

Epidemiological profile of *Trypanosoma cruzi*-infected mothers and live birth conditions in the State of Minas Gerais, Brazil

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ABSTRACT

Introduction: The aim of this study was to assess the epidemiological characteristics of *Trypanosoma cruzi*-infected mothers and the live birth conditions of neonates. **Methods:** A serological survey with IgG-specific tests was conducted using dried blood samples from newborn infants in the State of Minas Gerais. *T. cruzi* infection was confirmed in mothers through positive serology in two different tests, and infected mothers were required to have their infants serologically tested after the age of 6 months. The birth conditions of the neonates were obtained from the System of Information on Live Births database. **Results:** The study included 407 children born to *T. cruzi*-infected mothers and 407 children born to uninfected mothers. The average age of seropositive mothers was 32 years (CI95% 31.3-32.6), which was greater than the average age of seronegative mothers - 25 years (CI95% 24.8-25.2). The mothers' level of education was higher among uninfected mothers (41% had 8 or more years of education, versus 22% between the infected mothers). Vaginal delivery was more frequent among infected mothers. There was no evidence of inter-group differences with respect to the child's sex, gestational age, birth weight or Appearance, pulse, grimace, activity and respiration (APGAR) scores at 1 and 5 minutes. **Conclusions:** The level of education and the greater number of previous pregnancies and cases of vaginal delivery reflect the lower socioeconomic conditions of the infected mothers. In the absence of vertical transmission, neonates had similar health status irrespective of the infection status of their mothers.

Keywords: *Trypanosoma cruzi*. Newborn screening. Pregnant women.

INTRODUCTION

In 2006, the Pan-American Health Organization certified that Brazil had eliminated *Triatoma infestans*, the major Chagas disease vector, and thus managed to interrupt vector-based transmission¹. Given the control of both the primary vector and transmission via transfusion, vertical transmission has become one of the major modes of Chagas disease transmission². Most cases of probable congenital transmission in Brazil have been found in the States of Rio Grande do Sul and Minas Gerais, as shown in a serological survey carried out from 2001 through 2008¹.

Congenital transmission is related to the prevalence of *Trypanosoma cruzi* infection in the population. This type of transmission is affected by mothers' and fetuses' immunological factors and the mothers' parasitemia levels at delivery³. The early diagnosis and treatment of infected children are important

health public measures, and the cure index reach over 90% when treatment is started early⁴. Between 10 and 40% of non-treated children develop chronic cardiomyopathy and are subject to significant morbidity and mortality⁵. A study carried out with 422 *T. cruzi*-infected Bolivian mothers showed that maternal infection did not affect pregnancy and fetal development in the cases in which congenital transmission did not occur^{6,7}.

A serological survey of newborns participating in the Newborn Screening Program of Minas Gerais, Brazil, from August 2005 through October 2006 found a prevalence of 0.5% among mothers and only one congenitally infected neonate². The aim of this study was to assess the epidemiological characteristics of *T. cruzi*-infected mothers and the live birth conditions of neonates born to such mothers.

METHODS

The Newborn Screening Program of Minas Gerais carried out a serological survey in all 853 municipalities of the state from August 2005 through October 2006². The detection of anti-*T. cruzi* IgG antibodies in newborns' blood samples was used as an indirect marker of maternal infection. Enzyme-linked immunosorbent assay (ELISA) immunoglobulin G (IgG) tests have been performed in dried blood samples of newborns. In

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all the samples which presented positive or indeterminate IgG ELISA results and in 20% of the samples with a negative result, indirect immunofluorescence tests (IFAT-IgG) have also been applied. Cases that had discordant ELISA/IFA IgG results have been tested by indirect hemagglutination (IHA) to resolve the discordance. When serologic status on filter paper was positive (in two of the cases) or indeterminate (in one of them), a blood sample was collected by venipuncture from the mother and the son for the diagnosis to be confirmed. The methodology of this serological survey has been published in another article².

Chagas infection was confirmed in 532 mothers of 541 neonates (there were 9 cases of double pregnancy). The detection of *T. cruzi* in the mothers followed the World Health Organization (WHO) criterion of detecting seropositivity in at least two serological tests performed using different techniques. We collected new capillary blood samples on filter paper from children at ages of 6-8 months when the children had positive serology results for two different tests or when they had a positive serology result in one positive test and Chagas disease was confirmed in their mothers. No symptomatology was observed among the children.

Identification of children in the System of Information on Live Births

The study database was cross-referenced with the System of Information on Live Births database from 2005 through 2006 to obtain data on the birth conditions of infants born to infected and uninfected mothers in the State of Minas Gerais. The cross-referencing was based on the infants' mothers and was confirmed by both the residence municipality code and the birth date. This cross-referencing identified 407 children born to seropositive mothers and 6,198 children born to seronegative mothers in the System of Information on Live Births database. To match the number of children born to seropositive mothers, 407 children born to seronegative mothers were randomly selected to form the control group. The selection process considered the following age groups of mothers: ≤ 19 years; 20-29 years; 30-39 years; ≥ 40 years. The selection also considered the mesoregional origin of the neonates as a means of controlling for possible confounding factors. Most (73%) of the children lived in northern Minas Gerais, and the remainder came from other mesoregions.

Statistical analysis

The database was created in MS Excel, and the statistical tests were run in SPSS. The data were descriptively analyzed,

and the following parametric tests were performed to assess the correlations between dichotomous variables: the Pearson chi-square test and, whenever necessary, Fisher's exact test. Whenever relevant, the analysis involved odds ratios (ORs) at a 95% confidence interval (95% CI). The non-parametric Mann Whitney test was performed for non-normally distributed variables. To verify the correlation between *T. cruzi* infection and maternal epidemiological variables identified in the univariate analysis we initially used the Spearman coefficient, considering only statistically significant variables. In the final model of multivariate logistic regression analysis, only the variables with p value < 0.05 were considered. Hosmer and Lemeshow statistical analysis were used for the multiple regression model adjustments, with a p value = 0.673 indicating a good fit.

Ethical considerations

This study was approved by the ethics committee of the Federal University of Minas Gerais (ETIC 260-03-EX 01/07).

RESULTS

The study comprised a group of 407 children of chagasic mothers and 407 children of uninfected mothers (i.e., 401 infected mothers and 404 uninfected mothers because of double pregnancies).

Of the seropositive mothers, 73% lived in northern Minas Gerais, 78% had studied for no longer than 8 years (**Table 1**), and their average age was 32 years old (CI95% 31.3-32.6). The uninfected mothers had an average age of 25 years old (CI95% 24.86-25.2). **Figure 1** shows the distribution of infected and uninfected mothers according to the age group. The average number of children from previous pregnancies was 3.4 among infected mothers (CI95% 3.13-3.75) and 2.5 among uninfected ($p \leq 0.01$). The most frequent type of delivery was vaginal delivery (OR=1.5; 95%CI 1.09-2.06), and approximately 86 % of mothers attended at least four prenatal appointments. In the multivariate model low level of schooling (OR=2.61; 95% CI 1.82-3.74) and higher number of pregnancies (OR=1.06; 95%CI 1.0-1.12) remained associated with Chagas infection (**Table 1**).

There were no evidence of inter-group differences with respect to the child's sex, gestational age, birth weight or APGAR scores at 1 and 5min after birth.

TABLE 1 - Comparison of infected and non-infected mothers according to number of children in previous pregnancies and schooling, State of Minas Gerais, Brazil, 2006

Variables	Infected mothers		Uninfected mothers		p	Odd ratio (IC95%)
	n*	mean (SD) or %	n*	mean (SD) or %		
Number of children	379	3.44 (2.98)	395	2.47 (2.64)	< 0.01	1.13 (1.07-1.20)
Schooling (%)					< 0.01	
< 8 years	318	78.1	233	57.2		3.08 (2.23-4.25)
≥ 8 years	74	18.2	167	41.1		

*number of infected mothers and uninfected mothers for which the data found in the SINASC: System of Information on Live Births database; SD: standard deviation; IC95%: confidence interval 95%.

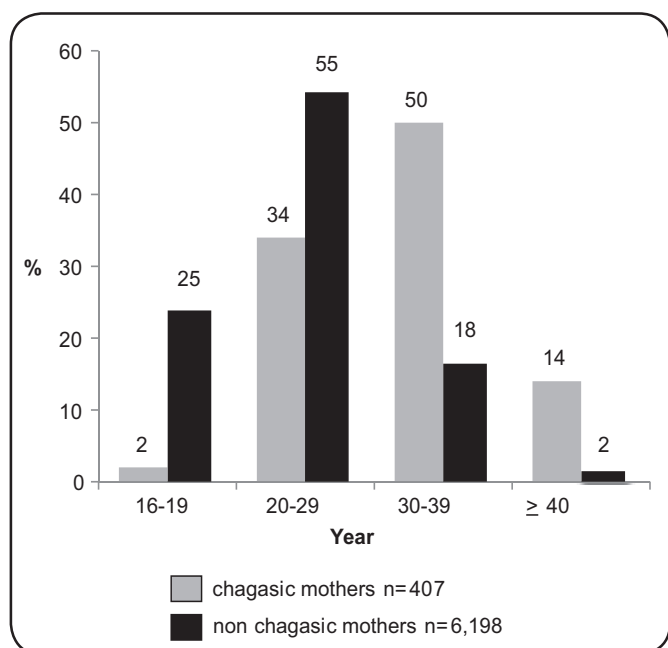


FIGURE 1 - Stratification by age of chagasic and non chagasic mothers identified in the System of Information on Live Births database, State of Minas Gerais, Brazil, 2006.

Clinical and serological assessment of infants after the age of 6 months

Among the 254 serological tests of children of Chagas-seropositive mother conducted at the age of 6-10 months (average age of 246 days, standard deviation of 78 days), only 6 (2%) of the IgG enzyme-linked immunosorbent assay (ELISA) results were positive, and 4 samples had indeterminate results. All immunofluorescence reaction (RIFI) and hemagglutination (HAI) tests were negative. After repetition of the serological tests on venous blood, only one result remained positive. All infants with positive or indeterminate IgG antibody results at age of 6-10 months were found to be seronegative before the age of 13 months. Despite the active search by health teams in the municipalities, 153 (38%) children with positive results in the first data collection could not be located for blood collection after the age of 6 months. The RIFI and HAI titers in the children's venous blood samples were lower than the mothers' titers. Four of the data losses were related to the death of the mother.

A case of congenital Chagas disease

A 33-year-old mother from the Municipality of Capitão Eneias had two previous pregnancies and was seropositive for *T. cruzi*. An asymptomatic child from the Municipality of Montes Claros was born at term by vaginal delivery with a weight of 3.2kg and a length of 50cm. His serology was positive, according to the RIFI IgG titer of 1/160 and the reactive ELISA and HAI assays carried out at age of 9 months. A polymerase chain reaction (PCR) assay was also positive. Blood cultures for *T. cruzi* were negative. The child was treated with benznidazole for 60 days, and his serology was negative after 6 months of specific treatment. The child's electrocardiogram, echocardiogram and fundoscopy were normal, and his serology remained negative at the age of 3 years.

DISCUSSION

This study is the first to assess the birth conditions of children of *T. cruzi*-infected mothers who participated in the Brazilian Newborn Screening Program.

The age of the infected mothers was 32 +/-6.5 years on average, which was higher than the mean ages in studies conducted in Argentina (29.9 years - CI95% 29.3-30.5), Bolivia (24.2 +/-6.8 years), and Peru (29.9 years - CI95% 29.3-30.5)⁸⁻¹⁰. A serological survey carried out in the State of Minas Gerais from 1995 through 1997 found an average age of 26.4 years for pregnant women¹¹. In this study, 63% of the infected mothers were older than 30 years. This difference is most likely to be the result of the efficiency of vector control in Brazil from the 1980s onwards, culminating in the 2006 PAHO/WHO certification of eliminated Chagas disease transmission through the main vector (*Triatoma infestans*) and transfusion. Over 36% of the infected mothers in this study were younger than 30 years old and can potentially transmit the disease vertically in future pregnancies.

The significant inter-group difference in the mothers' level of education reflects the lower socio-economical level of the infected mothers. Issues of social inequality are notable in the context of the transmission and control of Chagas disease. The disease primarily affects people in poor regions in Latin America, especially socially excluded people that have little political power and a low level of education¹². Other differences that may also reflect seropositive mothers' social vulnerability include the greater number of previous pregnancies and a greater number of cases of vaginal delivery among infected mothers. Women from families with higher incomes and higher levels of education had caesarean sections more often than other women^{13,14}.

A study in Brazil showed that cesarean delivery takes place 4.4 times more often in private maternity hospitals than in public maternity hospitals and that 77.9% of the mothers at public maternity hospitals had studied no longer than 8 years (versus 19.4% in at private maternity hospital)¹³.

Even though most mothers with Chagas disease had attended more than 4 prenatal appointments, the fact that serology is not a routine procedure, even in endemic areas, prevents adequate disease diagnosis. Although congenital transmission has low epidemiological relevance, it still has the ability to keep Chagas disease endemic in Brazil. Therefore, Chagas-specific serological surveys should be performed routinely at prenatal appointments, at least in the endemic areas⁴.

As in other studies, there were no significant inter-group differences in this study regarding prematurity, birth weight, or APGAR scores at 1 and 5min after birth^{15,16}. The sample size did not provide sufficient statistical power to show this association.

In our study, only 6 (2%) of the IgG enzyme-linked immunosorbent assay results were positive after the age of 6 months. In Paraguay, researchers found that 7% of children have maternal antibodies after the age of 6 months¹⁷.

A possible limitation of this study is the absence of serology for the children of 153 infected women at the age of 6 months. The major reasons for the impaired communication between health professionals and caregivers/parents were the absence

of a fixed residence or an incorrect address. Other studies have reported similar losses¹⁸. The need to confirm (via serology) the negative parasitological results at birth is the reason for the data loss and the underestimation of the incidence of congenital Chagas disease¹⁹. The possibility of congenital infection of these infants was not rejected in the study, but the titers found in the neonates' first samples were lower than those found in the mothers. All mothers received written reports of the positive results and recommendations for further follow up in their municipality within the scope of the National Policy of Basic Attention as developed by the Brazilian Unified Health System.

Another limitation is the losses related to the lack of retrieval of mothers' names from the System of Information on Live Births database, which occurred due to typos and a lack of data in some fields. Despite these limitations, this study was sufficiently comprehensive and included a population of uninfected infants, which is typically lacking in most related studies.

The age of the infected mothers was on average higher than the mean age of the uninfected mothers, but the study showed the existence of a number of infected women of reproductive age. The level of education and the greater number of previous pregnancies and cases of vaginal delivery reflect the lower socioeconomical conditions of the infected mothers. The investigation of Chagas infection should be a routine procedure during prenatal appointments in endemic areas. Treatment should be initiated after the confirmation of congenital infection. The mothers' infection did not affect the condition of the neonates at birth. Therefore, in the event of no vertical transmission, neonates will be subject to similar morbidity rates irrespective of the infection status of their mothers.

Providing assistance to infected patients and controlling of *T. cruzi* transmission to humans usually presuppose state initiatives and continued policies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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