

Serological screening for Chagas disease in an endemic region of Northern Minas Gerais, Brazil: the SaMi-Trop project

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ABSTRACT

Chagas disease (CD) is still a neglected disease. Infected individuals are diagnosed late, being treated in worse clinical conditions. Thus, this study aimed to analyze the prevalence and the factors associated with new confirmed cases of CD identified by serological screening in an endemic region of Minas Gerais State, Brazil. This is an analytical cross-sectional study with data from a project of the Research Center in Tropical Medicine of Sao Paulo- Minas Gerais (SaMi-Trop) conducted in two municipalities. Data collection included a questionnaire with closed questions, a venous blood collection and an ELISA serological test for CD. A total of 2,038 individuals with no previous diagnosis of CD participated in the study. The result of the serological test for CD was adopted as the dependent variable. The independent variables addressed personal issues, health conditions and lifetime housing. A descriptive analysis of individual variables was performed. Subsequently, a bivariate analysis was performed using the Pearson's chi-square test. Households sheltering individuals positive for CD were georeferenced, and the analysis of spatial distribution was performed using the quartic function to estimate the density of the nucleus. Among the participants, 188 (9.2 %) were positive for CD. The profile of participants with CD was associated with place of residence, age, relative/family member with CD and living conditions. It is noteworthy that there are still patients with CD who are unaware of their diagnosis in both, rural and urban areas.

KEYWORDS: Chagas disease. Serologic tests. Spatial analysis.

INTRODUCTION

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected disease¹. It still constitutes a major social and public health problem in endemic countries, and represents an emerging health problem in non-endemic countries^{2,3}. In both places of occurrence, there is inexperience of health professionals^{4,5} and a high rate of underdiagnosis^{2,5-7}.

It is estimated that 6 to 7 million people are infected worldwide⁸. In Brazil, it was estimated that between 1.3 and 3.2 million people would be infected with *T. cruzi* in 2020². However, the country does not have data on the prevalence of chronic CD. Although the notification of chronic cases of CD became mandatory in 2020⁹, the CD control program is disjointed and its actions are discontinuous¹⁰.

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Received: 6 April 2021

Accepted: 3 August 2021

From 2009 to 2018, 45,863 deaths were recorded in Brazil as a result of CD¹¹. Of these, 11,343 (24.7 %) were registered in the Brazilian Minas Gerais State, and the highest number of deaths, 2,300 or 20.2 %, were reported by the Northern health macro-region¹¹. Although much of Northern Minas Gerais has been identified as an area of high risk for vector transmission of CD or of reestablished home transmission¹², there is no investigation of CD in the prenatal care routine¹³ and many infected people are unaware of their condition¹⁴.

The late diagnosis of CD makes infected people arrive in worse clinical conditions to the health system, favoring the evolution of the disease, decreasing the quality of life of infected individuals, increasing their morbidity and mortality and causing a significant economic loss¹⁵⁻¹⁷. The absence of accessible diagnostic methods and the lack of specific information on CD by health professionals, leaves infected individuals unidentified, and therefore, untreated^{14,18}. This reality mainly affects vulnerable populations¹³. An early diagnosis allows the access to an appropriate treatment, minimizing complications of the disease, and lowering the corresponding mortality². In addition, it is recommended that the diagnosis be made in all suspected cases².

Thus, this study aimed to analyze the prevalence and the factors associated with new confirmed cases of CD identified by serological screening in an endemic region of Minas Gerais State, Brazil.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethics Committee of the State University of Montes Claros (CEP/UNIMONTES Nº 3.502.440/2018). All participants agreed to participate in this study and signed the Informed Consent Form.

Study design

This is a cross-sectional analytical study with data from a project performed at the Center for Research in Tropical Medicine of Sao Paulo-Minas Gerais (Centro de Pesquisa em Medicina Tropical de Sao Paulo-Minas Gerais - SaMi-Trop). This multicenter project is carried out by researchers from four Brazilian public universities in the States of Minas Gerais and Sao Paulo, Brazil. Its purpose is to develop and conduct research projects on CD.

Study area

This study was conducted in two municipalities

in the Northern region of the Brazilian State of Minas Gerais: Espinosa (Latitude: 15°56'55" South; Longitude 44°51'52" West) and Sao Francisco (Latitude: 15°56'41" South; Longitude: 44°51'14" West). The territorial extension of Espinosa is 1,884.23 km² and of Sao Francisco is 3,312.27 km². The population of Espinosa comprises 31,113 inhabitants and of Sao Francisco 53,828¹⁹. Both municipalities have high coverages of the Family Health Strategy (FHS) and more than 98% of the population depends on the Primary Health Care (PHC) offered by the Public Health System (Sistema Unico de Saude - SUS)²⁰.

Sample

Individuals over the age of 18 and belonging to the FHS coverage area were invited. The sample was defined in a non-probabilistic way. The exclusion criterion was: individuals with a previous diagnosis of CD.

A total of 2,056 individuals participated in the stage of sample collection. However, for this study, 18 individuals who reported having been diagnosed with CD were excluded from the analysis, thus 2,038 were eligible.

Data collection

A meeting was held with the managers and nurses responsible for the FHS of the respective municipalities in order to inform them on the planned intervention and request their consent to carry out the study. After their acceptance, the municipalities made available an accessible FHS unit to carry out the data collection. Nurses passed on the information to the community health workers (CHW). Then, upon a scheduled date, the CHW invited the community to participate in the study and informed that more than one family member could participate.

The data collection took place between February and November 2019, and included a questionnaire with closed questions and one collection of venous blood. The questionnaire was applied to the participants with the questions addressing personal information, health and housing conditions during their lives.

Serological screening

The collected venous blood samples were centrifuged the resulting serum was stored at -20 °C and subsequently sent in dry ice to the Institute of Tropical Medicine of the University of Sao Paulo (IMT-USP), where two enzyme linked immune sorbent assays (ELISA) tests were performed in duplicate, using commercial kits (Chagatest, Wiener Lab, Rosario, Argentina, and *Trypanosoma cruzi*

IgG, Euroimmun, Lübeck, Germany) according to the manufacturer's instructions. To confirm the serological diagnosis, it was necessary to obtain concordant results in at least two serological tests of different methodological principles or antigenic preparations².

Individuals identified with CD who authorized the notification of their condition, and those with inconclusive serology, were included in the flow of the Brazilian unified health system for the continuity of their health care and treatment of CD.

Variables

The results of the ELISA tests were adopted as the outcome (dependent variable), dichotomized into two categories (negative/inconclusive vs. positive). The independent variables were composed by city, age, gender, relative/family member with CD, knowledge on triatomine insects, have lived in an area with triatomine insects, have lived in a wooden/wattle and daub/adobe house. The age was calculated based on the date of birth of the participants and categorized as: up to 35 years, from 36 to 55 years, from 56 to 75 and 76 years or more.

Statistical analysis

The descriptive analysis of individual variables was performed, estimating absolute (n) and relative (%) frequencies for the categorical variables and calculating the central tendency and dispersion measures for the numerical variables. Subsequently, a bivariate analysis was performed using the Pearson's chi-square test, considering only the associations that maintained a significance level of up to 5 % ($p < 0.05$). The statistical software Statistical Package for the Social Sciences (SPSS) V.19 (SPSS Inc., IBM, Armonk, New York, USA) was used to carry out the analyzes conducted in this study.

The households that sheltered individuals serological positive for CD were georeferenced on site using a Global Positioning System (GPS) navigation device (Garmin Etrex® Garmin Ltd., Olathe, USA). In the Qgis 3.4® program (version 3.4 Madeira, QGIS Development Team), the spatial distribution analysis of the residences was carried out using the quartic function of the kernel density estimation (KDE)²¹, considering a bandwidth of 500 m. KDE is a non-parametric function that estimates probable occurrence values based on the density of upcoming events.

RESULTS

A total of 2,038 individuals participated in this study, of

which 188 (9.2 %) were serologically positive, 48 (2.6%) were inconclusive and 1,850 (90.8 %) were negative for CD. The inconclusive tests were repeated when these individuals were entered into the SUS, but for logistical and ethical reasons, we did not have access to these results. Among the participants, the majority live in the city of Sao Francisco, are female (63.5%), have a mean age of 48.18 years (± 15.43), with a minimum age of 18 and a maximum of 94 years. They reported to be related to people with CD (50.8%), not having been bitten by triatomine insects (60.3 %), having lived in an area with triatomine insects (87.1 %), and having lived in a wooden/wattle and daub/adobe house (72.2 %) (Table 1).

Among the individuals diagnosed with CD, the mean age was 56.19 (± 9.02) years with a minimum of 35 and a maximum of 86 years old. The majority of individuals with a diagnosis of CD lived in the city of Espinosa (12%), while the majority of respondents lived in the city of Sao Francisco. When analyzing the distribution of positive diagnoses in the cities studied (Figure 1, Table 2), it was observed that 75 % of positive cases live in the rural area of Espinosa and 55 % live in the urban area of Sao Francisco.

In the bivariate analysis, it was observed that the characteristics that were statistically associated with positive diagnoses were age, having a family member with CD, have been bitten by the triatomine insect, have lived in an area with triatomine insects, and have lived in a wooden/wattle and daub/adobe house (Table 1).

The individual variables that were statistically associated with the outcome were age, being related to people with CD, having been bitten by the triatomine insect, having lived in an area with triatomine insects, and have lived in a wooden house/wattle and daub/adobe.

DISCUSSION

The present study observed that the number of positive cases screened by serology corroborates the number of positive cases estimated by the high-risk population of the Clinical Protocol and Therapeutic Guideline for Chagas Disease (PCDT)²².

With the increase in migration and progressive changes in the rural economy, the epidemiological patterns of CD have been modified, with a transition from acute rural to chronic urban disease²³. In Minas Gerais State, it was observed that between 2001 and 2006, 84 cases of acute CD were reported, with 58% of the cases of acute CD being registered in the urban area²⁴, a significant data, since for decades CD was restricted to rural areas and was strongly marked by poverty². However, our study shows that it is not possible to generalize the epidemiological patterns of CD,

Table 1 - Descriptive and bivariate analysis of the diagnoses of Chagas disease (n = 2,038).

Characteristics	Descriptive n (%)	Bivariate		P-value ^a
		New positives		
		Negative/inconclusive n (%)	Positive n (%)	
Individuals				
City				
Espinosa	897 (44%)	789 (88%)	108 (12%)	<0.001
Sao Francisco	1,141 (56%)	1,061 (93%)	80 (7%)	
Age in years*				
Up to 35	454 (22.1 %)	453 (99.8 %)	1 (0.2 %)	<0.001
36 to 55	884 (43.4 %)	795 (89.9%)	89 (10.1%)	
56 to 75	614 (30.1%)	523 (85.2%)	91 (14.8%)	
76 of or more	84 (4.1%)	77 (91.7%)	7 (8.3%)	
Gender				
Male	743 (36.5%)	664 (89.4%)	79 (10.6%)	0.096
Female	1,295 (63.5%)	1,186 (91.6%)	109 (8.4%)	
Relative/family member with CD*				
No	846 (49.2%)	793 (93.7%)	53 (6.3%)	<0.001
Yes	875 (50.8%)	775 (88.6%)	100 (11.4%)	
Triatomine insect*				
No	786 (60.3%)	742 (94.4%)	44 (5.6%)	<0.001
Yes	518 (39.7%)	444 (85.7%)	74 (14.3%)	
Lived in an area with triatomine insects*				
No	256 (12.9%)	248 (96.9%)	8 (3.1%)	0.001
Yes	1,732 (87.1%)	1,556 (89.8%)	176 (10.2%)	
Lived in a wooden/wattle and daub/adobe house*				
No	565 (27.8%)	549 (97.2%)	16 (2.8%)	<0.001
Yes	1,469 (72.2%)	1,298 (88.4%)	171 (11.6%)	

*Variation of n = 2,038 due to missing information.

as the municipality of Sao Francisco presented an urban pattern, but the municipality of Espinosa presented a rural pattern. CD is still recognized as a neglected disease, which mainly affects vulnerable populations in need of public policies that promote access to health care¹.

Regarding the age, our study corroborates the results of others^{25,26} who observed a greater relationship between positive serology and older people, suggesting that the infection by *T. cruzi* has happened in the past. From the 1950s to the mid-1990s, campaigns to prevent CD were intensified, and since then, vector transmission has suffered a significant decrease. As a consequence, it is possible that the positive individuals in the study became infected in a period prior to the campaigns to combat CD^{2,26,27}. Another factor to consider would be the lack of adequate training of PHC professionals during their under graduation studies as well as their professional lives, leading to the failure to consider the suspicion of CD in their patients⁴, making the early diagnosis and treatment unfeasible.

Having a relative/family member with CD was associated with a positive diagnosis. Other studies have

also observed a history of CD in the families of diagnosed patients^{28,29}. The authors hypothesized that these infections occurred through vector transmission, due to their region of birth, being in the rural area or in an endemic area, and the presence of triatomine insects in the households^{28,29}. This demonstrates the relevance of medical care to the entire family group. In addition, another possibility of infection is through congenital transmission¹². A systematic review with meta-analysis carried out in Brazil, in 2014, reported a congenital transmission rate ranging from 0 to 5.2%³⁰. The WHO recommends that all pregnant women be subjected to serology to *T. cruzi*³¹, but this has not yet been achieved, and CD continues to be neglected and excluded from the prenatal care examinations¹³.

Having previous contact with the CD vector through a triatomine bite was associated with a positive diagnosis. In 2006, Brazil was certified for the interruption of the transmission of CD by its main vector, and also made progress in the transfusion screening³². Even in the face of these achievements, an alert is essential, as it is still possible to find residual foci in the Brazilian territory

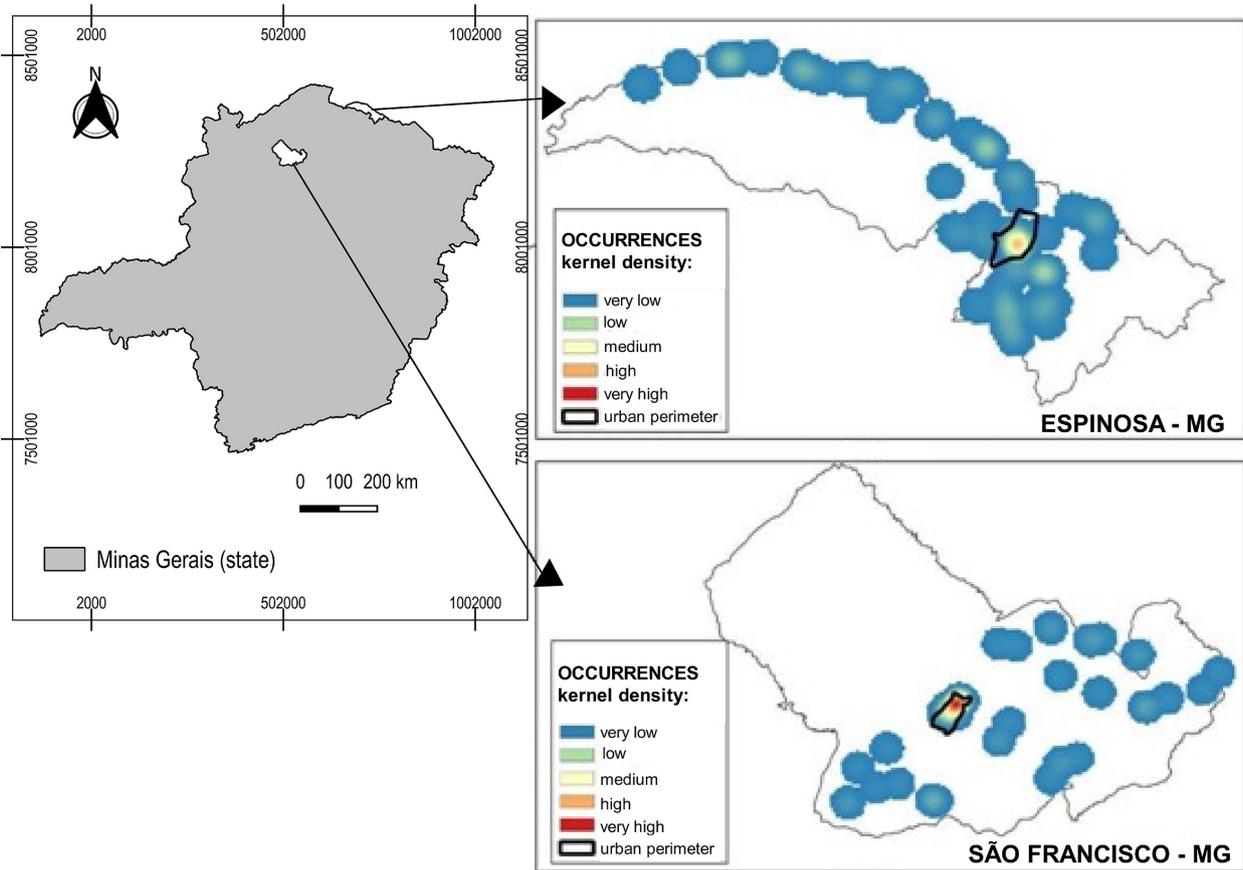


Figure 1 - Spatial distribution of cases diagnosed with Chagas disease in 2019, in the municipalities of Espinosa and Sao Francisco, Minas Gerais State (n = 2,038).

Table 2 - Description of the place of residence of the individuals participating in the study (n = 2,038).

Municipality	Individuals surveyed			Individuals diagnosed with CD		
	Rural zone	Urban zone	Total	Rural zone	Urban zone	Total
Espinosa	377 (42.03%)	520 (57.97%)	897 (44%)	81 (75%)	27 (25%)	108 (12%)
Sao Francisco	417 (36.55%)	724 (63.45%)	1141 (56%)	36 (45%)	44 (55%)	80 (7%)

capable of reintroducing the main vector of CD³². Bearing in mind that the studied municipalities are currently located in areas at risk of transmission of CD due to the presence of the vector, we cannot rule out the possibility of vector transmission in the individuals participating in the study, therefore continuous epidemiological surveillance actions are necessary¹². The CD control program is in the epidemiological surveillance phase, where the population has a fundamental role, but many people do not have knowledge on the management and identification of the vector, making this type of control vulnerable^{33,34}. A study carried out in Diamantina (Minas Gerais State) demonstrated that the population’s knowledge on the identification of the CD vector is indispensable for the correct referral of insects to the control service. In areas where people had sufficient knowledge to identify the vector, entomological

surveillance actions were more assiduous³⁵. This indicates the need to carry out health education activities, because, through alerting and bringing knowledge, it is possible to achieve an effective control.

Living in a wooden, wattle and daub, or adobe house at some point in life was related to positive serology. Housing characteristics have already been reported to be significantly associated with domestic infestation of the CD vector³⁶. Houses built with wood, wattle and daub, or adobe have refuges that are ideal shelters for vector infestation, thus making their residents a food source, increasing the chances of infection by *T. cruzi*³⁶. Despite the Federal Government’s incentive aimed to improve the civil construction in endemic regions of Brazil³⁷, it is still possible to find precarious households in rural communities in the country. Adequate housing infrastructure generates

positive impacts on health in general, thus the improvement of households is an important factor for vector control, as a long-term strategy³⁷.

In the development of this study, some limitations were observed, among them the cross-sectional design, which does not allow establishing causal relationships. IN addition, there is a limitation of temporality, as it is not possible to verify when these people were infected. However, this type of design allows to characterize the population and identify risk groups. One of the strengths of our study was the high detection rate of patients with CD who were unaware of their condition. The low visibility of these individuals in the society is notorious and our study shows the deficit in coping with CD along with the PHC, which is the closest health resource for the population. Although a PCDT for CD was instituted in 2018 by the Ministry of Health, there is still no specific line of care for chronic CD patients²². CD continues to be neglected even one hundred years after its discovery, demonstrating the importance of actively searching for new CD carriers in an endemic region.

CONCLUSION

The profile of participants with positive CD serology identified in this study is associated with place of residence, age, family and life conditions. Our study demonstrates that in both, rural and urban areas, there are still people with CD who are unaware of their diagnosis. CD remains a major challenge for the SUS, thus it is necessary that the PHC together with the epidemiological surveillance establish effective protocols for the screening and management of CD.

ACKNOWLEDGMENTS

We thank the SaMi-Trop project team, the patients in this study and the National Institutes of Health (NIH).

FUNDING

National Institutes of Health (NIH) (P50 AI098461-02).

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