SEROPREVALENCE AND RISK FACTORS OF CHAGAS DISEASE IN RURAL COMMUNITIES IN THE STATE OF PIAUÍ, BRAZILIAN SEMI-ARID REGION

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ABSTRACT: Objective: To investigate the seroprevalence of Chagas disease (CD) among the rural population of communities within the municipality of Simplício Mendes, located in the state of Piauí, Brazilian semiarid region. Methods: This is a cross-sectional study and serological survey conducted from 2022 to 2023 in 10 rural communities. Capillary blood samples were collected from consenting residents and screened for anti-*Trypanosoma cruzi* antibodies using the TR Chagas Bio-Manguinhos kit. Positive samples underwent confirmatory testing using ELISA and Indirect Immunofluorescence Assay techniques. Data on sociodemographic characteristics and potential risk factors for CD transmission were collected through a structured questionnaire. Results: The overall serologically confirmed positivity rate in the rural communities of Simplício Mendes municipality was 4.1% (8/195). Older age (> 40 years) and lower levels of formal education were associated with higher seropositivity rates. Triatomine infestation was reported in 71.3% of households, with higher prevalence during the dry season (69.2%). Conclusion: The study highlights the persistent threat of CD in rural communities of Simplício Mendes, Piauí, emphasizing the need for sustained surveillance and control efforts. Socioeconomic and environmental factors contribute to ongoing transmission cycles, underscoring the importance of community engagement and health education initiatives.

KEYWORDS: Chagas Disease; Triatomines; Vector Insects, Health Surveillance.

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SOROPREVALÊNCIA E FATORES DE RISCO DA DOENÇA DE CHAGAS EM COMUNIDADES RURAIS NO ESTADO DO PIAUÍ, REGIÃO SEMIÁRIDA BRASILEIRA

RESUMO: Objetivo: Investigar a soroprevalência da doença de Chagas (DC) entre a população rural de comunidades no município de Simplício Mendes, localizado no estado do Piauí, região semiárida brasileira. Métodos: Este é um estudo transversal e uma pesquisa sorológica realizada de 2022 a 2023 em 10 comunidades rurais. Amostras de sangue capilar foram coletadas de residentes que consentiram e rastreadas quanto a anticorpos anti-*Trypanosoma cruzi* usando o kit TR Chagas Bio-Manguinhos. Amostras positivas foram submetidas a testes confirmatórios usando técnicas de ELISA e Imunofluorescência Indireta. Dados sobre características sociodemográficas e potenciais fatores de risco para transmissão de DC foram coletados por meio de um questionário estruturado. Resultados: A taxa geral de positividade confirmada sorologicamente nas comunidades rurais do município de Simplício Mendes foi de 4,1% (8/195). Idade mais avançada (> 40 anos) e níveis mais baixos de educação formal estavam associados a taxas mais altas de soropositividade. A infestação por triatomíneos foi relatada em 71,3% dos domicílios, com maior prevalência durante a estação seca (69,2%). Conclusão: O estudo destaca a ameaça persistente da DC nas comunidades rurais de Simplício Mendes, Piauí, enfatizando a necessidade de esforços sustentados de vigilância e controle. Fatores socioeconômicos e ambientais contribuem para os ciclos de transmissão contínua, destacando a importância do envolvimento da comunidade e iniciativas de educação em saúde.
PALAVRAS-CHAVE: Doença de Chagas; Triatomíneos; Insetos Vetores; Vigilância em Saúde.

SEROPREVALENCIA Y FACTORES DE RIESGO DE LA ENFERMEDAD DE CHAGAS EN COMUNIDADES RURALES EN EL ESTADO DE PIAUÍ, REGIÓN SEMIÁRIDA DE BRASIL

RESUMEN: Objetivo: Investigar la seroprevalencia de la enfermedad de Chagas (EC) entre la población rural de comunidades en el municipio de Simplício Mendes, ubicado en el estado de Piauí, región semiárida de Brasil. Métodos: Este es un estudio transversal y una encuesta serológica realizada de 2022 a 2023 en 10 comunidades rurales. Se recogieron muestras de sangre capilar de residentes que dieron su consentimiento y se analizaron en busca de anticuerpos anti-Trypanosoma cruzi utilizando el kit TR Chagas Bio-Manguinhos. Las muestras positivas se sometieron a pruebas confirmatorias utilizando técnicas de ELISA e Inmunofluorescencia Indirecta. Se recopilaron datos sobre características sociodemográficas y posibles factores de riesgo para la transmisión de la EC mediante un cuestionario estructurado. Resultados: La tasa general de positividad confirmada serológicamente en las comunidades rurales del municipio de Simplício Mendes fue del 4,1% (8/195). La edad avanzada (> 40 años) y los niveles más bajos de educación formal estuvieron asociados con tasas más altas de seropositividad. Se informó de infestación por triatominos en el 71,3% de los hogares, con una mayor prevalencia durante la estación seca (69,2%). Conclusión: El estudio destaca la amenaza persistente de la EC en las comunidades rurales de Simplício Mendes, Piauí, enfatizando la necesidad de esfuerzos sostenidos de vigilancia y control. Los factores socioeconómicos y

ambientales contribuyen a los ciclos de transmisión continua, subrayando la importancia del compromiso comunitario e iniciativas de educación en salud.
PALABRAS CLAVE: Enfermedad de Chagas; Triatominos; Insectos Vectores; Vigilancia en Salud.

1. INTRODUCTION

Chagas disease (CD), also known as American trypanosomiasis, is caused by a flagellated protozoan parasite, *Trypanosoma cruzi*, and stands as one of the most Neglected Zoonotic Diseases (NZD). Recent data reveals an annual incidence of 30,000 new cases in 21 Latin American countries, impacting nearly six million individuals and resulting in an average of 12,000 deaths per year. Moreover, an estimated 8,600 newborns are infected during gestation. Over the past few decades, concerted efforts have led to successful vectorial control in Central America (Durães-Oliveira *et al*., 2024).

Nevertheless, despite the reduction in prevalence within endemic regions, there has been a notable surge in non-endemic countries attributable to the substantial migration of the infected population to Asia, North America, Oceania, and Europe, or the presence of the vector and parasite. This event has contributed to making the disease a global health issue (Navarro *et al*., 2022).

CD is transmitted primarily through infected triatomine bugs, which are commonly known as "kissing bugs" due to their tendency to bite around the lips and face (Rose Ghilardi *et al*., 2024). However, the disease can also be transmitted through transplacental transmission, orally by the ingestion of food or beverages contaminated with *T. cruzi* and, more rarely, through blood transfusion or organ transplantations, sexual transmission, laboratory accidents and inadequate management of infected animals (Rios *et al*., 2020).

In Brazil, oral transmission is the main route of human infection by *T. cruzi* in the last 15 years, followed by vector transmission. This transmission occurs through the consumption of raw foods contaminated by the pathogen such as açaí and sugarcane juice, especially in the Amazon region (Bruneto *et al*., 2021; Nascimento *et al*., 2021; Trovo *et al*., 2024).

Brazil is one of the most important CD endemic countries in Latin America. In Brazil, over a thousand cases of acute Chagas disease were confirmed between 2012 and 2016, resulting in an average annual incidence of 0.1 cases per 100,000 inhabitants (Fidalgo et *al*., 2021). However, there are no up-to-date estimates of the real prevalence

of CD at the national level in Brazil. Estimates based on theoretical modelling indicate that approximately 1.2 million people are infected with *T. cruzi* in Brazil. The only nationwide seroprevalence survey of human Chagas infection (1975–1980) estimated an average prevalence of infection in the general population residing in rural areas of 4.22 %. Mortality data at national and subnational levels have been the primary source of information on CD epidemiology in the country, with all the known inherent limitations (França *et al*., 2024).

The northeastern region of Brazil is recognized as an endemic area for CD. Within this region is the state of Piauí, where despite previous studies indicating a decrease in seroprevalence (4% to 1.9%) over the past decades, *T. cruzi* transmission still persists. This manifestation may be associated with the presence of secondary species of triatomines involved in the transmission process of CD. The health regions of Oeiras (5.8%), São João do Piauí (5.3%), and Picos (4.3%), which are all in the central-southern region of the state, present the highest prevalence rates for CD (Mendes-Sousa *et al*., 2020). A study in the state also uncovered elevated rates of CD seroprevalence among adults and elderly individuals, suggesting a longstanding history of vector-borne transmission in the region (Dos-Santos *et al*., 2020).

CD continues to pose a significant public health concern in the State of Piauí, being endemic in extensive areas, particularly in the southeastern region. The endemic nature of CD in Piauí is closely linked to inadequate surveillance and a considerable portion of the population residing in rural areas, often in homes lacking plastering. This circumstance increases the risk of insect vectors infiltrating and colonizing homes, as species capable of establishing themselves in human habitats. Seroepidemiological studies in endemic areas are relevant strategies to obtain updated information to support surveillance, control, and prevention measures.

This study aimed to investigate the seroprevalence of CD among the rural population of communities within the municipality of Simplício Mendes, located in the state of Piauí, Brazilian semiarid region.

2. METHODS

2.1 Methodological approach and study area

The methodological design underlying this study is a cross-sectional serological survey that aimed to investigate the seroprevalence of *T. cruzi* infection among the

population of rural communities in the municipality of Simplício Mendes (latitude 07º51'14" south and longitude 41º54'37" west, situated at an altitude of 302 meters), located in the southeastern region of the state of Piauí.

Simplicio Mendes is a municipality located in the Brazilian semi-arid region, a territorial area that spans nine states in the Northeast and part of northern Minas Gerais, with a population of approximately 28 million inhabitants distributed between urban (62%) and rural (38%) areas (INSA, 2024).

This municipality has 13,870 inhabitants and a semi-arid tropical climate, with high temperatures throughout the year and concentrated rainfall from August to November. Its vegetation is characterized by the typical resistance of the Caatinga biome, with thorny trees and bushes adapted to water scarcity. The topography varies from flat to slightly undulating terrain, with mountainous areas influencing the flow of rainfall and the distribution of vegetation. Although the majority of the population resides in urban areas, a significant portion lives in rural areas, primarily engaged in agricultural, livestock, or subsistence activities (IBGE, 2024).

The research was carried out in 10 rural communities (Barro Vermelho, Betânia, Cassange, Ligeiro, Manoel Joaquim, Monte Santo, Olho d'água, Saco, Sobradinho, and Tanquinho) during the period from 2022 to 2023 (**Figure 1**). The sites were selected based on data indicating a history of high infestation and colonization by triatomines or with characteristics that facilitate the domiciliation of the vector insect, according to the most recent records of local entomological surveillance. All investigated households were georeferenced with GPS for mapping purposes.

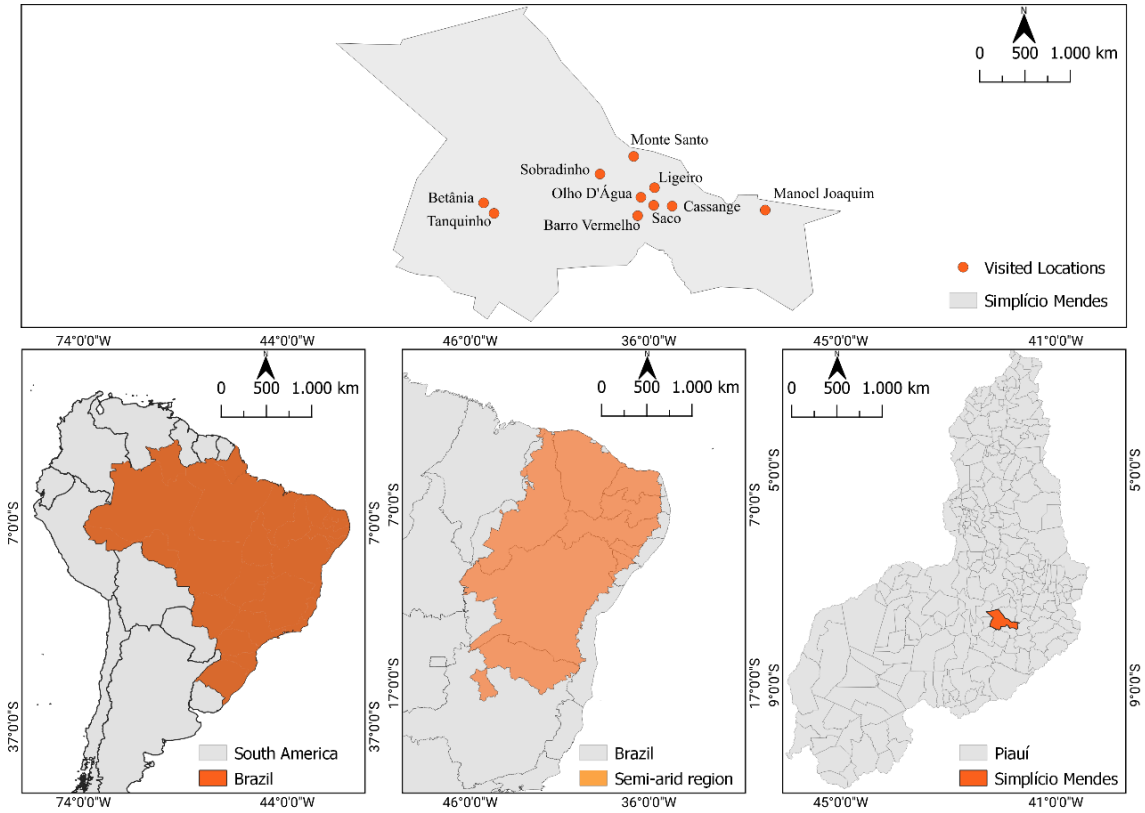

Figure 1: Map illustrating the geographic distribution of rural locations visited within the municipality of Simplício Mendes, Piauí, Brazilian semi-arid region.
Source: Own authorship.

2.2 Data collection and screening test execution

The serological survey was conducted among residents of the 10 selected rural communities, in collaboration with the municipality's Endemic Disease Control Agents. The recruitment strategy involved visiting all households with the aim of including all residents of the studied communities. However, residents who were not at home or those who refused blood collection were considered lost to follow-up.

During home visits, residents were invited to participate in the research after providing informed written consent. For the administration of the sociodemographic questionnaire and blood sample collection, this investigation adhered to ethical standards in scientific research, guaranteeing confidentiality.

Following the questionnaire administration, capillary blood samples from the pulp of the residents' fingers were performed for a qualitative screening test to detect antibodies for CD, TR Chagas Bio-Manguinhos kit (Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil). This test is based on immunochromatography technology and uses a lateral flow platform, with its principle involving the use of two distinct recombinant

antigens from the *T. cruzi* protozoan bound to a membrane (solid phase) and protein A conjugated with colloidal gold particles.

Before conducting the procedure, the integrity of all kit components was verified. At the time of sampling, each plate was correctly identified with the participant's full name, date of birth, location, and identification code. Blood sample collection was performed through digital puncture using a capillary tube after cleansing the fingertip with 70% alcohol, puncturing the cleansed area with lancets, and discarding the first drop of blood.

Subsequently, the samples were applied to the well, followed by the addition of a running buffer. The buffer facilitates the lateral flow of the conjugate components, revealing the interaction of the sample's specific antibodies with the antigen.

After the buffer addition, a 15-minute waiting period was allowed for reading and release of the results. Reactive results are evidence of exposure to the *T. cruzi* protozoan and can be used to support clinical diagnosis.

In cases of reactivity, a second blood sample (5ml) was collected through vacuum venous puncture. The serum obtained from this second sample was stored and transported under appropriate conditions for confirmation of chagasic diagnosis to the Public Health Central Laboratory of Piauí Dr Costa Alvarenga (LACEN-PI), located in Teresina, Piauí. All aforementioned procedures were performed by adequately trained professionals in proper protective attire.

2.3 Data collection and screening test execution

The laboratory diagnosis was performed using two different serological techniques for detection of anti-*T. cruzi* antibodies at the LACEN-PI. For the Enzyme-linked Immunosorbent Assay (ELISA) technique, the Recombinant Biolisa Chagas kit (Quibasa-Bioclin, Belo Horizonte, MG, Brazil) was used and for the Indirect Immunofluorescence Assay (IFA) technique. The IFA Chagas BioManguinhos kit (BioManguinhos, Rio de Janeiro, RJ, Brazil) was used.

The Recombinant Biolisa Chagas kit is an immunoenzymatic test used to detect *T. cruzi* antibodies in human serum or plasma. It has a clinical sensitivity above 99.9% and a specificity of 99.3%. The test works by binding these antibodies to specific *T. cruzi* antigens on a microplate. After several steps, including washing and adding enzyme-

linked antibodies, a color change occurs: blue indicates a positive result. A stop solution changes the color to yellow, which is then measured with a microplate reader.

The IFA Chagas Biomanguinhos kit uses a microscope slide coated with *T. cruzi* to detect antibodies in human samples. It has sensitivity and specificity of at least 90%. A fluorescent dye is used to make the reaction visible under UV light. Fluorescence indicates a reactive (positive) result, while no fluorescence indicates non-reactive (negative). Each slide includes positive and negative controls for reference.

Those individuals who tested positive in both techniques were deemed positive for CD. All residents received their test results and underwent clinical evaluation, as well as receiving guidance regarding treatment protocols.

2.4 Statistical analysis

Prevalence ratios and 95% confidence intervals (CI) were calculated. A descriptive analysis of the profile of the target population was conducted using R *software* version 4.3.1. Fisher's exact test was used considering a significance level of 0.05 to determine the potential associations between positive results for CD and independent variables.

2.5 Ethical Considerations

The current study underwent ethical review via the Brazil Platform by the Human Research Ethics Committee at the Oswaldo Cruz Institute (IOC/FIOCRUZ), and received approval under registration number CAAE No. 89970718.7.0000.5248, with evaluation No. 2,940,155.

3. RESULTS

The survey was conducted in a rural area comprising 263 households across 10 localities within the municipality of Simplício Mendes, Piauí. Some residences could not be surveyed due to either lack of authorization from occupants or the homes being closed during the visit (n=92). This situation primarily occurred in the communities of Manoel Joaquim and Olho d'água. Consequently, a total of 171 households were investigated, with 195 residents consenting to participate in the survey and underwent CD screening tests.

The age of the participants ranged from four to 104 years, including individuals of both genders and different educational levels. Among the research subjects, only 22 (11.3%) had previously undergone serological testing for CD, and all of them had previously received negative results. The overall positivity rate of rural communities surveyed regarding the screening test was 17/195 (8.7%), as shown in **Table 1**.

The communities of Monte Santo (33.3%) and Sobradinho (12.9%) exhibited higher rates of seropositivity for *T. cruzi* infection, with statistically significant p-values of 0.008 and 0.011, respectively. These results suggest possible clustering of cases related to environmental or vector-related factors in specific localities, warranting further spatial and entomological investigation. Regarding age groups, the positivity rate for CD was higher among participants aged over 40 years, with statistical significance observed among those over 80 years old ($p = 0.049$). Although other age groups over 40 also presented increased prevalence, the lack of statistical significance may reflect reduced power due to sample size limitations.

As for gender, the rates were similar between males and females (7.8% and 9.7%, respectively). Concerning level of education, individuals with less than 8 years of formal education showed higher positivity rates, at 11.2%. Although not statistically significant ($p = 0.086$), this finding points toward a potential association between lower educational attainment and greater vulnerability to Chagas disease, possibly reflecting broader socioeconomic inequalities.

While key variables such as income, housing conditions, occupation, and access to health services were not included in the present statistical analysis, their relevance to the social determination of health is well recognized. Including these indicators in future research could enhance the understanding of how socioeconomic factors influence *T. cruzi* transmission dynamics and population vulnerability.

Table 1: Prevalence and distribution of the surveyed population regarding Chagas infection in the rural communities of Simplício Mendes, from 2022 to 2023, assessed through screening test.

Variable	Positive ratio, n/N (%)	Prevalence ratio (95% CI)	*p-Value*
Age group (years)			
0 – 20	0/9	–	–
21 – 40	1/47 (2.1)	0.021 (-0.019 - 0.062)	0.124
41 – 60	10/86 (11.6)	0.116 (0.048 - 0.184)	0.336
61 – 80	3/43 (6.9)	0.069 (-0.006 - 0.145)	1.000

> 80	3/10 (3)	0.3 (0.015 - 0.584)	0.049
Gender			
Male	8/102 (7.8)	0.078 (0.026 - 0.130)	1.000
Female	9/93 (9.7)	0.096 (0.036 - 0.156)	0.712
Level of education			
≤8 years of formal education	16/143 (11.2)	0.111 (0.060 - 0.163)	0.297
>8 years of formal education	1/52 (1.9)	0.019 (-0.018 -0.056)	0.086
Community			
Barro Vermelho	1/12 (8.3)	0.083 (-0.073 - 0.239)	0.459
Betânia	1/30 (3.3)	0.033 (-0.030 - 0.097)	1.000
Cassange	2/36 (5.5)	0.055 (-0.019 - 0.130)	0.701
Ligeiro	1/28 (5.6)	0.035 (-0.033 - 0.104)	1.000
Monte Santo	3/9 (33.3)	0.333 (0.025 - 0.641)	0.008
Saco	0/8	–	–
Sobradinho	8/62 (12.9)	0.129 (0.045 - 0.212)	0.011
Tanquinho	1/10 (10)	0.1 (-0.085 - 0.285)	0.401

Legend: n: Number of sampled individuals positive for *T. cruzi* infection on the screening test; N: Total number of individuals who underwent the screening test; %: Positivity rate on the screening test; CI: Confidence intervals.
Source: Own authorship.

However, out of the 17 individuals seropositive on the screening test, only eight had serological confirmation for both ELISA and IFA techniques and as shown in **Table 2** and **Figure 1**. Among the seronegative blood samples, six tested non-reactive for both techniques, three samples were inconclusive (ELISA reactive and IFA non-reactive) and classified as seronegative. Thus, the overall serologically confirmed positivity rate in the rural communities of Simplício Mendes municipality was 4.1% (8/195). Except for the Monte Santo community, which previously showed high positivity rates in the screening test (33.3%), all other locations had at least one serological confirmation for CD infection. Among these rural communities, Sobradinho had the highest number of confirmatory serological tests for both techniques (n=3).

This discrepancy between screening and confirmation results, especially in Monte Santo, highlights the importance of confirmatory testing to avoid overestimation of disease prevalence based solely on screening tests.

Among the seropositive individuals, all claimed to be unaware of their *T. cruzi* infection status. Additionally, all individuals received guidance regarding their clinical report and were referred to specialized medical consultation within the municipality.

Table 2: Distribution of the population examined with confirmatory serology for CD using the Elisa and IFA techniques.

Community	**Gender**		**ELISA positive cases n/N**	**IFA positive cases n/N**
	Male	**Female**		
Barro Vermelho	**0**	**1**	**1/1**	**1/1**
Betânia	**1**	**0**	**1/1**	**1/1**
Cassange	**2**	**0**	**1/2**	**½**
Ligeiro	**0**	**1**	**1/1**	**1/1**
Monte Santo	**2**	**1**	**2/3**	**0/3**
Sobradinho	**3**	**5**	**4/8**	**3/8**
Tanquinho	**0**	**1**	**1/1**	**1/1**
Total	**8**	**9**	**11/17**	**8/17**

Legend: n: Number of blood samples positive for *T. cruzi* infection on the test; N: Total number of blood samples analyzed in the test; IFI: Indirect Immunofluorescence Reaction Technique.
Source: Own authorship.

Regarding the risk factors associated with increased vulnerability to CD, the majority of research participants stated that they had already seen triatomines inside their homes (71.3%), during the dry season (69.2%), at evening (50.2%). Additionally, most of the population claims to be able to identify these vector insects (91.8%) and to have close contact with animals in their homes (84.6%). However, despite being able to identify triatomines, the majority consider killing these insects (42.2%) as an appropriate practice if they find them inside the household (**Table 3**).

While the study gathered valuable information on vector exposure and related behaviors, statistical associations between these factors and seropositivity were not explored in this analysis. Investigating such relationships in future studies may uncover important behavioral or environmental determinants, contributing to more effective health education and vector control initiatives.

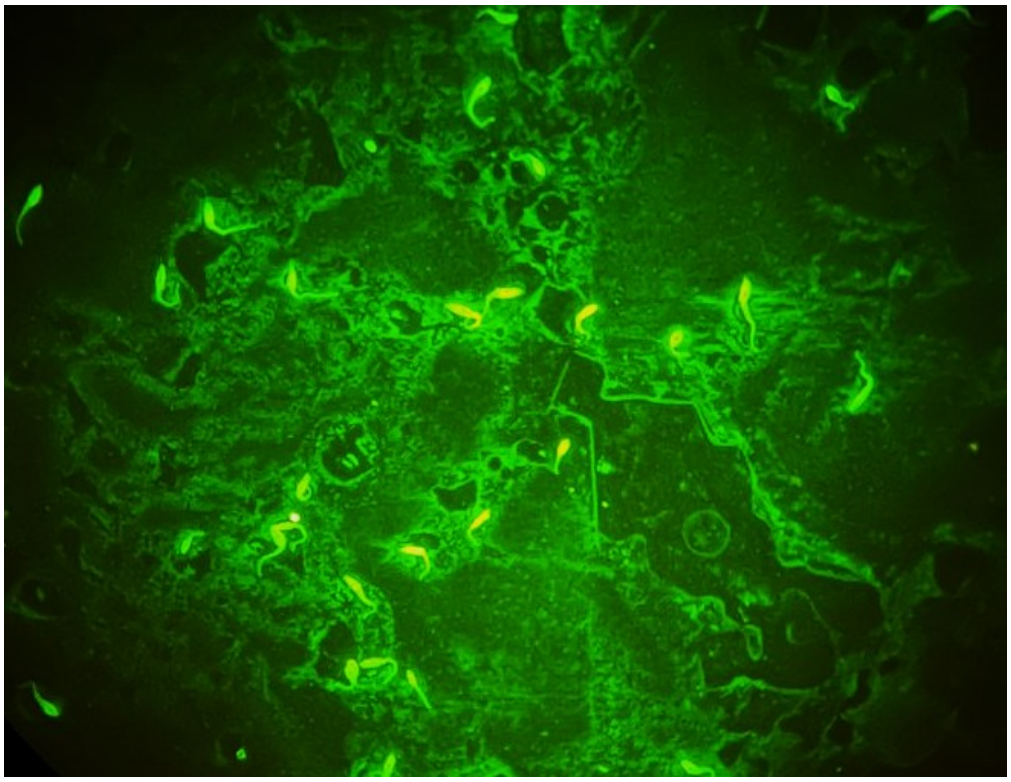

Figure 2: Positive slide of reagent sample for *T. cruzi* by Indirect Immunofluorescence Assay (IFA) technique.
Source: Own authorship.

Table 3: Distribution of risk conditions associated with CD in the rural communities of Simplício Mendes, Piauí.

Characteristics	**N**	**%**
Can identify the triatomine bug		
Yes	179	91.8%
No	16	8.2%
Knows the season of the year when the bug is most frequently present		
Rainy	44	22.6%
Dry	135	69.2%
Does not know	16	8.2%
Knows it transmits Chagas disease		
Yes	179	91.8%
No	16	8.2%
Reported the bug's presence in the house		
Yes	139	71.3%
No	56	28.7%
If you've already seen the insect, what time was it?		
Morning	47	24.2%
Afternoon	34	17.4%
Evening	98	50.2%
Never seen	16	8.2%
What to do on finding the bug inside the house?		
Kill it	82	42.2%

Capture it and advise the health center	55	28.2%
Others	58	29.6%
Has the house been sprayed with insecticides by the Endemic Disease Control Agents due to the presence of triatomines?		
Yes	139	71.3%
No	56	28.7%
Has animals inside the house or surrounding area		
Yes	165	84.6%
No	30	15.4%

Legend: n: Number of individuals who participated in the questionnaire; %: Frequency.
Source: Own authorship.

4. DISCUSSION

CD remains a significant public health issue in Brazil, particularly in the northeastern state of Piauí, as evidenced by the findings of this study. The seroprevalence rates observed in rural communities within the municipality of Simplício Mendes (4.1%) corroborate previous research indicating the endemicity of the disease in this region. Similarly, studies such as those conducted by Dos-Santos *et al*. (2020), Mendes *et al*. (2020) and Silva *et al*. (2024) have highlighted the persistence of CD in the state of Piauí over the past decade, emphasizing the need for continued surveillance and intervention efforts. These findings reinforce a historical pattern of endemicity in the region, which has been documented for over a century.

In Piauí, the initial reports of CD emerged in 1916 in the municipalities of Parnaguá, Correntes, and São Raimundo Nonato, where the populace presented symptoms of dysphagia (megaesophagus) and cardiac issues (Neiva e Penna, 1916). In 1975, the first native cases occurred among the residents of Oeiras, Bom Jesus do Gurgueia, and Castelo do Piauí cities (Figueirêdo, Lima e Nunes, 1975). These historical records provide important context for understanding the current epidemiological scenario.

Since then, the State of Piauí has been characterized as an endemic territory for CD. In 2002, a serological survey was also conducted to assess the epidemiological situation of CD in Piauí. It covered all 224 municipalities and involved the examination of 36.399 residents, revealing a prevalence rate of 1.9%. In the municipality of Simplício Mendes, 157 samples were analyzed, revealing a positivity rate of 3.8% (Borges-Pereira *et al*., 2006). When compared to the present study's positivity rate of 4.1%, these findings suggest relative stability in disease prevalence over the past two decades, despite ongoing surveillance efforts.

In the present study, 195 samples were analyzed with an infectivity rate of 4.1%. According to the local entomological surveillance reports, despite the municipality maintaining a consistent operation of surveillance, control, and combat against the main vectors of *T. cruzi*, the risk of disease transmission persists. This is mainly due to factors such as poor living conditions of the population, the presence of triatomines both peri- and intradomiciliary, and the involvement of secondary species in disease transmission. This scenario may facilitate the maintenance of the *T. cruzi* cycle in these rural areas. Such persistence underscores the importance of examining not only biological vectors but also the broader environmental and social determinants of transmission.

The socioeconomic and cultural factors associated with CD, exacerbated by poverty not only increase the risk of disease transmission in urban and periurban areas, but also contribute to its spread in rural populations. This is primarily because rural dwellings are typically constructed with plant materials, which are conducive to triatomine infestation. Additionally, customary practices such as stockpiling materials and establishing animal enclosures in peridomiciliary areas further elevate the risk. Moreover, the proximity of rural households to wilderness environments, where activities like agriculture, cattle farming, and extractivism are common, facilitates contact with the vector (Paixão *et al*., 2024). These social and environmental vulnerabilities converge to create a high-risk setting for sustained transmission, as also supported by entomo-epidemiological studies in the region.

The entomo-epidemiological context of CD in rural communities of Piauí, as explored by Santos *et al*. (2020) and Mendes-Sousa *et al*. (2020), underscores the complex interaction between environmental factors and disease transmission dynamics. This interplay likely contributes to the observed spatial variability in seroprevalence rates across different localities within Simplício Mendes.

A study conducted in Campinas do Piauí, a neighboring municipality of Simplício Mendes, showed an infectivity rate of 5.1% for CD among residents of rural areas. Furthermore, this study indicates that CD remains actively transmitted in the region, as evidenced by a case occurring in a resident under 10 years old (Aquino Santana *et al*., 2021). This finding is particularly relevant when interpreting age-specific patterns of infection observed in the present study.

The age-related pattern of CD prevalence observed in this study is consistent with findings from other areas of Brazil, as demonstrated by studies conducted by Costa *et al*.

(2018) in the state of Ceará (Brazil) and Nobre *et al*. (2021) in the Capital of Brazil (Brasília). This suggests that older individuals, who may have experienced prolonged exposure to vector habitats, are at increased risk of infection. Such age associations may reflect cumulative exposure over time, highlighting the importance of long-term surveillance even in areas with apparent stability in transmission rates.

The similarity in seroprevalence rates between genders agrees with previous studies such as those conducted by Santana, Souza-Santos e Almeida (2018), indicating a relatively uniform distribution of the disease among male and female populations in the northeastern region.

Individuals with lower levels of formal education demonstrated higher rates of seropositivity, suggesting a potential link between socioeconomic status and disease prevalence. This finding is consistent with studies conducted in other endemic regions, such as the study by Fidalgo *et al*. (2021) in Ceará, which reported similar associations between socioeconomic factors and CD prevalence. These associations underline the role of structural determinants, such as education and income, in shaping individual and collective vulnerability to CD.

However, it is essential to acknowledge the limitations of the diagnostic tests used in this study and their potential impact on the interpretation of results. The discrepancy between screening test positivity and serological confirmation highlights the challenges associated with false-positive results, which can arise due to cross-reactivity with other pathogens endemic to the region such as *Leishmania spp*, as noted by Barcelos *et al*. (2021). Additionally, the variability in test performance observed across different geographical regions, as discussed by Sáez-Alquezar *et al*. (2021), underscores the importance of considering the geographical origin of patients when interpreting test results.

Moreover, the use of a non-probabilistic sampling method may limit the extrapolation of findings to the entire rural population of the municipality, and the possibility of underreporting cannot be fully ruled out, especially in households that were unavailable or declined participation. Nevertheless, the results presented offer valuable insights into the local epidemiological scenario of Chagas disease and contribute meaningfully to the understanding of its persistence in endemic rural areas, particularly where surveillance data are often scarce. Thus, diagnostic limitations must be carefully

considered in both clinical practice and epidemiological surveillance, particularly in regions where multiple parasitic diseases co-exist.

Critical conditions of social vulnerability in rural areas in the northeastern region of Brazil reinforce the need to strengthen actions for disease surveillance and control and to integrate human and social development efforts. The high proportion of participants reporting the presence of triatomines (71.3%) inside their homes underscores the ongoing risk of vector-borne transmission within these communities. Their presence within households indicates a potential source of transmission, particularly during the nighttime when they are most active (50.2%). These behavioral and ecological factors directly influence the dynamics of transmission, emphasizing the need for community-based strategies.

The observation that triatomines are more commonly seen during the dry season (69.2%) is in line with known ecological patterns of these vectors. Triatomines thrive in warm and dry conditions, making their presence more prevalent during periods of reduced precipitation. This seasonal variation in vector abundance highlights the importance of targeted vector control efforts during peak transmission periods (Honorato *et al*., 2021; Lilioso *et al*., 2020).

The majority of participants (84.6%) reported having close contact with animals in their homes, which can increase the risk of exposure to triatomines and subsequent transmission of *T. cruzi*. Animals, particularly domesticated species such as dogs and cats, can serve as reservoir hosts for the parasite, further perpetuating the transmission cycle within communities (Barreto *et al*., 2019; Matos *et al*., 2023; Pinotti *et al*., 2021).

Despite the ability to identify triatomines (91.8%), a concerning finding is that a significant proportion of participants (42.2%) consider killing these insects as an appropriate practice if found indoors. Although killing triatomines may provide immediate relief, it does not address the root cause of *T. cruzi* infestation. Furthermore, many residents reported using insecticides independently to eliminate triatomines and other insects, which may inadvertently contribute to insecticide resistance or disrupt the natural balance of the ecosystem (Lima-Neiva *et al*., 2021; Rabinovich, 2021). These findings reflect a need for improved health literacy and integrated vector management, supported by continued education and community participation.

This study emphasizes the significant need to promote health education initiatives for the population of Simplício Mendes, covering general aspects of CD and the

ecological characteristics of triatomines. Greater community engagement through more proactive involvement in surveillance activities is crucial.

The clinical course, progression, and survival of CD are highly variable and poorly understood. The severity of this disease is associated with complex interactions among the genetic diversity of the parasite, host, and environmental factors. The disease has few treatment alternatives and high mortality rates that vary between countries and regions (Olivera *et al*., 2021). These differences in mortality rates could indicate changes in the effectiveness of control measures, transmission dynamics, *T. cruzi* genetic diversity, unequal recognition of the disease, and inequalities in care and diagnostic capabilities.

5. CONCLUSSION

The findings of this study underscore the persistent threat of CD in rural communities, particularly in the municipality of Simplício Mendes, located in the state of Piauí, Brazilian semiarid region. Despite decades of surveillance and control efforts, the disease continues to affect populations, with evidence suggesting ongoing transmission cycles in this region. Socioeconomic and environmental factors exacerbate the risk, highlighting the need for comprehensive interventions that address both the ecological and social determinants of CD. In this context, the results presented can serve as a basis for strengthening local public health policies, particularly by guiding the prioritization of surveillance activities, the allocation of resources for vector control, and the expansion of early diagnosis and treatment services in the most affected communities.

The study also emphasizes the importance of community engagement and health education initiatives to empower residents in recognizing and mitigating the risks associated with CD transmission. These findings support the implementation of intersectoral strategies that integrate health, education, and social protection policies to reduce vulnerability and promote sustained control of the disease. Furthermore, the complexities surrounding CD, including its limitations of diagnostic tests, variable clinical course and the challenges in treatment and care, emphasize the necessity of continued research and investment in health surveillance to combat this neglected tropical disease effectively.

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AUTHORSHIP CONTRIBUTION

Darwin Renne Florencio Cardoso: Study conception and design, data collection and analysis, original drafting of the manuscript, and critical revision of the intellectual content.

Simone Patrícia Carneiro de Freitas: Co-supervision, support in data interpretation, and critical review of the final version of the text.

Ranieri Flávio Viana de Sousa: Contribution to the literature review, support in data analysis and contributions to the discussion of the results.

Jéssica Milena Moura Neves: Support in data collection and organization of bibliographic references.

Amália Ramos de Sousa: Contribution to the literature review and formatting of the manuscript according to journal guidelines.

Roberto Coelho de Farias: Technical support in data organization and language editing of the final version of the article.

Jacenir Reis dos Santos Mallet: Research supervision, methodological guidance, critical review, and substantial contributions to the final version of the manuscript.