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Original article

Seroprevalence of *Toxoplasma gondii* Infection and Its Association with Gender and Age among Patients Attending Al-Bayda Medical Center, Libya

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Abstract
Toxoplasma gondii is a globally prevalent intracellular protozoan parasite and a significant public health concern due to its asymptomatic nature, lifelong persistence, and potential to cause severe disease in vulnerable populations. This hospital-based cross-sectional study aimed to determine the seroprevalence of T. gondii infection and its association with age and gender among patients attending Al-Bayda Medical Center, Libya, in 2025. A total of 124 patients (94 females, 30 males) aged <1–51 years were included. Venous blood samples were collected, and anti-T. gondii IgG and IgM antibodies were measured using the Maccura i1000 Automatic Chemiluminescence Immunoassay System. Results indicated that 32 participants (25.8%) were IgG positive, reflecting past or chronic infection, while 92 participants (74.2%) were seronegative. No IgM-positive cases were detected, suggesting the absence of acute infection. Statistical analysis revealed no significant association between seroprevalence and gender ($p > 0.05$), whereas age was significantly associated with IgG positivity ($p < 0.05$), with older individuals more likely to exhibit past infection. The high proportion of seronegative individuals highlights a large at-risk population, especially among women of childbearing age. These findings provide baseline epidemiological data for T. gondii infection in eastern Libya and underscore the importance of preventive measures, including public awareness campaigns and routine serological screening, to reduce the risk of primary infection and potential congenital toxoplasmosis. The study emphasizes age as a key determinant of exposure, while gender appears not to influence seropositivity in this population.
Keywords. *Toxoplasma gondii, Seroprevalence, IgG antibodies, IgM Antibodies, Age, Libya.*

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Introduction

Toxoplasma gondii is a globally distributed obligate intracellular protozoan parasite and is considered one of the most prevalent zoonotic pathogens affecting humans worldwide. Despite its wide distribution, toxoplasmosis has been described as an underestimated public health threat due to its often-asymptomatic nature, lifelong persistence, and potential to cause severe disease in vulnerable populations [1]. The parasite has a complex life cycle in which felids act as definitive hosts, shedding environmentally resistant oocysts. At the same time, a broad range of warm-blooded animals, including humans, serve as intermediate hosts [2]. Human infection commonly occurs through ingestion of oocysts contaminating food, water, or soil, consumption of undercooked meat containing tissue cysts, or vertical transmission from mother to fetus during primary infection [3].

In immunocompetent individuals, *T. gondii* infection is frequently asymptomatic or presents with mild, nonspecific symptoms. However, the parasite has the capacity to establish lifelong chronic infection through the formation of tissue cysts, particularly within neural and muscular tissues [4]. In immunocompromised individuals, reactivation of latent infection or primary exposure can result in severe clinical manifestations, including toxoplasmic encephalitis, ocular disease, and disseminated infection [5,6]. Infection during pregnancy represents a major clinical concern, as it may induce inflammatory pathways that lead to miscarriage, stillbirth, or congenital toxoplasmosis with long-term neurological and ocular sequelae in affected infants [7].

At the molecular and immunological levels, the pathogenicity of *T. gondii* is mediated by a wide range of virulence factors that facilitate host cell invasion, immune evasion, and intracellular survival [8,9]. Host defense against the parasite relies on a finely regulated immune response, particularly involving cell-mediated immunity, which is essential for controlling parasite replication while limiting tissue damage [4,10]. These complex host–parasite interactions contribute to the heterogeneous clinical spectrum of toxoplasmosis and underscore the importance of accurate diagnosis and epidemiological surveillance. Serological testing remains the cornerstone for the diagnosis and epidemiological assessment of toxoplasmosis. The presence of anti-*T. gondii* IgG antibodies indicate previous exposure or chronic

infection, whereas IgM antibodies are generally associated with recent or acute infection [11]. Advances in serological and molecular diagnostic techniques have improved sensitivity and specificity; nevertheless, population-based seroprevalence studies remain essential for understanding exposure patterns and transmission dynamics [12,11].

Globally, seroprevalence rates of *T. gondii* vary widely across regions and populations, reflecting differences in environmental conditions, dietary habits, hygiene practices, and socioeconomic factors [1,12]. Demographic variables such as age and gender have been frequently evaluated as potential determinants of infection. Increasing age is consistently associated with higher seroprevalence, likely due to cumulative exposure over time, whereas gender-related differences have been inconsistently reported across studies [10,12].

In Libya, available data on toxoplasmosis remain limited and are largely restricted to specific high-risk groups. Studies among pregnant women in El-Beida City and women with a history of abortion in eastern Libya have reported considerable rates of chronic *T. gondii* infection, highlighting its public health importance in the region [13,14]. Additionally, serological studies among male blood donors in southern Libya indicate ongoing exposure within the general population [15]. However, data on toxoplasmosis among hospital-attending patients in eastern Libya are still scarce.

Al-Bayda Medical Center is a major referral hospital serving eastern Libya and provides an important setting for evaluating the epidemiological characteristics of infectious diseases within the region. Hospital-based studies are particularly valuable for generating locally relevant data and identifying demographic patterns associated with infection, as demonstrated in previous epidemiological investigations conducted at the same center [16]. Therefore, the present study aimed to determine the seroprevalence of *Toxoplasma gondii* infection among patients attending Al-Bayda Medical Center, Libya, in 2025, and to assess its association with gender and age. The findings of this study are expected to contribute to a better understanding of toxoplasmosis epidemiology in Libya and support evidence-based public health interventions.

Methods

Study Design and Setting

A hospital-based cross-sectional study was conducted in 2025 at Al-Bayda Medical Center, a major tertiary and referral healthcare facility serving Al-Bayda City and surrounding areas in eastern Libya. The study aimed to assess the seroprevalence of *Toxoplasma gondii* infection among patients attending the medical center during the study period.

Study Population

A total of 124 patients of both genders and varying age groups were included. Patients were selected based on the availability of adequate serum samples for serological testing and complete demographic data (age and gender). Patients with insufficient samples or incomplete records were excluded from the analysis.

Sample Collection and Processing

Venous blood samples were collected under aseptic conditions by trained laboratory personnel. Samples were allowed to clot at room temperature and subsequently centrifuged to separate serum. Serum samples were analyzed immediately or stored under appropriate conditions according to the manufacturer's recommendations until testing.

Serological Testing for Toxoplasma gondii

Detection of anti-*Toxoplasma gondii* IgG and IgM antibodies was performed using the Maccura i1000 Automatic Chemiluminescence Immunoassay System (Maccura Biotechnology Co., Ltd., China). This fully automated analyzer utilizes magnetic microparticle–based technology combined with Acridinium Ester (AE) direct chemiluminescence, providing high analytical sensitivity, specificity, and reproducibility.

The following reagent kits were used in accordance with the manufacturer's instructions:

- TOXO IgG Reagent Kit
- TOXO IgM Reagent Kit

The assay principle involves the binding of *T. gondii*–specific antibodies in patient serum to antigen-coated magnetic microparticles. After washing to remove unbound components, an acridinium ester–labeled conjugate is added. The resulting chemiluminescent signal is measured by the analyzer and is directly proportional to the antibody concentration in the sample.

Interpretation of TOXO IgG and IgM Results

Serological results were automatically generated and interpreted by the Maccura i1000 system based on calibration curves and cut-off values provided by the reagent manufacturer. In addition, interpretation was aligned with internationally accepted clinical serology guidelines.

IgG Antibody Interpretation (Marker of Past or Chronic Infection)

- Negative: Antibody levels below the assay-specific cut-off, indicating no serological evidence of prior exposure
- Equivocal (Borderline): Values within a narrow range around the cut-off; such results may reflect early seroconversion or analytical variation and require repeat testing
- Positive: Antibody levels equal to or exceeding the defined cut-off, indicating past exposure or chronic infection

IgG antibodies are known to persist for life and therefore serve as markers of previous infection rather than acute disease.

IgM Antibody Interpretation (Marker of Recent or Acute Infection)

- Negative: Values below the assay cut-off, indicating no serological evidence of recent infection
- Equivocal: Borderline values requiring follow-up testing
- Positive: Values above the cut-off, suggesting recent or acute infection

IgM antibodies typically appear early after infection; however, they may persist for prolonged periods or yield false-positive results. Therefore, IgM positivity must be interpreted cautiously, particularly in pregnant or immunocompromised individuals.

Combined Serological Patterns

IgG and IgM results were interpreted together to determine overall Toxoplasma serostatus:

- IgG− / IgM−: No evidence of *T. gondii* infection (susceptible individual)
- IgG+ / IgM−: Past infection with immunological memory
- IgM+ / IgG−: Possible very recent infection or false-positive IgM; repeat testing recommended
- IgG+ / IgM+: Suggestive of recent infection or reactivation; confirmatory testing (e.g., IgG avidity) recommended

In the present study, no equivocal or IgM-positive cases were detected; therefore, participants were classified as either No exposure (IgG−/IgM−) or Past infection (IgG+/IgM−).

Quality Control

Internal quality control procedures were performed in accordance with the manufacturer's guidelines. Calibrators and control sera supplied with the reagent kits were included in each analytical run to ensure assay accuracy and reliability. All tests were performed by experienced laboratory technologists under standardized laboratory conditions.

Statistical Analysis

Data were entered, cleaned, and analyzed using the R statistical software environment. A total of 124 participants were included in the analysis. Categorical variables, including gender, *Toxoplasma gondii* IgG serostatus, IgM serostatus, and overall serological status, were summarized as frequencies and percentages. Continuous variables such as age, TOXO IgG concentrations, and TOXO IgM concentrations were expressed as mean ± standard deviation (SD), as well as median and interquartile range where appropriate. The Chi-square goodness-of-fit test was used to evaluate the distribution of categorical variables, including gender and IgG serostatus. The overall seroprevalence of *T. gondii* infection was assessed based on combined IgG and IgM results, and differences in proportions were tested using the Chi-square test. When expected cell counts were small, Fisher's exact test was applied to assess the association between serostatus and gender. The Shapiro–Wilk test was performed to assess the normality of continuous variables (age, TOXO IgG, and TOXO IgM levels). As TOXO IgG and IgM concentrations showed significant deviation from normal distribution ($p < 0.001$), appropriate non-parametric tests were considered when necessary.

Comparisons of mean TOXO IgG and TOXO IgM levels between males and females were conducted using the Welch two-sample t-test, which does not assume equal variances. The association between age and *T. gondii* serostatus (no exposure vs. past infection) was evaluated using the Wilcoxon rank-sum test, given the non-normal distribution of age

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across serological groups. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with accepted ethical standards for biomedical research. Patient data were anonymized prior to analysis, and laboratory results were used exclusively for research purposes. As the study relied on routinely collected diagnostic data, no additional interventions were performed.

Results

Study Population Characteristics

A total of 124 patients attending Al-Bayda Medical Center in 2025 were included in this study. The study population consisted of 94 females (75.8%) and 30 males (24.2%), indicating a predominance of female participants. The age of participants ranged from less than one year to 51 years, with a mean age of 18.2 ± 15.9 years, reflecting a heterogeneous population including both pediatric and adult individuals. Regarding serological status, 32 participants (25.8%) tested positive for anti-Toxoplasma gondii IgG antibodies, indicating past or chronic exposure, whereas 92 participants (74.2%) were IgG-negative, suggesting no previous infection. All study participants tested negative for IgM antibodies, demonstrating the absence of acute or recent toxoplasmosis infection at the time of sampling. Accordingly, the overall serostatus distribution in the study population showed that 74.2% had no prior exposure, while 25.8% had evidence of past infection.

Gender Distribution and Serostatus

Among females, 26 (27.7%) tested positive for IgG, while 68 (72.3%) were seronegative. In contrast, among males, 6 (20.0%) were IgG positive and 24 (80.0%) were seronegative. Despite the slightly higher percentage of IgG positivity observed in females, Fisher's exact test indicated no significant association between gender and overall serostatus (p-value > 0.05; odds ratio = 0.66, 95% CI: 0.20–1.90). These findings are consistent with the frequency patterns illustrated in Figure 1. No significant differences in TOXO IgG concentrations were observed between females (mean = 1.14 ± 1.79 IU/mL) and males (mean = 0.88 ± 1.52 IU/mL; t-test, p-value > 0.05). Similarly, TOXO IgM concentrations did not differ significantly by gender (females: 0.16 ± 0.13 AU/mL; males: 0.17 ± 0.13 AU/mL; p-value > 0.05).

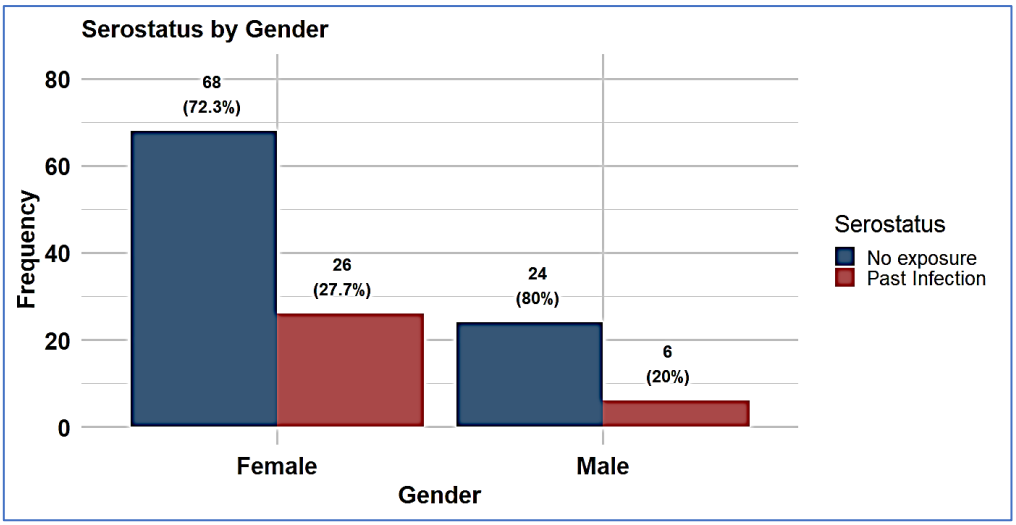

Figure 1. Grouped bar plot showing the frequency and percentage distribution of Toxoplasma serostatus by gender. The plot compares "No exposure" and "Past infection" across male and female participants, highlighting gender-related variation in serostatus distribution.

Age Distribution and Toxoplasma gondii Serostatus

Participants who exhibited signs of previous infection were typically older than those who had no exposure. The comparison of ages indicated a notable difference among the serological groups (Wilcoxon rank-sum test, p-value < 0.05), implying that the cumulative exposure to T. gondii tends to increase with advancing age. Figure 2 illustrates a violin plot that includes overlaid boxplots and jittered data points, depicting the age distribution across different

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serostatus categories. This plot emphasizes the density, median, interquartile range, and individual variations, indicating that past infections were more prevalent among older participants.

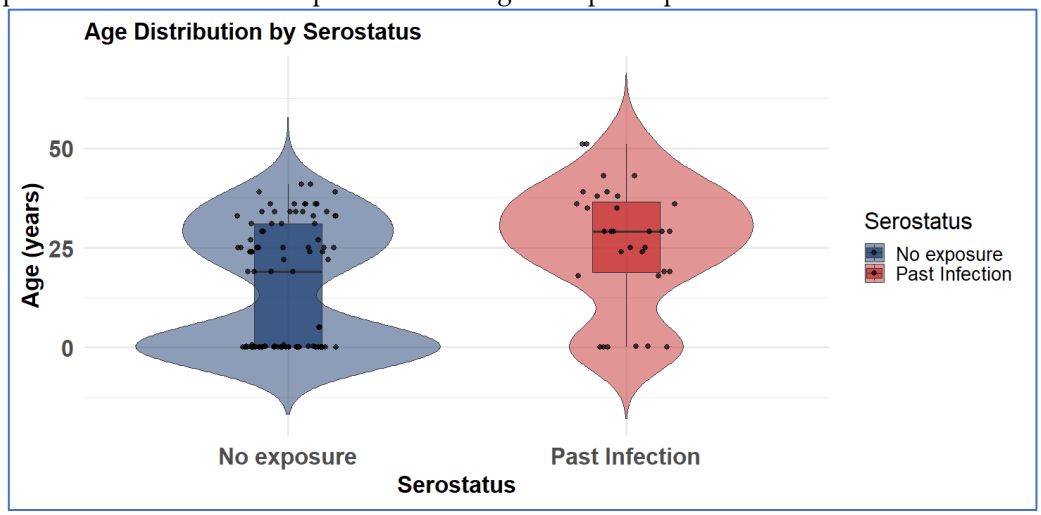

Figure 2. Age distribution by serostatus: violin plot with overlaid boxplot and jittered observations showing the density, summary statistics, and individual data points for age in each serostatus group

TOXO IgG and IgM Concentrations

The mean TOXO IgG concentration across all participants was 1.08 ± 1.72 IU/mL, ranging from 0.00 to 6.72 IU/mL. IgG levels were higher among participants classified as having past infection, reflecting immunological memory from prior exposure. The mean TOXO IgM concentration was 0.16 ± 0.13 AU/mL, with all values below diagnostic thresholds, confirming the absence of acute infection. Shapiro-Wilk normality tests indicated that both IgG and IgM distributions were non-normally distributed ($W = 0.618$, $p < 0.001$ for IgG; $W = 0.794$, $p < 0.001$ for IgM).

Overall Serological Classification

Integration of IgG and IgM results allowed participants to be categorized into four distinct serological profiles (Table 2):

- Profile 1 (IgG– / IgM–): 92 participants (74.2%) had no detectable antibodies, indicating susceptibility to primary infection.
- Profile 2 (IgG+ / IgM–): 32 participants (25.8%) exhibited past or chronic infection, with persistent IgG antibodies and no IgM positivity. The median age in this group was higher, suggesting cumulative lifetime exposure.
- Profiles 3 and 4 (IgM positive): No participants were positive for IgM alone or with IgG, indicating absence of recent or reactivated infection.

Statistical analysis confirmed that age was significantly associated with serological profile (Wilcoxon test, p-value < 0.05), whereas gender had no significant effect (Fisher's exact test, p-value > 0.05).

Table 1. Combined IgG and IgM Serological Profiles and Population Distribution (n = 124)

Serological Profile	IgG Status	IgM Status	Interpretation	Frequency	Percentage
Profile 1	Negative	Negative	No prior exposure; susceptible	92	74.2 %
Profile 2	Positive	Negative	Past or chronic infection	32	25.8 %
Profile 3	Negative	Positive	Recent infection (none observed)	0	0
Profile 4	Positive	Positive	Recent or reactivated infection (none observed)	0	0

Public Health Implications

- The high proportion of seronegative participants indicates a large at-risk population, particularly among women of childbearing age.

- The 25.8% IgG-positive population represents individuals with long-term immunity, though reactivation risk remains for immunocompromised patients.
- No IgM positivity suggests low active transmission within this population during the study period, providing useful baseline data for local infection control and awareness programs.

Discussion

This study provides a comprehensive analysis of the seroprevalence of *Toxoplasma gondii* among patients attending Al-Bayda Medical Center in 2025, highlighting the influence of age and gender on infection patterns. Our findings indicate that 25.8% of the participants had IgG antibodies against *T. gondii*, consistent with past or chronic infection, whereas 74.2% were seronegative, suggesting susceptibility to primary infection. No participants were positive for IgM, confirming the absence of acute or recent infections at the time of sampling.

The overall seroprevalence observed in this study aligns with reports from other regions in Libya. Previous studies among pregnant women in El-Beida City and women with a history of abortion reported comparable IgG positivity rates, emphasizing that chronic *T. gondii* infection is an ongoing public health concern in eastern Libya [13,14]. The high proportion of seronegative individuals in our study (74.2%) underscores that a majority of patients remain at risk of primary infection, particularly women of childbearing age, which is critical given the potential for congenital toxoplasmosis [9].

Globally, seroprevalence varies widely, with higher rates reported in Latin America and parts of Europe, often reflecting differences in environmental exposure, dietary habits, hygiene, and cultural practices [1,12]. Our study confirms that even within hospital-attending populations, local epidemiology must be assessed to guide targeted interventions. A key finding of this study is the significant association between age and IgG seropositivity. Individuals with past infection were generally older, with a median age higher than that of seronegative participants. This is consistent with the hypothesis that T. gondii exposure accumulates over a lifetime due to environmental contact, dietary practices, and repeated exposure to oocysts [10,12]. The Wilcoxon rank-sum test confirmed that age differences between serostatus groups were statistically significant (p-value < 0.05), supporting the concept of age-related cumulative risk. This trend is also reflected in the violin plot of age distribution (Figure 2), which clearly demonstrates a higher density of past infection among older participants.

Although females in this study exhibited a slightly higher rate of IgG positivity (27.7%) compared to males (20%), no statistically significant association between gender and serostatus was detected (Fisher's exact test, p-value > 0.05). This finding aligns with some international studies suggesting that gender may not be a consistent determinant of infection, despite occasional reports of higher seroprevalence in females due to exposure during food preparation or household activities [1,12]. The observed odds ratio of 0.66 (95% CI: 0.20–1.90) further indicates no substantial difference in infection risk between sexes. The mean TOXO IgG concentration of 1.08 ± 1.72 IU/mL reflects a wide range of antibody titers, likely influenced by differences in the timing of initial infection and individual immune responses. Participants classified with past infection exhibited markedly higher IgG levels than seronegative individuals, consistent with long-term immunological memory [4]. The absence of IgM positivity across all participants confirms that active transmission was low during the study period and supports the reliability of IgG as a marker of past exposure in this population.

The Shapiro–Wilk test confirmed that IgG and IgM distributions deviated from normality ($p < 0.001$), justifying the use of non-parametric tests in age comparisons. No significant differences in IgG or IgM levels were observed between genders (p-value > 0.05), indicating that antibody concentrations are not influenced by sex in this population.

Classification into combined IgG/IgM serological profiles (Table 2) revealed that 74.2% of participants had no prior exposure (Profile 1), while 25.8% had past or chronic infection (Profile 2). Profiles indicative of recent or reactivated infection (Profiles 3 and 4) were absent, highlighting low active transmission. The significant association between age and serological profile (Wilcoxon test, p-value < 0.05) reinforces the role of cumulative exposure over time, whereas gender had no significant effect (p-value > 0.05). From a public health perspective, the predominance of seronegative individuals underscores the need for preventive measures, particularly educational campaigns aimed at women of reproductive age, immunocompromised patients, and other high-risk groups. Such measures could include proper food handling, avoidance of undercooked meat, and minimizing contact with soil contaminated with cat feces [2,3].

Limitations

This study has several limitations. Being hospital-based, the findings may not be fully generalizable to the broader community. The cross-sectional design provides a snapshot of seroprevalence but cannot establish temporal trends or causality. Additionally, while serological testing is highly sensitive for detecting IgG and IgM antibodies, confirmatory molecular assays such as PCR were not performed, which could have detected low-level or latent infections [11,17].

Conclusion

This study demonstrates a moderate seroprevalence of Toxoplasma gondii (25.8%) among patients attending Al-Bayda Medical Center, with the majority of participants remaining susceptible to infection. Age was identified as a significant determinant of past infection, while gender was not. The absence of IgM-positive cases suggests low recent transmission, but the high proportion of seronegative individuals emphasizes the importance of preventive strategies to mitigate the risk of primary infection. These findings provide valuable epidemiological insight to guide local public health interventions and awareness programs in eastern Libya.

Conflict of interest. Nil

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