

Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection in People Living with HIV: A Cross-Sectional Study from Maputo Central Hospital, Mozambique

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Background: This study aimed to determine the seroprevalence of toxoplasmosis in people living with HIV (PLWH) in Maputo, Mozambique, exploring the interactions between HIV/acquired immunodeficiency syndrome (AIDS) and toxoplasmosis, including HIV-related factors such as the World Health Organization (WHO) HIV/AIDS clinical stage, degree of immunosuppression based on CD4⁺ T-cell count, and associated risk factors. Additionally, it aimed to assess the prevalence of neurological and psychiatric disorders (NPD) among study participants and its possible association with toxoplasmosis seropositivity.

Methods: We conducted a descriptive, cross-sectional study of 200 patients aged >18 years who were admitted to Maputo Central Hospital, Maputo, Mozambique, between March 2020 and October 2021. The participants were recruited by convenience, regardless of the reason for their admission. Sociodemographic and clinical data, such as age, sex, WHO HIV/AIDS stage, and CD4⁺ T-cell count, were collected. NPD disorders were assessed using the International Classification of Diseases criteria. Venous blood (5 mL) was obtained from each participant to determine anti-*Toxoplasma gondii* IgM and IgG antibodies using commercial enzyme-linked immunosorbent assay.

Results: Participants were aged 18–72 years, with the majority being female (64%) and unemployed (57%). Overall, 54.5% of patients tested positive for at least one anti-*Toxoplasma gondii* IgG (52%) or IgM (6.5%). Risk factors for *Toxoplasma gondii* infection ($p < 0.05$) were associated with age group 18–28 years, being male and unemployed. Moreover, 68.5% of the participants had NPD and of those, 65.1% exhibited anti-*Toxoplasma* antibodies. We found a significant association between anxiety and IgM seropositivity for $p = 0.016$. Though three out of four participants with positive anti-*Toxoplasma gondii* IgG had mood disorders, no significant association was found between *Toxoplasma gondii* infection with mood disorders, nor with other NPD assessed (56% depression, 33% motor disorder, 25.5% psychosis, 17% cognitive impairment, 7.5% mental retardation).

Conclusion: Toxoplasmosis may contribute to NPD in PLWH patients. Further studies are recommended to better understand the complex interactions between *Toxoplasma gondii*, NPD disorders, and HIV.

Plain Language Summary: Toxoplasmosis is a disease caused by the zoonotic and food-borne parasite, *Toxoplasma gondii*. It predominantly manifests in immunocompromised individuals, such as those living with human immunodeficiency virus (PWH), causing neurological and psychiatric impairments due to brain infections. The few existing studies in Mozambique on the burden of neurological and psychiatric disorders have not yet assessed the profile of neurological and psychiatric disorders in patients with HIV or the possible role that this parasite might play as a causative agent. The present study revealed that at least 54.5% of PWH were seropositive for *Toxoplasma gondii* antibodies and 68.5% had neurological and psychiatric disorders. Additionally, the majority (65.1%) of the patients with neurological and psychiatric disorders were seropositive for *Toxoplasma gondii* antibodies. Our findings highlight the need to screen for this parasite to clarify its role in the etiology of neurological and psychiatric disorders in both PWH and people without HIV for better healthcare delivery and management.

Keywords: latent and acute toxoplasmosis, co-infection *Toxoplasma gondii* and HIV, *Toxoplasma gondii* IgG, *Toxoplasma gondii* IgM, toxoplasmosis and neurological and psychiatric disorders, Mozambique

Background

Toxoplasmosis is caused by *Toxoplasma gondii* (*T. gondii*), an intracellular water and foodborne parasite that infects organs, including the muscle and brain, of a wide range of mammals by different routes of transmission and by more than one infective stage of the parasite.¹ Cats are definitive hosts for the sexual phase of the parasite and infectious oocysts are excreted into the environment. *T. gondii* is also an opportunistic parasite that can cause disease in individuals with compromised immune systems, as is the case in people infected with human immunodeficiency virus (HIV). In humans, infection can occur (i) after ingestion of oocysts from water, food, or soil contaminated with feline feces; (ii) from cysts containing bradyzoites through organ transplantation or ingestion of raw meat (muscle); and (iii) tachyzoites through blood transfusion or by vertical transmission from the mother to the fetus.^{2–4} Worldwide, nearly one-third of the general population is infected with this parasite, although 80% remain asymptomatic.⁵ The prevalence of infection varies according to region, culture, hygiene, and dietary habits.⁶ In low-income, middle-income, and high-income countries, the prevalence of HIV-*T. gondii* coinfection ranges from 44.9% to 55%, 34% to 35.8%, and 26%, respectively.⁷

It is well documented that *T. gondii* infection can persist for years (latent toxoplasmosis) in immunocompetent individuals, as defined by the presence of *Toxoplasma* IgG antibodies in the absence of any signs or symptoms of the disease. However, latent infection has also been linked to a variety of neurological and psychiatric disorders, including depression, cognitive impairment, anxiety, epileptic seizures, and schizophrenia in addition to attention-deficit hyperactivity disorder, which is common in children and adolescents.^{5,8–10} In people living with HIV (PWH) and advanced immunosuppression, or individuals undergoing immunosuppressive therapy for other disorders, *T. gondii* infection reactivation or acute infection may trigger an acute disease (*Toxoplasma gondii* encephalitis) associated with high anti-*Toxoplasma gondii* antibody levels and focal encephalitis syndrome with headache, confusion, motor weakness, and fever.^{3,11} According to Azovtseva et al,¹⁰ in PWH the reactivation of bradyzoites in latent toxoplasmosis can occur when the CD4⁺ T cell count drops below 200 cells/μL. *Toxoplasma* encephalitis or acute toxoplasmosis is often used as a diagnostic criterion for HIV and acquired immune deficiency syndrome (AIDS) in countries with high HIV burden, limited diagnostic resources, and reduced access to antiretroviral therapy (ART).^{11–13} However, neurological and psychiatric disorders such as depression, aggression, psychosis, epileptic seizures, delirium, memory loss, dementia, mood disorders, cognitive impairment, schizophrenia, and anxiety in PWH are highly prevalent,¹⁴ and may occur as a primary manifestation of HIV and immunosuppression.^{15–17} These conditions may result from a variety of HIV-related factors, including direct effects of HIV replication in brain immune cells, adverse effects of medications, or comorbidities such as toxoplasmosis and several other infectious diseases.^{11,15,18,19}

Multiple tools are available to aid in the diagnosis of toxoplasmosis, which include a combination of clinical findings, serology, molecular techniques and neuro-radiographic imaging.⁶

Studies on the interactions between *T. gondii* infection and HIV and *T. gondii* infection and neurological and psychiatric disorders are scarce, especially in sub-Saharan Africa and Mozambique. A few studies published in Mozambique used serologic testing or minimally invasive autopsies and found that the prevalence of toxoplasmosis in

PWH and without HIV varied from 5% to 46%^{20–22} and up to 10.9%, respectively.^{6,20} However, despite that neurological and psychiatric disorders are highly prevalent in Mozambique, to our knowledge there are no studies reporting the relationship between *Toxoplasma gondii* infection and any neurological and/or psychiatric disorder, in both HIV infected and non-HIV infected patients, raising the possibility that *T. gondii* may be implicated in the etiology of these disorders.^{14,23–25}

As Mozambique has one of the highest prevalence rates of HIV (12.1%) among the adult population in the region^{26,27} with approximately 2.2 million PWH, of whom only 1.8 million (82%) received ART in 2022,^{28,29} there are rising concerns about the possibility of cases of latent toxoplasmosis that suddenly reactivates and becomes acute with negative outcomes for PWH. Thus, we propose that the prevalence of *T. gondii* is high among PWH and that neurological and psychiatric disorders occurring in some PWH might be related to previously unrecognized *T. gondii* infections.

Therefore, this is the first study to be carried out in Mozambique to determine the baseline seroprevalence of anti-*T. gondii* antibodies among PWH hospitalized at Maputo Central Hospital, and to assess the association between seropositivity to *T. gondii* infection and HIV-related factors, including the degree of immunosuppression assessed by the CD4⁺ T cell count, World Health Organization (WHO) HIV/AIDS clinical stage, and risk factors for *T. gondii* infection. We also sought to assess the prevalence of neurological and psychiatric disorders among study participants and their possible association with *T. gondii* infection.

Materials and Methods

Study Setting, Design and Methods

This study was conducted in Maputo City, the capital of Mozambique, which has approximately 1.12 million inhabitants. Approximately half of the population resides in unplanned settlements characterized by high population density, poor sewage drainage, and inadequate access to safe drinking water.³⁰

This descriptive exploratory cross-sectional study was conducted between March 2020 and October 2021 at Maputo Central Hospital, the main referral hospital for Maputo City and across the country.³¹

Recruitment of Study Participants and Sample Size

We enrolled a convenience sample of 200 participants who were selected from among patients hospitalized in the Department of Medicine at Maputo Central Hospital. Participants were eligible if they met the following criteria: a) admitted to the medicine wards of the Maputo Central Hospital, b) at least 18 years of age or more, c) confirmed seropositive for HIV, and d) willing to participate in the study and provide informed consent. As the study took place during the peak of the SARS-COV-19 pandemic in Mozambique, the enrollment of participants was slowed owing to restrictions imposed on circulation within the hospital wards.

We estimated the sample size using the following formula: $n = Z^2 p(1 - p)/d^2$, where n is the sample size, Z is the critical value of the normal distribution for a confidence level of 95% (1.96), p is the expected seroprevalence of latent toxoplasmosis in PWH, and d is the error.³² Studies in Mozambique have reported the prevalence of latent toxoplasmosis among PWH to range from 5% to 46%, with an average of 26%.^{20–22} Based on this prevalence, we calculated a minimum sample size of 200 participants to achieve our study goals.

Prior to enrollment, two investigators (LM and ICR) explained to the potential participants the aims of the study and the methodology to be followed to obtain written informed consent from the study participants.

Data Collection

Sociodemographic data such as age, sex, education, employment, marital status, and pet ownership were collected. We also recorded HIV-related factors, such as WHO HIV/AIDS clinical stage (a tool commonly used in resource-limited settings, to provide prognostic insights into morbidity and mortality risks for PWH),³³ CD4⁺ T cells count, ART regimen, time on ART, and treatment with trimethoprim-sulfamethoxazole (TMP-SMX) used to prevent opportunistic diseases.

Assessment of Neurological and Psychiatric Disorders

Neurological and psychiatric disorders were assessed using the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and International Classification of Diseases, Tenth Revision (ICD-10). This was done by the research doctors, an internal medicine specialist (AC) and general physician (SJM).

Blood Sample Collection and Laboratory Processing

A 5 mL of venous blood samples were collected from each participant. Four mL of whole blood were drawn into a Gel⁺Clot Activator tube (Biota, Istanbul, Turkey), placed in a cool box on ice, and immediately transported to the Laboratory of Parasitology, Faculty of Medicine at Eduardo Mondlane University. The samples were then centrifuged at 3000 rpm for 10 min, and the serum was kept at -80°C until further use for the detection of anti-*Toxoplasma gondii* IgM and IgG.

Serological Screening for *Toxoplasma gondii* IgM and IgG Antibodies

To examine the presence of anti-*T. gondii* IgM and IgG antibodies, we used the commercial serological enzyme-linked immunosorbent assay (ELISA) Human TOXO IgG and Human TOXO IgM (Gesellschaft für Biochemical und diagnostical mbH, Wiesbaden, Germany) according to the manufacturer's instructions. The optical density (OD) was measured using a Microplate ELISA reader (Thermo Scientific Multiskan FC).

Data Analysis

We double-entered all data from the questionnaires and laboratory tests into a Microsoft Excel and exported them to SPSS version 29 for statistical analysis. Participants were stratified according to age (18–28, 29–38, 39–49, >50 years), CD4⁺ T-cell count (<200, 200–500, >500 cells/ μL), and WHO HIV clinical stage (I–IV).

Categorical variables were summarized as frequencies and percentages, while quantitative data were analyzed using mean and standard deviation (SD). We applied the chi-squared (χ^2) or Fisher's exact test to assess the associations between categorical variables. *T. gondii* seropositivity for IgM and/or IgG were used as the dependent variable.

Additionally, we conducted univariable and multivariable logistic regression analyses to examine the associations between independent variables (sociodemographic factors, clinical profile, and HIV-related factors) and *T. gondii* seropositivity. The results were presented as odds ratios (OR) with 95% confidence intervals (CI), considering a *p*-value <0.05, as statistically significant. To prevent overfitting in the multivariable logistic regression model, we limited the inclusion to 10 independent variables, based on their known or hypothesized associations with *T. gondii* seropositivity.³⁴

Results

Seroprevalence of *Toxoplasma gondii* According to Sociodemographic and HIV-Related-Factors

We included 200 participants in the study, ranging in age from 18 to 72 years old, with an average age of 41.5 years [Standard Deviation (SD) \pm 12.1].

Table 1 summarizes the overall seroprevalence of anti-*T. gondii* IgM and IgG according to the sociodemographic and HIV-related factors.

Overall, 64 (30%) of participants were in the aged group 29–38 years, 128 (64%) were female, 170 (85%) lived in urban area, 114 (57%) were unemployed. Concerning domestic animals, 120 (60%) of participants owned pets, of which 82 (41.0%) were cats. Furthermore, all participants referred to ingest raw food.

Regarding *T. gondii* serology, we found that out of 200 samples tested, 109 (54.5%) were positive to at least one anti-*T. gondii* antibody IgM or IgG; 13 (6.5%) had circulating IgM and 104 (52%) had circulating IgG. Higher seropositivity for anti-*T. gondii* antibodies were found in female 58 (55.8%) and in unemployed 12 (92.3%) participants for IgG and IgM respectively, and these differences were statistically significant ($p=0.01$), Table 1.

Concerning HIV-related factors, the study participants had CD4⁺ T cell counts average was 363.1 cells/ μL , ranging from 2 to 2297 cells/ μL (SD \pm 353.8); the majority, 81 (40.5%) had CD4⁺ T cell counts <200 cells/ μL and 138 (69%) were in WHO HIV/AIDS clinical stage IV. Furthermore, among those receiving ART 195 (97.5%), only 35 (17.9%) were

Table 1 *Toxoplasma gondii* Seroprevalence of by Sociodemographic and HIV-related Factors of the Study Participants

Variable	Category	N=200 (%)	Toxo-IgM ⁺ N=200 (%)	p-value	Toxo-IgG ⁺ N=200 (%)	p-value	Toxo IgG ⁺ or IgM ⁺ N=200 (%)	p-value
Age group in years	18-28	30 (15)	13 (6.5)	0.67	104 (52.0)	0.26	109 (54.5)	0.25
	29-38	64 (30)	3 (23.1)		20 (19.2)		21 (19.3)	
	39-49	58 (29)	3 (23.1)		32 (30.8)		33 (30.3)	
	> 50	48 (24)	4 (30.8)		26 (25.0)		28 (25.7)	
Sex	Female	128 (64)	3 (23.1)	0.38	58 (55.8)	0.01	62 (56.9)	0.02
	Male	72 (36)	10 (76.9)		46 (44.2)		47 (43.1)	
Marital status	Single	106 (53)	6 (46.2)	0.69	58 (55.8)	0.8	60 (55.0)	0.86
	Married	73 (36.5)	5 (38.5)		36 (34.6)		39 (35.8)	
	Divorced	5 (2.5)	0 (0.0)		2 (1.9)		2 (1.8)	
	Widowed	16 (8)	2 (15.4)		8 (7.7)		8 (7.4)	
Residence	Urban	170 (85)	11 (84.6)	0.70	86 (82.7)	0.34	91 (83.5)	0.51
	Rural	30 (15)	2 (15.4)		18 (17.3)		18 (16.5)	
Education	Illiterate	30 (15)	0 (0.0)	0.47	17 (16.4)	0.51	17 (15.6)	0.66
	Elementary school	85 (42.5)	6 (46.2)		48 (46.2)		50 (45.9)	
	Secondary school	73 (36.5)	6 (46.2)		33 (31.7)		36 (33.0)	
	High school	12 (6)	1 (7.7)		6 (5.8)		6 (5.5)	
Employment	Unemployed	114 (57)	12 (92.3)	0.01	60 (57.7)	0.84	64 (58.7)	0.59
	Employed	86 (43)	1 (7.7)		44 (42.3)		45 (41.3)	
Domestic animals	Yes	120 (60)	10 (76.9)	0.20	66 (63.5)	0.30	70 (64.2)	0.18
	No	80 (40)	3 (23.1)		38 (36.5)		39 (35.8)	
Source of drinking water	Cats	82 (41.0)	6 (54.5)	0.42	44 (62.9)	0.47	45 (60.8)	0.18
	Piped	120 (60)	8 (61.5)		76 (73.1)		78 (71.6)	
	Hole/River	80 (40)	5 (38.5)		28 (26.9)		31 (28.4)	
CD4⁺ T cells count	<200	81 (40.5)	3 (23.1)	0.38	42 (40.4)	0.32	47 (43.2)	0.41
	200-500	67 (33.5)	5 (38.5)		39 (37.5)		37 (33.9)	
	>500	52 (26.0)	5 (38.5)		23 (22.1)		25 (22.9)	
WHO HIV/AIDS stage	I	15 (7.5)	0 (0.0)	0.45	9 (8.7)	0.67	9 (8.2)	0.68
	II	32 (16.0)	3 (23.1)		14 (13.5)		15 (13.8)	
	III	15 (7.5)	0 (0.0)		7 (6.70)		7 (6.4)	
	IV	138 (69.0)	10 (76.9)		74 (71.20)		78 (71.6)	
ART intake	Yes	195 (97.5)	12 (92.3)	0.22	100 (96.2)	0.20	105 (96.3)	0.25
	No	5 (2.5)	1 (7.7)		4 (3.8)		4 (3.7)	
TMP-SMX prophylaxis	Yes	65 (32.5)	3 (23.1)	0.45	33 (31.7)	0.06	33 (30.3)	0.46
	No	135 (67.5)	10 (76.9)		71 (68.3)		76 (69.7)	

Abbreviations: N (%), number and percentage of participants; n, Toxo, *T. gondii*; IgM, Immunoglobulin M; IgG, Immunoglobulin G; HIV, human immunodeficiency virus; WHO, World Health Organization; AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; TMP-SMX, trimethoprim/sulfamethoxazole.

on ART less than six months and none of them tested positive for IgM. No other statistically significant associations were found regarding other sociodemographic variable or HIV-related factors, ART and TMP-SMX prophylaxis with anti-*T. gondii* antibodies seropositivity either for IgM, IgG or for both antibodies.

Toxoplasma gondii Serology and Neurological and Psychiatric Disorders

Table 2 summarizes *T. gondii* serology and neurological and psychiatric disorders assessed in the study participants. Overall, 137 (68.5%) patients had neurological and psychiatric disorders (an average of 1.46 diagnosis per patient) and of those 71 (65.1%) tested positive for at least one anti-*T. gondii* antibody. A statistically significant association was found between anxiety and anti-*T. gondii* IgM seropositivity for $p = 0.016$. Although not statistically significant, we found that three out of four patients with mood disorders, tested positive for anti-*T. gondii* IgG. No other associations were found between neurological and psychiatric disorders identified like depression (56%), motor disorders (33%), psychosis (25.5%), and cognitive impairments (17%), mental retardation (7.5%), seizures (1%) and seropositivity to any of anti-*Toxoplasma gondii* antibodies.

Table 2 *Toxoplasma gondii* Serology and Neurological and Psychiatric Disorders of the Study Participants

Variable	Category	N=200 (%)	Toxo-IgM ⁺ N (%)	p-value	Toxo-IgG ⁺ N (%)	p-value	Toxo IgG ⁺ or IgM ⁺ N (%)	p-value
Neurological and Psychiatric disorders		137 (68.5)	7 (5.1)	0.24	69 (50.4)	0.5	71 (65.1)	0.26
	Depression	112 (56.0)	7 (6.3)	0.87	54 (51.9)	0.23	56 (51.4)	0.15
	Motor disorder	66 (33.0)	3 (6.1)	0.9	34 (51.5)	0.92	36 (33.0)	0.99
	Psychosis	51 (25.5)	1 (7.7)	0.13	25 (49.0)	0.62	25 (22.9)	0.36
	Cognitive impairment	34 (17.0)	2 (5.9)	0.87	18 (52.9)	0.9	16 (14.7)	0.34
	Mental retardation	15 (7.5)	2 (13.3)	0.26	14 (46.7)	0.4	9 (8.3)	0.66
	Anxiety	7 (3.5)	2 (28.6)	0.016	3 (2.9%)	0.92	3 (2.8)	0.53
	Seizures	2 (1.0)	0 (0.0)	0.59	1 (14.3)	0.04	0 (0.0)	0.04
	Mood disorders	4 (2.0)	0 (0.0)	0.59	3 (2.9)	0.35	3 (2.8)	0.41

Abbreviations: N (%), number and percentage of participants; NPd, neurological and psychiatric disorders; Toxo, *T. gondii*; IgM, Immunoglobulin M; IgG, Immunoglobulin G.

Risk Factors Associated with Seropositivity to at Least One Anti-*Toxoplasma gondii* Antibody

Table 3 summarizes the risk factors associated with *T. gondii* infection in the study participants. In the multivariable analysis, males were nearly threefold more likely to be infected than females (OR = 2.99; 95% CI 1.46–6.15; $p = 0.01$).

Moreover, individuals aged 18–28 years old were more likely to be infected compared to age groups 39–49 (OR: 0.34; 95% CI 0.12–0.96; $p = 0.04$) and above 50 years of age (OR: 0.34; 95% CI 0.12–1.01; $p = 0.05$).

Table 3 Univariable and Multivariable Logistic Regression Analyses of Risk Factors Associated with Seropositivity to at Least One Anti- *Toxoplasma gondii* Antibody

Variables	Univariable Analysis OR 95% CI	P-value	Multivariable Analysis OR 95% CI	p-value
Age (Years)				
18–28	Ref		Ref	
29–38	0.46 (0.18–1.15)	0.10	0.40 (0.14–1.0)	0.08
39–49	0.40 (0.16–1.02)	0.06	0.34 (0.12–0.96)	0.04
>50	0.55 (0.21–1.15)	0.23	0.34 (0.12–1.01)	0.05
Gender				
Female	Ref			
Male	2.0 (1.10–3.63)	0.02	2.99 (1.46–6.15)	< 0.01
Residence				
Urban	Ref		Ref	
Rural	1.39 (0.59–2.87)	0.51	1.61 (0.65 –3.96)	0.30
Education				
Illiterate	Ref		Ref	
Elementary school	1.09 (0.47–2.53)	0.27	1.32 (0.53–3.30)	0.55
Secondary school	0.74 (0.32 –1.75)	0.50	0.55 (0.21 –1.47)	0.24
High school	0.77 (0.20–2.93)	0.70	0.86 (0.20–3.75)	0.85
Employment				
Unemployed	Ref		Ref	
Employed	0.86 (0.49–1.50)	0.59	0.62 (0.31–1.23)	1.72
Pets				
Yes	1.47 (0.83–2.60)	0.18	1.46 (0.78–2.73)	0.24
No	Ref		Ref	
CD4⁺ T cells count				
<200	Ref		Ref	
200–500	1.25 (0.65–2.4)	0.51	1.38 (0.66–2.90)	0.39
>500	0.78 (0.39–1.57)	0.48	0.59 (0.27–1.28)	0.18

(Continued)

Table 3 (Continued).

Variables	Univariable Analysis OR 95% CI	P-value	Multivariable Analysis OR 95% CI	p-value
WHO HIV/AIDS stage				
I	Ref		Ref	
II	0.59 (0.17–2.04)	0.40	0.48 (0.12–1.91)	0.30
III	0.58 (0.14–2.48)	0.47	0.44 (0.09–2.20)	0.32
IV	0.87 (0.29–2.57)	0.80	0.73 (0.22–2.47)	0.62
ART intake				
Yes	0.29 (0.03–2.66)		0.37 (0.03–4.07)	
No	Ref	0.27	Ref	0.42
TMP-SMX prophylaxis				
Yes	0.80 (0.44–1.45)		0.79 (0.41–1.44)	
No	Ref	0.46	Ref	0.49

Abbreviations: Ref, Reference category; OR, Odds Ratio; CI, confidence interval; ART, Antiretroviral therapy. WHO, World Health Organization. HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; TMP-SMX, trimethoprim-sulfamethoxazole.

Discussion

Our results indicated that anti-*T. gondii* antibodies IgM and IgG were present in 54.5% of the study participants, with 52% testing positive for IgG and 6.5% for IgM. Male sex, younger age (18–28 years), and unemployment have emerged as the key risk factors for *T. gondii* infection. Moreover, we found that among the study participants the prevalence of neurological and psychiatric disorders was 68.5% and of those 65.1% exhibited at least one anti-*T. gondii* IgM or IgG antibodies. Significant association was observed between anxiety and IgM seropositivity for $p = 0.016$, although other neurological and psychiatric disorders presented no significant association with *T. gondii* infection.

These findings suggest that *T. gondii* infection is highly prevalent in our study population, as are the neurological and psychiatric disorders regardless of seropositivity to toxoplasmosis.

Similar to other studies done in Mozambique in HIV-infected pregnant women (31.3%)²⁰ and HIV adult patients (46%),^{20,21} our study found also higher seroprevalence of toxoplasmosis antibodies. These findings are consistent with studies done in other settings like Ghana (57.6%),³⁵ Ethiopia (87.4%)³⁶ Marroccos (62.1%),³⁷ Brazil (80%),³⁸ and Iran (45.9%)³⁹ some of them reporting even higher prevalences, but lower than those found in Japan (8.3%)⁴⁰ and Singapore (23.7%).⁴¹ These global disparities likely result from a combination of local epidemiology factors which includes environmental exposures, dietary practices, and regional variations in public health interventions.^{34,42}

Although the IgM rate found in our study is comparable to the ones reported in South Africa (9.8%),⁴³ Ethiopia (10.7%)³⁶ it was notably higher than the one observed in countries like Iran 2.6%³⁹ and China (1.2%).⁴⁴

A serological survey performed on inhabitants of the city of Telangana state (India) found that among PWH anti-*T. gondii* IgG seropositivity was more common in women (39%) than in men (29%).⁴⁵ In contrast, our study found that although women had a higher overall seroprevalence of anti-*T. gondii*, the risk of testing positive for acute or latent toxoplasmosis was two to three-fold higher in men than in women. This sex disparity could be influenced by biological differences, such as hormonal and genetic factors, including those linked to sex chromosomes that affect the immune response, as suggested by Gay et al.⁴⁶ Additionally, lifestyle factors, occupational exposure, comorbidities, and social inequalities may contribute to an elevated risk of infection in men.^{6,42,46}

In our study, we observed a notably high prevalence of acute toxoplasmosis among PWH, particularly among unemployed participants, underscoring the complex interplay between socio-economic status and health risk. Studies have shown that poverty and financial hardship heightens the risk of contracting and spreading infectious diseases including HIV. Indeed a study from Brazil has identified unemployment as a significant risk factor for *T. gondii* seropositivity.⁴⁷

A previous study from northern South Africa by Ngobeni and Samie, 2017, reported that *T. gondii* IgG-positive serology was more frequent in PWH who were not receiving ART than in those who were taking ART in disagreement

with the findings of the current study.⁴⁸ However, most participants on ART in our study had low CD4⁺ T cell counts and were in WHO HIV clinical Stage IV, which may have resulted in a poor immune response against *T. gondii*. Thus, despite receiving ART, PWH in our study may have had latent toxoplasmosis or an increased risk of acute toxoplasmosis due to impaired immunocompetence. A meta-analysis performed by Yenilmez and Çetinkaya concluded that PWH with low CD4⁺ T cell counts have an increased epidemiological and immunological risk of acquiring toxoplasmosis.⁴⁹ None of 35 participants on ART less than six months had positive *T. gondii* serology for IgM. This might be in part because our study participants in general were not seriously immunocompromised as per their CD4⁺ cell count average.

Additionally, our findings suggest that younger age (18–28 years) is a significant risk factor for *T. gondii* infection among PWH. Several hypotheses may account for this finding, including hygiene practices, dietary habits, working in high-exposure environments such as food handling, agriculture activities and contact with the soil or cats.⁶ These considerations highlight the need for targeted prevention efforts in younger populations at risk.

Interestingly, our analysis only identified a significant association between anxiety and *T. gondii* IgM seropositivity, though this finding should be interpreted with caution, as factors such as HIV serostatus, associated comorbidities, and hospitalization could also contribute to anxiety.^{50–52}

No significant association was found between neurological and psychiatric disorders identified (68.5%) and *T. gondii* seropositivity (65.1%), despite prior research suggesting a connection.^{13,53,54} Nonetheless, among participants with mood disorders, 3 out of 4 had positive serology for anti-*T. gondii* IgG, all with CD4⁺ counts <200 cells/μL and WHO HIV clinical Stage IV. This highlights the need for studies using larger sample sizes, robust and sensitive tools to assess the potential causal relationship between mood disorders and other neurological and psychiatric disorders as found in many other studies.^{8,11–13,15} Additionally, this absence of a correlation may result from several factors, such as ART-related immune reconstitution, which could reduce *T. gondii* reactivation and the potential obscuring effects of HIV or medication side effects on neurological and psychiatric symptoms.

Reports from the Ministry of Health in Mozambique indicate that the most common reason for psychiatric consultations was epilepsy (57%), followed by psychosis (15%) and mood disorders (6%). A few studies in different settings in Mozambique found that depressive disorders (19%), schizophrenia (14%), psychosis (18.3%), chronic headache (49.5%), and epilepsy (27%) comprised the majority of case presentation.^{14,23–25} The varying severity and onset of neurological and psychiatric disorders assessed in our study may also complicate the detection of subtle or slow-acting effects of *T. gondii*, particularly in a cross-sectional design lacking long-term follow-up. Future studies with larger samples and longitudinal follow-up are needed to better explore these complex relationships and confirm our findings.

Strengthens and Limitations

The strengths of this study include its novelty as the first assessment of neurological and psychiatric disorders in PWH with positive serological results for *T. gondii* in Mozambique. This study provides updated baseline information on the seroprevalence of *T. gondii* among PWH, contributing crucial data to an under-researched area. Additionally, the study comprehensively examined various factors, such as immunosuppression, WHO HIV/AIDS clinical stage, ART intake and TMP-SMX prophylaxis, to enhance our understanding of disease dynamics. Furthermore, these findings have important public health implications, offering valuable insights for healthcare professionals and policymakers to improve prevention, detection and management of *T. gondii* infections and neurological and psychiatric disorders in both PWH and the general population.

However, some limitations should be noted. This study was conducted at a single urban referral hospital, limiting the generalizability of findings to rural or other non-referral healthcare settings. The cross-sectional design prevents causal inference. Diagnostic limitations included reliance on ELISA without confirmatory tests such as avidity assay or polymerase-chain reaction (PCR), which could have better distinguished acute from chronic infection. While our study was sufficiently powered to estimate seroprevalence and perform multivariable analysis, the relatively small sample may have limited our ability to detect associations with less frequent neuropsychiatric outcomes or interaction effects. Finally, the assessment tools used for neurological and psychiatric disorders may have led to an underestimation of their prevalence.

Conclusions

Our study revealed a high seroprevalence of *T. gondii* antibodies (54.5%) as well as high prevalence of neurological and psychiatric disorders (68.5%) among PWH in Mozambique. Nonetheless, the seroprevalence of *T. gondii* antibodies was only associated with younger age, unemployment and anxiety.

Given the high prevalence of *T. gondii* and its potential implications for health outcomes, it is essential to enhance awareness among healthcare providers and policy makers regarding prevention, diagnosis and management of both *T. gondii* infections, and neurological and psychiatric disorders in this vulnerable population. Further research, particularly longitudinal studies with larger sample sizes, is recommended to better understand the complex interactions between *T. gondii*, neurological and psychiatric disorders, and HIV infection.

Data Sharing Statement

All data generated during this study will be available from the corresponding author if needed.

Ethics Approval and Informed Consent

The study was approved by the National Bioethics Committee of Mozambique. Administrative approval was obtained from Maputo Central Hospital. Informed consent was obtained from all subjects involved in the study. All personal information from the research participants was confidentially maintained, and the principles of the Declaration of Helsinki were followed.

Consent for Publication

All authors have consented to the publication of this article.

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Author Contributions

Robert T. Schooley, Gabriela Maria Santos Gomes, Emília Virgínia Noormahomed, and Constance A. Benson contributed equally as senior mentors and senior authors. All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All the authors have read and approved the final version of the manuscript. In addition, RTS and EVN were responsible for funding acquisition.

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Disclosure

The authors report no conflicts of interest in this work.

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