

Serological Detection of Human Toxoplasmosis by using IgG and IgM Antibodies Among Suspected Individuals from Selected Regions of Islamabad, Pakistan

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ABSTRACT

Objective: To determine the prevalence rate of human toxoplasmosis using IgG and IgM antibodies to identify potential risk factors from selected regions of Pakistan.

Methodology: A total of 352 suspected individuals were included in the study, with clinical and demographic data collected retrospectively from medical records (2019–2023) and blood samples collected between January 2024 and December 2024 from Rawal General and Dental Hospital, Islamabad. Serological testing for *T. gondii* IgG and IgM antibodies was performed using chemiluminescent microparticle immunoassay (CMIA). The data were analyzed using SPSS, applying the chi-square test to determine associations, with a significance level of $p < 0.05$.

Results: This study discovered that the prevalence of Toxoplasma varied by age and gender, occurring in those aged ≤ 10 years and > 40 years at rates of 11.5% and 17.6%, respectively (p -value = 0.031). The prevalence of toxoplasmosis infection was higher in females (12.9%) than in males (7.8%) (p -value = 0.030).

Conclusion: Toxoplasmosis remains a significant public health concern in urban areas of Pakistan, particularly among vulnerable populations such as pregnant women and older adults. These findings highlight the need for routine screening and preventive measures. Further multicenter studies are recommended to validate these findings and identify additional risk factors like foodborne transmission, environmental exposure, cat contact, demographics, etc.

Keywords: Epidemiology, IgG, IgM, Seroprevalence, Toxoplasma gondii, Toxoplasmosis.

Authors' Contribution:

^{1,2}Conception; Literature research; manuscript design and drafting; ^{3,4}Critical analysis and manuscript review; ^{5,6}Data analysis; Manuscript Editing.

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Article info:

Received: February 19, 2026
Accepted: March 03, 2026

Cite this article. Bibi R, Zaidi SHA, Arif M, Bibi N, Ahmed T, Ashir A. Serological Detection of Human Toxoplasmosis by using IgG and IgM Antibodies Among Suspected Individuals from Selected Regions of Islamabad, Pakistan. J Islamabad Med Dental Coll. 2026; 15(1): 72-77.

DOI: <https://doi.org/10.35787/jimdc.v15i1.1557>

Funding Source: Nil

Conflict of interest: Nil

Introduction

Toxoplasmosis, caused by the protozoan parasite is a zoonotic disease that poses significant health risks globally, especially for pregnant women and immunocompromised individuals. The parasite is primarily transmitted through the exposure to infected cat faeces, consumption of undercooked

meat, drinking contaminated water or through congenital transmission from mother to child during pregnancy.¹ The disease often presents as asymptomatic or with mild flu-like symptoms in immune-competent individuals, but it can cause severe complications including congenital defects, miscarriage, encephalitis; particularly those with

HIV/AIDS.^{2,3} Detection of toxoplasmosis typically involves serological tests that identify the presence of *T. gondii* specific antibodies, primarily IgG and IgM, which indicate past or recent infection respectively.⁴ Persons living in low socioeconomic conditions are more prone to infection and delay in diagnosis results in complications and even worse outcome. Early diagnosis of women especially pregnant is necessary to treat ocular lesions in infants. If delayed, then the quality of life and behavioural changes results.¹⁵

The disease burden of toxoplasmosis is high in developing countries, where risk factors such as close contact with cats, consumption of undercooked meat, and limited access to sanitation exacerbate its prevalence.^{5,6} In Pakistan, toxoplasmosis remains a neglected disease¹⁶ despite its potential for causing significant public health issues, especially among women of childbearing age and causing behavioural changes in children like impaired judgment, delirium, autism¹ and psychosis if left untreated. In infected children it causes retarded cognition¹⁵.

In recent years, the serological detection of IgG and IgM antibodies has been widely adopted for diagnosing *T. gondii* infections. IgG antibodies are typically associated with past infections more than 2-3 months, while IgM antibodies are a marker of recent infection within 2-3 months, which is crucial for diagnosing acute cases and preventing vertical transmission during pregnancy.⁷ ELISA (IgG and IgM) is preliminary test and ELISA positive individuals should be screened by more specific tests (IgG avidity test PCR testing) to rule out other infections.⁴

The detection of *T. gondii* antibodies is particularly important in populations at high risk, including pregnant women and individuals with compromised immune systems. Recent studies have highlighted the significance of screening for toxoplasmosis in pregnant women, as the infection can result in severe outcomes, including miscarriage, stillbirth, and decreased cognition in children with congenital

abnormalities¹. There is need to raise awareness among general population to adopt preventive measures and health authorities to implement routine screening of *T. Gondii* for vulnerable population especially pregnant to reduce the risk of vertical transmission.³

The **objective and rationale** for conducting this study is to assess the seroprevalence of *T. gondii* IgG and IgM antibodies among individuals suspected of having toxoplasmosis in selected areas of Islamabad and Rawalpindi Pakistan. By determining the extent of exposure, identifying demographic areas and risk factors, this research aims to provide valuable insights into the epidemiology of toxoplasmosis in Pakistan. Additionally, understanding the prevalence in Islamabad and Rawalpindi will help to design effective prevention and control strategies, particularly in vulnerable populations.⁹

Methodology

This cross-sectional study was conducted to investigate the prevalence of *Toxoplasma gondii* infections among patients visiting Rawal General and Dental Hospital from selected areas of Rawalpindi and Islamabad over a study period lasting from January 3rd, 2024, to December 31st, 2024. The geographical focal points, Rawalpindi (GPS coordinates 33° 37' 33.8052" N, 73° 4' 17.1912" E) and Islamabad (33° 44' 16.9620" N, 73° 5' 4.1568" E), were mapped using ArcGIS software and were chosen due to their mixed urban and rural population, which presents a high likelihood of exposure to risk factors for toxoplasmosis. The sample size was calculated based on the estimated prevalence in the target population to ensure accurate representation, encompassing a varied demographic of both healthy and immunocompromised individuals. Suspected individuals of both genders and all age groups presenting with relevant signs and symptoms were included to capture a comprehensive view of prevalence across the general population, including

vulnerable groups such as pregnant women, transplant recipients, HIV/AIDS patients, and newborns. Individuals without suspicion of toxoplasmosis or those from outside the defined geographical region were excluded from the study. For sample collection, a 5 ml blood sample was drawn from the cubital vein of each suspected individual using a gel tube. These samples were appropriately labelled, stored at 2-8°C, and later centrifuged at 3000 to 4000 rpm for 10 minutes before being analysed using the Alinity i CMIA machine following the manufacturer's instructions. All medical waste was discarded in accordance with established biohazard protocols. The collected data were analysed using SPSS Statistics and Microsoft Excel, with descriptive statistics computed to summarize demographic information. The chi-square test was employed to assess associations between toxoplasmosis prevalence and various demographic factors, with statistical significance set at a p-value of less than 0.05. Graphical representations of the data were created using GraphPad software to assist in the interpretation of results.

The study received **ethical approval** from the IRB of Rawal institute of health sciences (RISH/IRB/36/2024) and strictly adhered to ethical standards, ensuring respect for participant autonomy and confidentiality by obtaining informed consent from all patients or their legal guardians prior to sample collection.

Results

A total of 352 suspected individuals were analysed, included both males and females across various age groups, with clinical data collected retrospectively from January 2019 to December 2023, and blood samples collected from January 2024 to December 2024, as per the study's timeline. The primary objective was to determine the seroprevalence of *Toxoplasma gondii* infection using serological tests

for IgG and IgM antibodies, which indicate past and recent infections, respectively.

Prevalence of Toxoplasmosis According to different Risk Factors:

The prevalence of *T. gondii* infection varied significantly across different age groups and between genders. The age group under 10 years exhibited prevalence at 11.5% including neonates. The prevalence decreased progressively with age up to 40 years and then highest in adults more than 40 years: 6.6% in the 11–20 years, 10.8% in 21–40 years and 17.6% above 40 years. Chi-square test revealed a significant correlation between age and the prevalence of toxoplasmosis (p-value = 0.031). In genders females had a higher prevalence (12.9%) compared to males (7.8%), and this difference was statistically significant (p-value = 0.030) (Table 1).

Variables	Category Level	N	Infected	%	Chi-Square	p-value
Age	≤ 10 Years	26	3	11.5	4.72	0.031
	11–20 Years	15	1	6.6		
	21–30 Years	170	19	11.1		
	31–40 Years	101	11	10.8		
	> 40 Years	40	9	17.6		
Gender	Male	51	4	7.8	1.063	0.030
	Female	301	39	12.9		

Gender-wise prevalence of toxoplasmosis: In terms of gender differences, males exhibited a lower prevalence as compared to females. Throughout the study record from 2019–2024, females showed higher prevalence and from 2019–2021 no recorded male cases. In 2024, females showed the highest rate i.e., 47.6% whereas it was 14.2% in males.

Prevalence of Toxoplasmosis Infection in Relation to Age: When considering age as a risk factor, the study revealed varied prevalence rates across

different age groups. In 2019, individuals under 10 years had the highest prevalence at 25%. However, over the years, the highest prevalence shifted to older age groups, with 2020 seeing the highest prevalence of 23.5% in the 31–40 years group. In 2021, individuals over 40 years exhibited the highest prevalence at 30%, and in 2024, individuals aged 21–30 years had the highest prevalence at 66.6%. The findings suggest that age plays an essential role in determining the susceptibility to *Toxoplasma gondii* infection, with children and older adults being more affected in specific years.

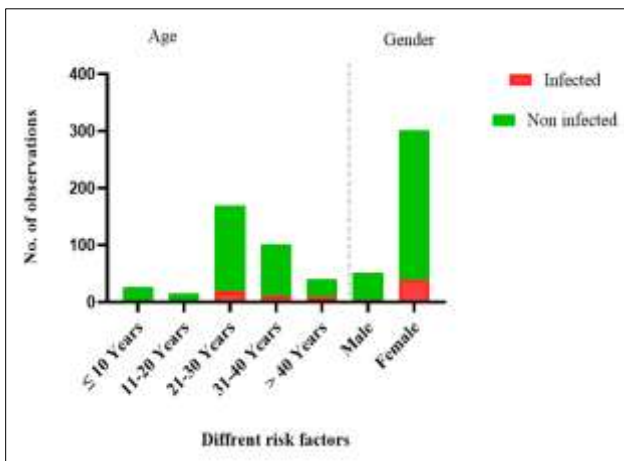


Figure 1. Prevalence of Toxoplasmosis Infection According to Different Risk Factors

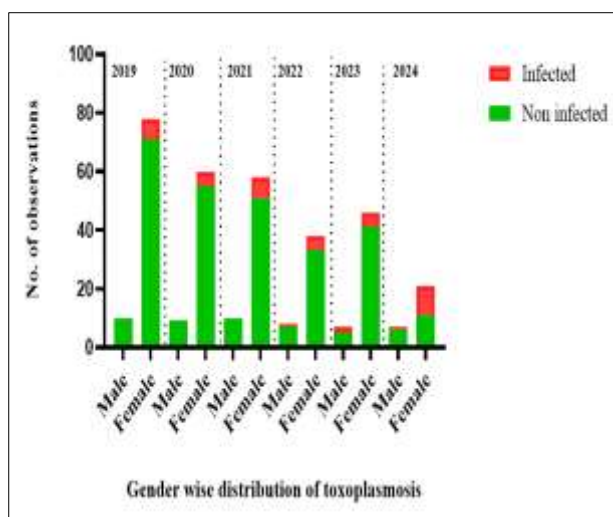


Figure 2 Yearly Gender-wise Prevalence of Toxoplasmosis Infection in Suspected Individuals

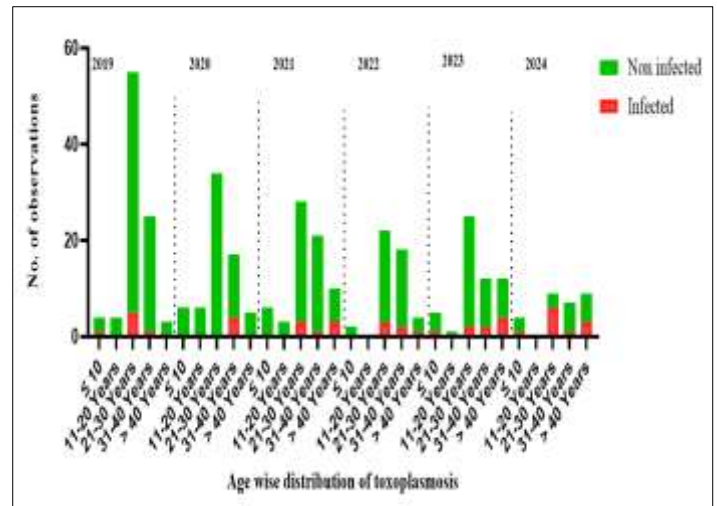


Figure 2 Prevalence of Toxoplasmosis According to Age Group

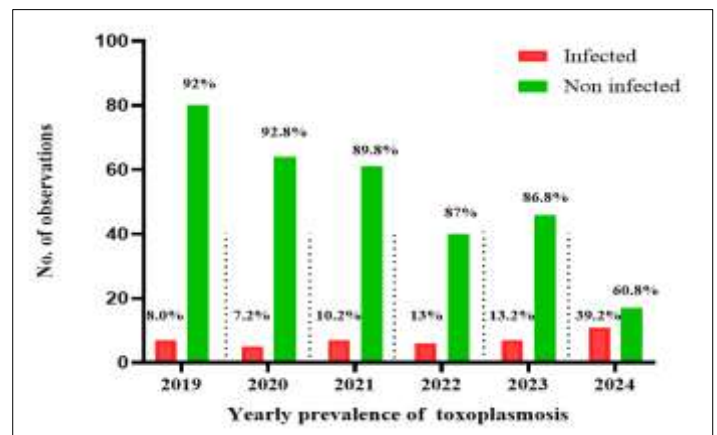


Figure 3 Yearly Prevalence of Toxoplasmosis Infection in Suspected Individuals

Yearly Prevalence of Toxoplasmosis Infection: In the study period from 2019 to 2024, the overall prevalence of toxoplasmosis showed a rising trend. The prevalence in 2019 was 8%, increasing to 39.2% in 2024, indicating a significant rise in infections over the years. The data highlight a steady increase in the incidence of toxoplasmosis, suggesting a growing burden of the disease within the study area.

Discussion

This study discovered that the prevalence of *Toxoplasma* varied by age and gender, occurring in those aged ≤10 years and >40 years at rates of 11.5%

and 17.6%, respectively. The prevalence of toxoplasmosis infection was higher in females (12.9%) than in males (7.8%). Of particular significance, the study found that the seroprevalence of IgG (26.4%) was substantially higher than that of IgM (6.4%). The results suggest that toxoplasmosis is a significant public health concern in the region, with varying degrees of infection across different demographic groups.

This study contributes to the body of knowledge by providing data on the prevalence of *T. gondii* infection in Pakistan, a region with limited comprehensive research on this zoonotic disease. Previous studies in Pakistan, such as Maqsood et al. (2021), have indicated regional variations in the seroprevalence of toxoplasmosis, particularly in rural areas.¹¹ But this is one of the few studies focused on a relatively urbanised region like Rawalpindi and Islamabad. This research presents unique insights into how the infection varies by gender and age, which has been under-explored in the local context.

Globally, toxoplasmosis remains a major public health issue, with varying prevalence rates depending on geographical location, environmental factors, and healthcare infrastructure. In a study conducted in Goiania, Brazil, found a prevalence rate of 65.8% among women of reproductive age.¹² Similarly, research from Nigeria reported a seroprevalence of 10.8% in pregnant women, showing a comparison to our findings with women being particularly vulnerable.¹³ Study in Iraq aligns with our study showing higher prevalence of IgG⁷ and in Saudi Arabia a higher prevalence of IgM in pregnant similar to our results².

Review of toxoplasmosis seroprevalence in Pakistan indicated an average national prevalence of 24%, with regional differences across provinces.¹¹ In Khyber Pakhtunkhwa (KPK), the prevalence was found to be higher at 40.6%, comparable to our study among older populations, particularly those over 40 years. In European countries (Spain), seroprevalence is 32.3%¹⁴, lower prevalence in our

study may be due less awareness of public. The findings of this study have significant implications for clinical decision-making, especially in urbanised regions of Pakistan and suggests that targeted screening in the vulnerable population can significantly reduce the risk of infection and complications including behavioural changes¹⁵.

Future studies should also explore the molecular epidemiology of *T. gondii* in Pakistan to identify the strain types present in the population, as well as potential resistance patterns to treatment. This would provide more targeted insights into effective prevention and treatment strategies.

Limitations and Future Directions: While this study provides valuable data, it does have several limitations. First, the study relied on a retrospective collection of medical data from clinical records, which may have resulted in missing information or under-reporting of cases. Additionally, while the study was conducted in urban areas, it is not clear whether the results are generalisable to rural populations in Pakistan, where the risk of exposure to *T. gondii* might be higher. Future research should consider a broader geographical scope, including rural areas, to better understand regional disparities in prevalence. Furthermore, longitudinal studies could help clarify the dynamics of *T. gondii* infections and their potential links to environmental factors and changes in public health strategies over time. Future studies should also explore the molecular epidemiology of *T. gondii* in Pakistan to identify the strain types present in the population, as well as potential resistance patterns to treatment.

Conclusion

This study aimed to determine the prevalence of *Toxoplasma gondii* infection in Rawalpindi and Islamabad, Pakistan, and assess its association with age, gender, and other risk factors. The findings revealed a 12.2% overall prevalence, with significant variations across age groups and genders. Females had a higher prevalence compared to males, and

individuals under 10 years and over 40 years exhibited the highest infection rates. The IgG seroprevalence was notably higher than IgM, indicating a greater exposure to past infections. These results highlight the ongoing public health concern posed by toxoplasmosis in Pakistan, particularly among vulnerable populations such as pregnant women and the elderly. The study underscores the importance of incorporating routine screening for toxoplasmosis in clinical practice, especially for high-risk groups. To build on these findings, future research should include larger, multicentre prospective studies to confirm these results and explore the underlying factors contributing to the infection's prevalence in the region.

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