldia village), 45/452 individuals were seropositive (9.9%). In Berilo City 6.8% of the residents studied were seropositive. None of 232 subjects younger than 20 years examined in the urban area were seropositive. Here the seroprevalence was only observed in those over 40 years old.

Of 1,809 rural area residents studied, 373 (20.6%) were considered seropositive. There was a clear increment in the prevalence rates after 30 years of age, reaching 56.1%.

Among the population over 30 years old, seropositivity to *T. cruzi* infection was 53.9% in women and 45.9% in men, this difference was statistically significant ( $p = 0.036$ ).

The seropositivity rates in individuals younger than ten years old during the decades from 1920 to 1990 were calculated as an approximate evaluation of the transmission of *T. cruzi* infection in each decade. Figure

2 shows a reduction in *T. cruzi* infection prevalence among those born after 1960.

Table 2 compares the results of the IFAT performed on filter paper in 1987 and 1997 in residents of Berilo City and seven rural communities of Berilo. In Berilo City the seropositivity rate had a reduction from 9.7 to 6.8% in ten years. In contrast, among residents from rural communities the seroprevalence rate was higher in 1997. With respect to age-specific comparisons, it should be taken into account that about three children younger than ten years old who were positive by IFAT on filter paper in 1997, were negative by serology. On the other hand, two of the children younger than ten years old in 1987 that were positive by IFAT on filter paper (Table 3), were confirmed as positive by serology. Given these considerations, cross-sectional comparison shows a decrease in the proportion of infected subjects in the 0-9 years old group. The cohort analysis indicated an increase in the proportion of *T. cruzi* infection in various age groups.

Table 3 compares the results of the IFAT performed on filter paper in 1983 and 1997 in residents of rural area of Berilo. Cross-sectional comparison shows a decrease in the proportion of infected subjects in the various age groups. The cohort analysis indicates a concordance in

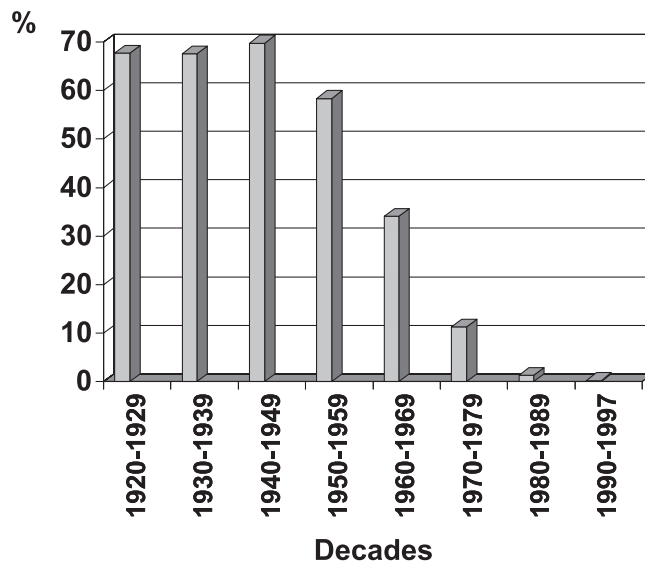


Fig. 2 - Seropositivity rates in individuals considered to have spent most of the first ten years of their lives in a particular decade since 1920 to 1990.

**Table 1**  
Serology for anti - *T. cruzi* antibodies in residents of Berilo, MG in 1997

Age groups (Years)	Urban Area			Rural Area			Total		
	Exam	Pos	%	Exam	Pos	%	Exam	Pos	%
0-9	102	-	-	464	1	0.2	566	1	0.2
10-19	130	-	-	502	7	1.4	632	7	1.1
20-29	67	7	10.4	226	19	8.4	293	26	8.9
30-39	61	8	13.1	198	76	39.4	259	84	32.4
40-49	33	12	36.3	164	90	54.8	197	102	51.8
50-59	26	8	30.7	133	94	70.6	159	102	64.1
60 +	33	10	30.3	122	86	70.4	155	96	61.9
<b>Total</b>	<b>452</b>	<b>45</b>	<b>9.9</b>	<b>1809</b>	<b>373</b>	<b>20.6</b>	<b>2,261</b>	<b>418</b>	<b>18.4</b>

**Table 2**  
Comparison of *T. cruzi* infection\* rates in 1987 and 1997 surveys, according to age group. Berilo, MG

Age groups (Years)	Urban area						Rural area					
	1987			1997			1987			1997		
	Exam	Pos	%	Exam	Pos	%	Exam	Pos	%	Exam	Pos	%
0 - 9	120	1	<b>0.8</b>	74	-	-	146	3	<b>2.05</b>	117	3	<b>2.5</b>
10-19	128	4	<b>3.1</b>	85	2	<b>2.3</b>	201	14	<b>6.9</b>	178	1	<b>0.5</b>
20-29	71	5	<b>7.0</b>	53	4	<b>7.5</b>	68	19	<b>27.9</b>	76	7	<b>9.2</b>
30-39	60	10	<b>16.7</b>	40	3	<b>7.5</b>	38	17	<b>44.7</b>	79	23	<b>29.1</b>
40-49	45	13	<b>28.9</b>	21	4	<b>19.0</b>	39	17	<b>43.5</b>	48	26	<b>54.1</b>
50-59	49	12	<b>24.5</b>	23	5	<b>21.7</b>	17	6	<b>35.3</b>	40	25	<b>62.5</b>
60 +	33	4	<b>12.1</b>	26	4	<b>15.3</b>	17	2	<b>11.7</b>	36	18	<b>50.0</b>
<b>Total</b>	<b>506</b>	<b>49</b>	<b>9.7</b>	<b>322</b>	<b>22</b>	<b>6.8</b>	<b>526</b>	<b>78</b>	<b>14.8</b>	<b>574</b>	<b>103</b>	<b>17.9</b>

\* IFAT on filter paper

**Table 3**

*T. cruzi* infection\* prevalence rates in residents of rural area of Berilo in 1983\*\* and 1997 and age-adjusted prevalence rates using the 1991 Berilo rural population as the standard

Age Group	1983			1997		
	Examined	Positive	%	Examined	Positive	%
0-9	245	25	10.2	464	6	1.3
10-19	490	107	21.8	502	12	2.4
20-29	142	70	49.2	226	20	8.8
30-39	118	80	67.7	198	75	37.8
40-49	92	59	64.1	164	88	53.6
50-59	65	48	73.8	133	92	69.2
60 +	44	33	75.0	122	85	69.7
<b>Total</b>	1196	422	35.2***	1809	378	20.9***
<b>Adjusted rate</b>			36.4****			17.9****

\* IFAT on filter paper; \*\* (DIAS *et al.*, 1985); \*\*\* Crude prevalence rates; \*\*\*\* Age-adjusted prevalence rate using the 1991 Berilo rural population as the standard.

seropositivity rates. Standardization of rates shows that if the age distribution of population was similar since 1983 (1991 IBGE census), the prevalence rate in rural area would have diminished from 36.4% in 1983 to 17.9% in 1997, since individuals studied in both surveys were representative of rural area inhabitants.

## DISCUSSION

This study shows a high prevalence of *T. cruzi* infection among residents of Berilo over 30 years of age. Studies carried out between 1976 and 1988<sup>1,2,11,12</sup>, also showed a high *T. cruzi* infection rate in adults in this region.

The analysis of *T. cruzi* infection prevalence rate in rural area was based on the study of 23 non randomly selected communities. Although we did not detect any bias introduced by the selection criteria, the non random selection of the communities studied should not be ignored. Even though the characteristics of a sampling process (non probabilistic sample) make it impossible to perform a precise statistical analysis of the estimated seropositivity rates of *T. cruzi* in Berilo, we consider that the rates verified for the rural residents studied do in fact provide an approximation of the magnitude of the endemia in this county.

A higher proportion of seropositivity among women was also registered by AGUILAR<sup>1</sup> in Berilo rural population and by several other workers in surveys carried out in unselected populations in various municipalities of Minas Gerais, Piauí, Paraíba and Bahia states<sup>3,7,15,20</sup>. There is still no convincing explanation for this difference.

The low *T. cruzi* infection rate in children younger than ten years old was to be expected, because in the last decade The National Health Foundation (NHF) has been carrying out programs to control the triatomine bugs in this region. Serologic surveys performed by NHF in schoolchildren, show low seropositivity rates<sup>18,21</sup>.

The high prevalence rates of *T. cruzi* infection in persons older than 30 years old from Berilo represent a serious public health problem. In Berilo, AGUILAR<sup>1</sup> found low rates in the over 60s and suggested that this indicates a high specific mortality due to Chagas disease. Likewise, MOTT *et al.*<sup>19</sup> did not find differences in complement fixation geometric mean titer in persons over age 55 years and those between ages 25 and 55 years and concluded that the decrease in prevalence rates in older people should be associated with increased mortality and not to a decrease in complement fixation reactivity with age. In the serologic survey carried out in Berilo in 1983<sup>11</sup>, the prevalence rate among those over 50 years of age was high. In Virgen da Lapa, MG, a Berilo contiguous municipality and in Iguatama and Pains, in another region of Minas Gerais state, the prevalence rates were also high in persons over 60 years old<sup>5</sup>.

In 1987 the vectorial transmission of Chagas infection in Berilo was considered low<sup>1</sup>. However, based on the finding of infected children younger than four years old, AGUILAR<sup>1</sup> concluded that this form of transmission was still active at that time. Ten years later, in the same communities, we did not find any seropositive individuals younger than 20 years old. Of 566 children younger than ten, only one was seropositive, and we consider that this was probably a case of congenital transmission. All these findings indicate a much reduced vectorial transmission of Chagas disease in Berilo since 1987.

We observed a poor concordance in the cohort analysis when we compared the age specific prevalence rates obtained in 1987 and 1997 surveys. While interpreting these findings it should be considered that the sample size was reduced when the analysis was limited to communities that were included in both surveys and the numbers were even more reduced when the compared populations were classified into age groups. For these reasons, and taking into account that in 1997 we could not find seropositive children younger than ten years old, we consider that the increase observed in prevalence rates in some age groups is not evidence of vectorial transmission in Berilo during the last ten years.

In order to analyze the evolution of *T. cruzi* infection transmission, we calculated the prevalence rate among individuals of some age groups according to the decade in which they probably were infected. We considered the fact that in areas with high vectorial transmission, when the density of infected triatomine bugs colonizing dwellings was high, the first contacts with the vector generally occurred in the first ten years of life<sup>4,6,16</sup>. In the interpretation of prevalence rates in Figure 2, as indicators of the intensity of vectorial transmission, one must take into account the chagasic mortality and late infections. In 1947 in Bambuí, MG, the seroprevalence rate in children younger than ten years old was 47% and based on demographic data an annual incidence rate of 5% was calculated<sup>6</sup>.

According to AGUILAR<sup>1</sup>, the occurrence of Chagas disease in this region appears to be relatively recent, occurring probably in the last 100 years. Apparently, vectorial transmission in Berilo was more intense between 1950 and 1970, because the main vector species (*Triatoma infestans*) was introduced in the region after 1950<sup>16</sup>. According to a population survey of triatomine bugs carried out in Minas Novas region (in which Berilo is situated) in 1951, *P. megistus* was the only species of triatomine bug found inside dwellings<sup>20</sup>. Serological studies show that the transmission was still important between 1957 and 1967 and it is

probable that some individuals that were born before 1960 were infected between 1960 and 1970. There is a clear reduction of *T. cruzi* transmission in Berilo after 1970.

Since 1960 vectorial control activities have been carried out in Berilo. However, the control program functioned in an interrupted form between 1970 and 1980. In 1982, a triatomine survey performed in the municipality found that 42.2% of communities and 4.4% of dwellings were positive. Since 1982, *P. megistus* has been the triatomine bug species most frequently captured in Berilo. In 1985 and 1986 almost 100% of dwellings in Berilo were sprayed with insecticides. The infestation rates decreased significantly between 1982 and 1987. During the last ten years, the National Health Foundation has continued to carry out control actions and reports that *T. infestans* is no longer detected in the county.

A good concordance in prevalence rates estimated for age cohorts in an age specific analysis of 1983 and 1997 surveys was verified. The standardized rates show a decrease in prevalence rate over the last 15 years.

The decrease in prevalence rates since 1960 and 1970 appears to be correlated with the intervention of control programs. Furthermore since 1970, some social factors that lead to important improvements in the living conditions of rural populations, could have also contributed to reducing *T. cruzi* infection transmission.

It is not possible with this study to attribute the reduction in infection rates to one or another fact, nor to measure the control program impact. In a study designed with the objective of evaluating the efficacy of control programs, the authors compared three municipalities of Minas Gerais State with different intervention periods. Although the overall rate of seropositivity to *T. cruzi* increased in an area with more prolonged intervention (ten years), the authors found an association between the duration of the intervention and the reduction in prevalence rates among children younger than ten years old.

If control activities in the region determined the decrease in infection rates, their absence between 1978-80 and 1985 apparently may be associated with the maintenance of transmission. Taking into account that after 1986 transmission apparently was not important, the seven seropositive individuals younger than 20 years old detected in this survey, may have become infected between 1977-80 and 1985. In a triatomine survey carried out in 1982, triatomine bugs were found in the communities where six of these individuals were born.

The presence is noteworthy of seropositive individuals between 10 and 20 years old and the higher seroprevalence rate among those between 20 and 40 years old in some communities in the north with respect to other communities studied. These differences could be evidence of a more intense transmission of *T. cruzi* infection in some communities of this region between 1957-60 and 1985. Three facts, could explain these findings: delayed or lower intensity control actions, slower social changes and/or the presence of *T. infestans* in these communities between 1957-60 and 1985.

*T. infestans* colonized the north of Minas Gerais from the south of the state after 1950<sup>11</sup>. In control activities carried out in Berilo during 1982, *T. infestans* was found in 13 communities, all situated in the north

of the municipality. Between 1975 and 1983, *T. infestans* was found in municipalities north and east of Berilo. While in Chapada do Norte and Minas Novas (in the south) as in other municipalities situated south of the Jequitinhonha river these species of triatomine bugs were not found<sup>22</sup>.

The congenital transmission of *T. cruzi* infection is most probably infrequent in Berilo. Although especially designed studies are necessary to quantify the frequency of this type of transmission, it is possible to reach an approximate indication of the low rate of this event by comparing the low *T. cruzi* infection rate (0.4%) among children younger than 15 years of age with the prevalence of 30% found in women between 20 and 50 years old, or that is the age group that should include their mothers.

In eight of the nine seropositive individuals younger than 20 years old, we also verified seropositivity in their mothers. However, taking into account the high seropositivity in women over 35, we consider that this fact is not relevant in relation to vectorial transmission.

Although vectorial transmission of *T. cruzi* is apparently controlled in Berilo, there is a permanent risk of colonization of peridomestic areas of dwellings by native triatomines, especially *P. megistus*. In 1997, the NHF found triatomine bugs in 21/102 communities examined in Berilo (21.5%). The majority of captures were in peridomestic areas and 84.6% of the captured insects were *P. megistus*. The reinfestation of peridomestic areas by this species is a common process<sup>14</sup> and frequently there is associated natural *T. cruzi* infection<sup>15</sup>. This condition can determine the maintenance of the domestic cycle of *T. cruzi* and constitute a bridge between the domestic and sylvatic cycle<sup>13</sup>. Since it is a native species, the elimination of *P. megistus* is impossible<sup>21</sup>, thus one of the most important goals of the Chagas Disease Control Program is an improvement in the methods and strategies to detect and control peridomestic infestation by native species<sup>8</sup>.

## RESUMO

### **Doença de Chagas em uma comunidade do Sudeste do Brasil. I. Seguimento sorológico em uma área com controle vetorial**

A prevalência da infecção pelo *Trypanosoma cruzi* foi avaliada no município de Berilo, Minas Gerais, Brasil, no período de janeiro a julho de 1997. Uma amostra de 2.261 indivíduos foi estudada sorologicamente mediante o teste de imunofluorescência indireta em sangue coletado em papel de filtro. A taxa de prevalência foi de 18% no total da população estudada e 50% em pessoas da área rural maiores de 30 anos. A percentagem de soropositividade foi 0,17% entre os menores de 10 anos estudados, o que sugere que a transmissão vetorial está controlada na área. Observamos uma diminuição na taxa de soroprevalência entre as pessoas nascidas após 1960 e 1970 o que teria relação com o início das ações de controle. Observou-se também uma redução na taxa de infecção pelo *T. cruzi* quando comparamos os nossos achados com as estimativas de infecção registradas em um estudo sorológico realizado em Berilo em 1983.

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