Scientific Article



Prevalence of anti-Toxoplasma gondii antibodies in quilombola communities of the Pajeú and Mata Meridional Pernambucana microregions, Brazil

Prevalência de anticorpos anti-Toxoplasma gondii em comunidades quilombolas da microrregião do Pajeú e Mata Meridional Pernambucana, Brasil

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Resumo

Abstract

Pernambuco has 195 quilombos, yet little is known about the epidemiology of human toxoplasmosis in these communities. This study aimed to determine the seroprevalence of Toxoplasma gondii in guilombola humans. Blood samples were collected from 82 individuals (28 men and 54 women) from eight quilombola communities located in the Pajeú and Mata Meridional Pernambucana microregions, Brazil. The samples were subjected to the Indirect Fluorescent Antibody Test (IFAT) to detect anti-Toxoplasma gondii IgG and IgM antibodies, using cutoff point of 1:16. To elaborate the geographic distribution of the seroprevalence, the communities' coordinates were obtained using GPS equipment. A seroprevalence of 54.9% (45/82) was observed among guilombolas in Pernambuco state. Notable, 62.5% (51/82) of the communities had guilombolas testing reagent for anti-T. gondii IgM. Studying the seroprevalence of *T. gondii* infection in guilombolas provides essential insights into the epidemiology of toxoplasmosis in these communities. The findings can be used for planning disease control strategies, the development of health services and technical assistance and rural extension services. Additionally, they support the creation of public health policies aimed at improving water access and implementing social technologies tailored to each community.

Pernambuco possui 195 quilombos e pouco se sabe sobre a epidemiologia da toxoplasmose humana nessas comunidades. O objetivo foi determinar a soroprevalência de *Toxoplasma gondii* em seres humanos quilombolas. Foram coletadas amostras de sangue 82 indivíduos (28 homens e 54 mulheres), procedentes de oito comunidades quilombolas distribuídas na microrregião do Pajeú e Mata Meridional Pernambucana, Brasil. As amostras obtidas foram submetidas à Reação de Imunofluorescência Indireta (RIFI) para detectar anticorpos IgG e IgM anti*Toxoplasma gondii*, considerando ponto de corte 1:16. Para elaborar a distribuição geográfica da soroprevalência, foram obtidas coordenadas geográficas das comunidades com equipamentos de GPS. Foi observada uma

soroprevalência de 54,9% (45/82) em quilombolas no estado de Pernambuco. É importante destacar que 62,5% (51/82) das comunidades tinham quilombolas reagentes para IgM anti-*T. gondii*. O estudo da soroprevalência da infecção por *T. gondii* em quilombolas permitirá compreender a epidemiologia da toxoplasmose nessas comunidades. Os resultados podem orientar estratégias de controle da doença, o desenvolvimento de serviços de saúde e a implementação de programas de assistência técnica e extensão rural, além disso apoiam a criação de políticas de saúde pública voltadas para a melhoria do acesso à água e a implementação de tecnologias sociais adaptadas a cada comunidade.

Pavaras-chave: Epidemiologia; Vigilância; Comunidades Tradicionais; Sorologia; Saúde Única.

1 | Introduction

Quilombos are historically recognized for their history of resistance and struggle against slavery and the slave system that placed black people in a condition of subjugation (BRASIL, 2017). Health for quilombolas is seen in an integral way, in its entirety, as in the case of healthy diet, and the relationship between health, work and nature (Gomes et al., 2021a). Thus, the understanding of health among quilombolas is related not only to the biological dimension, but also incorporates social. psychological, political and contextual issues. The lack of access to public policies and resources such as water directly affects the health of guilombola communities, impacting their lifestyle, traditional economic, cultural, and social activities, and the health of the population (Neves-Silva et al., 2024).

The population of quilombola communities of Pernambuco, faces issues characterized by constant social vulnerability, resulting in frequent violations of their rights to access health care, such as the shortage of human and structural resources in the health units (Matias and Cunha, 2024). In this environment of unfavorable socioeconomic conditions, and lack of access to basic health and sanitation services, observed in quilombola communities, diseases caused by helminths and protozoa become especially relevant (Santos et al., 2024).

Approximately one-third of the world's population may have already been infected by *T. gondii*, an obligate intracellular protozoan that presents three distinct infective morphological stages: tachyzoites, bradyzoites, and oocysts (Dubey et al., 1998). The prevalence of infection by this pathogen varies from 1% to 78%, and these rates are even higher in Latin American countries (Pappas et al., 2009). In 2018, the largest outbreak of human toxoplasmosis ever described in the literature was reported in the municipality of Santa Maria, Rio Grande do Sul State, Brazil (Fernandes et al., 2023). The occurrence of this outbreak justifies the need to

increase the monitoring of toxoplasmosis, especially in vulnerable situations, such as quilombola communities. Most outbreaks were due to contamination of water, fruits, and vegetables by oocysts (Balbino et al., 2021).

Environmental contamination by T. gondii oocysts in rural areas and urban environments has already been reported as a real risk for infection of animals and humans. The permanence of oocysts is linked to the environmental conditions necessary for them to establish themselves in a new population and thus cause an outbreak, or even an epidemic (Shakibaa et al., 2021). Being able to persist in adverse environments, oocysts remain in dry seasons and dissipate through water runoff when the rains arrive (Dubey, 1998; Dumètre et al., 2013). In Pernambuco, the climate is tropical with two welldefined seasons: a rainy season, from January to August, and a dry season for the rest of the year, with an average temperature of 25°C and a relative humidity of 81.5% (APAC, 2023). In a study conducted by Oliveira et al. (2021) in Recife, the capital of the state of Pernambuco, the potential of soil in public environments as a source of *T. gondii* infection was demonstrated through the identification of protozoa DNA in soil samples.

Individuals residing in rural areas may face increased exposure to T. gondii due to several factors, including vulnerable living conditions, a diverse range of domestic and wild intermediate hosts, an unspayed or unneutered cat population, and limited access to healthcare (Mahdy et al., 2017; Araújo et al., 2018; Panazzolo et al., 2023). Doline et al. (2023) evaluated the prevalence of anti-T. gondii antibodies in dogs and indigenous people in Paraná and reported a prevalence of 38.0% (97/253) and 49.0% (225/463), respectively, in addition to describing the water source as a risk for human and canine toxoplasmosis in the sample universe studied. In addition to water, contaminated food is a source of infection as reported by Panazzolo et al. (2023) who, when studying the risk factors associated with T.

gondii infection in a quilombola community in Paraná, reported that the consumption of raw/undercooked game meat by quilombola individuals may have contributed to greater exposure. In a seroepidemiological survey carried out in the remaining Kalunga quilombo community, in the cerrado biome, an occurrence of 8.93% (137/1533) of anti-*T. gondii* antibodies was reported in cattle (Gomes et al., 2021b).

In the researched literature, no studies were found regarding the seroprevalence of T. gondii infection in quilombolas in Pernambuco, Brazil. As such, there is a need for more specific studies, since remaining quilombolas may be considered as a group of greater socioeconomic vulnerability than others located in rural areas (Campos, 2008). Highlighting the significance of research in this area, which supports the development of health prevention programs, it is evident that countries with congenital toxoplasmosis prevention programs exhibit a low prevalence of the disease, underscoring the critical role of prevention (Lopes-Mori et al., 2011). In France, the introduction of a primary prevention program was associated with a decrease in toxoplasmosis prevalence among pregnant women, from 84% in 1960 to 54% in 1995, and further to 44% in 2003 (Lopes-Mori et al., 2011). The present study aimed to determine the seroprevalence of Toxoplasma gondii in quilombola communities in the state of Pernambuco, Brazil.

2 | Materials and Method

2.1 | Sampling

The data collection was conducted across eight distinct communities within the state of Pernambuco, located in the northeast region of Brazil, which covers an area of 98,312 km² and is situated between the latitudinal coordinates 7° 15' 45" N and 9° 28' 18" S, and the longitudinal coordinates 34° 48' 35" E and 41° 19' 54" W (Alvares et al., 2013). The sampling of community residents was based on individual interest, which emerged following discussions about toxoplasmosis, preventive measures, and the significance of serological testing for anti-T. gondii antibodies. Three of these guilombola communities are located in the city of Carnaíba (Abelhas, Travessão do Caroá, and Brejinho de Dentro), one in Iguaracy (Varzinha dos Quilombolas), one in Flores (Cavalhada), and one in Triunfo (Águas Claras), all of which belong to the Pajeú microregion of Pernambuco. Additionally, two communities in the city of Rio Formoso (Engenho Siqueira and Demanda) are included, which are part of the Mata Meridional Pernambucana microregion.

Blood samples were collected from 82 participants (ageing between 40 and 60 years). The collections were performed by a nursing technician and a nurse after explaining the reason and purpose of the samples and requesting the authorization of each participant by signing the informed consent form. Approximately five mL of blood was collected by puncture of the brachial vein using disposable needles and syringes, then transferred to tubes with clot separator gel (BD Vacutainer[®], USA). The samples were kept at room temperature (25°C) until visible clot retraction.

The biological material was identified and sent in isothermal boxes, containing recyclable ice, to the Infectious Diseases Laboratory of the Federal Rural University of Pernambuco for processing. To obtain the blood serum, properly labeled tubes were centrifuged for five minutes at 1500×g. Two aliquots of serum were taken from each sample, which were identified and stored at -20°C in 1.5mL polypropylene tubes, until the time of serological analysis for detection of IgG and IgM anti-*T. gondii*.

2.2 | Serological diagnosis

Serum samples were evaluated by the indirect fluorescent antibody test (IFAT). Initially, the slides were sensitized with suspensions of tachyzoites of T. gondii, strain ME49 (107 tachyzoites/mL), distributed on glass slides (10 µL/ well), dried at room temperature and then fixed with refrigerated acetone for 30 minutes. Sera, as well as, known positive and negative controls, were diluted in Phosphate-Buffered Saline -PBS (NaCl 8g, KCl 0.2g, Na2HPO4 1.44g and KH2PO4 0.24g in 1000mL of sterile distilled water, 7.2 pH) (Carmichael, 1975) to the cutoff point of 1:16 (Jones and Dubey, 2010). Slides were then incubated in approximately 80% humidity at 37°C for 30 minutes, then washed with PBS. The slides were then incubated with a rabbit Anti-Human IgG and IgM antibody - FITC (Sigma-Aldrich, St. Louis, MO) diluted in PBS with 0.02% Evans blue, with conditions similar to those of the first reaction. Slides were evaluated using solution of glycerol + PBS in a 50:50 ratio and visualized in an epifluorescence microscope (OLYMPUS BX60-FLA, USA). Samples were considered positive when whole peripheral surface fluorescence showed at least 50% of the tachyzoites in the well (Jones and Dubey, 2010). Positive and negative controls were provided by the Central Public Health Laboratory of Pernambuco (LACEN/PE).

2.3 | Statistical analysis

The research was carried out through descriptive analysis in eight quilombola communities in Pernambuco - Brazil. For the calculation of the sample, a prevalence of 74.7% (Porto et al., 2008) for infection by *T. gondii* in humans was considered, with a confidence level of 95% and error of 10%, which determined a minimum "n" sample of 73 quilombolas. The EpiInfo 3.5.2 program was used to perform statistical calculations.

2.4 | Spatial analysis of the occurrence of *T. gondii*

To elaborate the figures with the seroprevalence of *T. gondii* infection (positive and negative), geographic coordinates of the quilombos analyzed were collected with the aid of a GPS (Global Position System) equipment. The georeferenced data was launched in the QGIS application.

3 | Results and Discussion

This study determined the seroprevalence of *T. gondii* infection in quilombola communities, being important for the residents of these communities to be able to know the epidemiological status and to seek improvements for the health, production and hygiene conditions that interfere in the occurrence and control of this illness.

study Through this we detected а seroprevalence of IgG antibodies to T. gondii of 54.9% (45/82) in guilombola communities in the state of Pernambuco. In the Pajeú microregion quilombola communities presented the following seroprevalences, 80% (4/5) in the guilombola community of Abelhas, 60% (3/5) in Brejo de Dentro, 16.7% (1/6) in Travessão do Caroá, 30% (3/10) in Varzinha dos Quilombos, 37,5% (3/8) in Cavalhada, and 47.6% (10/21) in Águas Claras 47.6% In the Mata Meridional Pernambucana microregion, the seroprevalence of 70% (14/20) was observed in Povoado Demanda, and a IgM seroprevalence of 28.6% (2/7) of antibodies anti-T. gondii was obtained in Engenho Sigueira (Table 1, Figure 1). Also, residents reported the presence of domestic cats in these two communities. It is noteworthy that 62.5% (5/8) of the communities had guilombolas positive for IgM anti-T. gondii antibodies, and a total seroprevalence of IgM antibodies to T. gondii of 9.76% (8/82) (Table 1) was obtained in our study, indicating the occurrence of recent infection at the time of the survey, of which 87.5% (7/8) were simultaneously seropositive for both IgG and IgM (Table 2).

Communities	Microregion	Ν	lgG		lgM	
			Positive	Negative	Positive	Negative
Abelhas	Pajeú	5	4 (80.0%)	1 (20.0%)	1 (20.0%)	4 (80.0%)
Águas Claras	Pajeú	21	10 (47.6%)	11 (52.4%)	3 (14.3%)	18 (85.7%)
Brejo de Dentro	Pajeú	5	3 (60.0%)	2 (40.0%)	0%	100%
Cavalhada	Pajeú	8	3 (37.5%)	5 (62.5%)	1 (12.5%)	7 (87.5%)
Travessão do Caroá	Pajeú	6	1 (16.7%)	5 (83.3%)	0%	100%
Varzinha dos Quilombos	Pajeú	10	3 (30.0%)	7 (70.0%)	1 (10.0%)	9 (90.0%)
Engenho Siqueira	Mata Meridional Pernambucana	7	0%	100%	2 (28.6%)	5 (71.4%)
Povoado Demanda	Mata Meridional Pernambucana	20	14 (70.0%)	6 (30.0%)	0%	100%
Total		82	45 (54.9%)	37 (45.1%)	8 (9.8%)	74 (90.2%)

 Table 1. Seroprevalence of IgG and IgM anti-Toxoplasma gondii antibodies in quilombola communities in the Pajeú

 microregion and southern Pernambuco Forest, Brazil

N: number of samples

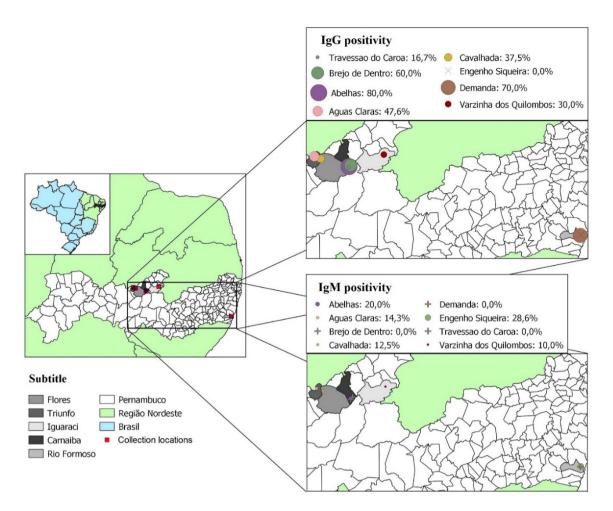


Figure 1. Distribution of the seroprevalence of Toxoplasma gondii infection in quilombola communities in Pernambuco, Brazil.

Table 2. Serological profile of IgM and IgG antibodies to *Toxoplasma gondii* in individuals from quilombola communities in the state of Pernambuco, Brazil

Serology	IgG			
lgM	Positive	Negative		
Positive	87.5% (7/8)	12.5% (1/8)		
Negative	51.4% (38/74)	48.6% (36/74)		

The studied communities are located in rural areas, near mountains and forests, being part of the Atlantic Forest biome in the case of communities located in the microregion of southern Pernambuco's Atlantic Forest, and the Caatinga biome in the case of communities located in the rural area of the Pajeú microregion). In these areas, the presence of wild felids (Prist et al., 2020), especially ocelots (Leopardus pardalis) and pumas (Puma concolor), is widely reported by the residents of these communities.

Besides the wild felids, it is common to find domestic cats (Felis silvestris catus), which means that they have access to the external and internal environment of the household, being free to hunt small animals. This behavior is a factor that contributes to the spread of *T. gondii*, because when consuming prey with tissue cysts, they can start releasing infectious oocysts to the environment through their feces (Jones and Dubey, 2010).

Faced with the semi-domiciled breeding of domestic cats, the risk of infection also presents itself through water contamination. As in most rural parts of Brazil, quilombola communities lack basic sanitation and access to piped water, which is a risk factor (Walker et al., 1997).

The stored water can be contaminated by cat feces or sporulated oocysts present in the soil and, during rainwater harvesting, either on roofs or sidewalk-type cisterns, these contaminants can be carried into the reservoirs. During dry periods, it is common to purchase water trucks, which are a risk factor for infections by *T. gondii* (Martinez et al., 2020). Although chlorination is a common practice in communities, it is known to be inefficient to control infectious oocysts (Martinez et al., 2020).

Besides the cisterns, which are common in the Pajeú microregion, water is collected through artesian wells, rivers, dams and springs (cacimbas), which are very common in the Zona da Mata. It is known that the sporulated oocyst of *T. gondii* can remain for long periods in the soil or in water (Martinez et al., 2020), offering a risk of infection to quilombolas who consume contaminated water and foods irrigated with it. The Povoado Demanda, located in Rio Formoso, has most of the water coming from wells or springs and presented a 70% seroprevalence for toxoplasmosis in this study, which may be related to this type of water source.

Regarding the higher seroprevalence of toxoplasmosis obtained in this study, it can be said that it comes from the Pajeú microregion, reaching 80% positivity for IgG and 20% for IgM in Abelha, a quilombo located in the municipality of Carnaíba. In the present study, the seroprevalence for IgM anti-*T. gondii* was 9.76%.

With the exception of Engenho Siqueira and Povoado Demanda (located near the urban area of Rio Formoso-PE), the other communities are located in rural areas far from urban ones (especially Abelha) (Figure 1), making access to public health services difficult, including visits from Community Family Health Agents. It is also observed that although Engenho Siqueira and Povoado Demanda are located close to the urban area, there is little access to the health network and there is no preparation of health teams to work with specific variables of a traditional people.

In the communities studied, Quilombola Health Services were not observed. The establishment of a Quilombola Health policy, respecting the specificities of each quilombola community, could contribute to the reduction of the seroprevalence of toxoplasmosis in this population, generating work and empowering the inhabitants, since they would be agents of change in their communities. Furthermore, because they know their culture and territory well, it would be possible to access the most distant homes, respecting more than anyone else, the culture and traditional knowledge of their people (BRASIL, 2003). From the creation of this public health service, it would enable the population to obtain information about personal hygiene, care with food, filtering water for family consumption and prenatal care.

The creation of specific technical assistance and water and sanitation access policies for quilombolas (BRASIL, 2010), could assist in the control of this and other pathologies based on the construction of knowledge about care in animal husbandry, care in slaughter, storage and preparation of their animals food, as well as care in capturing water, storing and maximizing its use, always providing quality supplies for families and for their agricultural production.

This is the first study that determined the seroprevalence of *T. gondii* infection in remaining quilombola people, which enabled a better understanding of the epidemiology of toxoplasmosis in quilombola territories in the state of Pernambuco, indicating a seroprevalence of 54.9%.

The results can be used to support the planning of disease control actions, the creation of quilombola health services, as well as Technical Assistance and Rural Extension, which take into account the popular and cultural knowledge of these traditional people.

4 | Conflict of Interest Statement

The authors have no relevant financial or non-financial interests to disclose.

5 | Ethics Committee

This research was submitted and approved by the National Research Ethics Commission (CONEP), being approved under CAAE 89887918.5.0000.5207. In addition, this project proposal was presented to community leaders and quilombola associations in each of the studied territories. Each participant signed the Free and Informed Consent Form (TCLE).

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