

Original article

Integrated Statistical Evaluation of IgG/IgM Serostatus Patterns and Cross-Infection Correlations among TORCH Pathogens in a Libyan Clinical Population

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Toxoplasma Gondii, Rubella
Virus, Cytomegalovirus,
Seroprevalence.**ABSTRACT**

TORCH infections, including Toxoplasma gondii (TOXO), Rubella virus (RUB), and Cytomegalovirus (CMV), remain a major public health concern due to their potential to cause severe outcomes in susceptible individuals. This cross-sectional analytical study evaluated seroprevalence, age- and gender-specific patterns, and co-infection correlations of TORCH pathogens among 61 participants attending Al-Bayda Teaching Medical Center, Libya, in 2025. Venous blood samples were analyzed using chemiluminescent immunoassays to detect IgG and IgM antibodies. Results revealed that TOXO IgG seropositivity was 26.23% with no IgM positivity, indicating low historical exposure and absence of acute infection. Rubella IgG and IgM positivity were 85.25% and 60.66%, respectively, highlighting widespread immunity alongside substantial recent infection. CMV IgG and IgM positivity were 78.69% and 62.30%, respectively, demonstrating persistent circulation and ongoing infections. Correlation analyses identified moderate associations between IgG and IgM within each pathogen and notable co-occurrence of recent Rubella and CMV infections ($r = 0.51$, $p < 0.001$). Age significantly influenced TOXO and CMV serostatus, whereas Rubella immunity was largely age-independent. No significant gender differences were observed. These findings provide a comprehensive epidemiological profile, emphasizing the importance of integrated monitoring and targeted public health interventions.

Introduction

TORCH infections, including Toxoplasma gondii, Rubella virus, and Cytomegalovirus (CMV), remain a significant public health concern worldwide, affecting both males and females of all ages [1,2]. These pathogens are capable of causing acute or chronic infections that can lead to systemic complications, immune modulation, and increased susceptibility to other infections [3,4]. Understanding the epidemiological characteristics of these infections requires assessing seroprevalence across different age groups, genders, and demographic settings, as well as identifying patterns of co-infection and cross-immunity [5,6].

Toxoplasma gondii, a protozoan parasite with a complex life cycle, is widely distributed and can establish latent infections in immunocompetent hosts, while causing severe complications in immunocompromised individuals [7,8]. Infection is acquired via ingestion of contaminated food, water, or contact with feline feces, and its prevalence varies across age groups, gender, and regional factors [1,9]. Studies in Libya demonstrate that T. gondii exposure is common among both men and women, with some studies highlighting higher seroprevalence in adults compared to adolescents [10-12]. Mapping these seroprofiles and correlations with demographic variables provides critical insights into population-level risk factors and informs targeted public health interventions.

Rubella virus infection, though widely controlled in regions with vaccination programs, still presents a risk in populations with immunity gaps, including males and females of reproductive and non-reproductive age [13,14]. Rubella infection in the general population can cause mild systemic illness in children and adults, while in pregnant women, it may result in congenital rubella syndrome [15,16]. Therefore, assessing population-wide seroprevalence across ages and sexes is essential for understanding herd immunity, vaccination coverage gaps, and potential outbreaks.

Cytomegalovirus (CMV) is another highly prevalent pathogen, capable of causing latent, persistent infections in both males and females [2,17]. CMV infection in healthy adults is often asymptomatic but can have serious consequences in immunocompromised individuals. Population-based studies demonstrate that CMV seroprevalence increases with age and varies according to socioeconomic and environmental factors, highlighting the importance of demographic stratification and seroprofile analysis [18-20].

Understanding the distribution of TORCH infections in the general population requires rigorous statistical approaches, including cross-sectional serological surveys, correlation analyses, and evaluation of co-infection patterns [5,21,15]. Age, gender, occupation, geographic location, and seasonal variations are key factors that shape infection dynamics. For instance, demographic and seasonal trends observed in *Helicobacter pylori* infections at Al-Bayda Teaching Medical [22] suggest that similar analyses can be applied to TORCH pathogens to identify high-risk populations and transmission hotspots.

In the Libyan context in 2025, evaluating TORCH infections among males and females of all age groups is crucial to developing comprehensive prevention and monitoring strategies. Incorporating modern serological and molecular diagnostic methods [23,3] ensures accurate detection, enabling the identification of latent infections, co-infection patterns, and age- or gender-specific trends. Such population-wide studies are essential to inform public health policies, immunization strategies, and education programs, reducing the burden of TORCH infections across the community.

In summary, TORCH infections represent a persistent, widespread health concern that affects males and females across the lifespan. Population-level studies that integrate seroprevalence, demographic correlations, co-infection analysis, and seasonal trends are essential for understanding the epidemiology of these pathogens in Libya. This study aims to provide a comprehensive assessment of TORCH infections across different ages and genders, offering insights into risk factors, infection patterns, and potential public health interventions to mitigate disease burden in 2025.

Methods

Study Design and Population

This study was conducted as a cross-sectional analytical investigation that included participants ranging from children to older adults. Participants were recruited from routine laboratory visitors and patients attending Al-Bayda Teaching Medical Center between January and December 2025.

Eligibility criteria

Inclusion criteria encompassed both genders and all age groups, while exclusion criteria were immunodeficiency, recent chemotherapy, or blood transfusion, which could interfere with accurate serological detection.

Sample Collection and Processing

Demographic data, including age, gender, and relevant clinical history, were collected using a pre-structured, validated questionnaire. The study population included participants across the full age spectrum, ranging from infants younger than one year (<1 year) to adults aged up to 51 years.

Venous blood samples were collected under aseptic conditions using serum-separating tubes (SSTs). After allowing samples to clot at room temperature, serum was separated by centrifugation at 3000 rpm for 10 minutes, then aliquoted into sterile cryovials for storage at -20°C until analysis. Quality assurance measures included duplicate testing for randomly selected samples, monitoring of storage conditions, and strict adherence to standard operating procedures (SOPs) during collection, handling, and storage. These steps ensured the reliability and reproducibility of serological results.

Serological Testing and Reagents

Serological assays were conducted using the Maccura i1000 automated immunoassay analyzer, a high-throughput platform based on chemiluminescent immunoassay (CLIA) technology. This system allows rapid detection of antibodies with high sensitivity and specificity.

The study employed commercial immunoassay kits for the detection of IgM and IgG antibodies against *Toxoplasma gondii*, rubella virus, and cytomegalovirus. For each pathogen, both IgM and IgG reagent kits were used to differentiate between recent infection, past exposure, and immune status.

Serological results were classified into four categories for each pathogen: No Prior Exposure, Past Infection, Ongoing Infection, and Acute Infection. These categories provided the basis for evaluating population-level immunity and for correlating serostatus patterns with demographic variables such as age and gender.

Data Management and Variable Definition

Data were organized into categorical variables (e.g., gender, serostatus for TOXO, RUB, CMV) and continuous variables (e.g., age, antibody titers). Each participant was assigned a unique study ID to maintain confidentiality. Data entry was performed in Microsoft Excel, followed by validation and cleaning to correct errors or inconsistencies. Outliers and missing values were addressed using standard statistical procedures to maintain data integrity.

Statistical Analysis

Statistical analyses were performed using R software, with a focus on descriptive, inferential, and graphical methods to fully characterize seroprevalence patterns. Categorical variables were summarized using frequency counts and percentages to describe gender distribution and serostatus across TORCH infections. Continuous variables were presented using measures of central tendency and variability, including mean, standard deviation, standard error, and 95% confidence intervals.

Associations between categorical variables, such as gender and TORCH serostatus, were examined using Fisher's exact test. Independent t-tests were applied to compare means between two groups, while one-way analysis of variance (ANOVA) was used for comparisons involving three or more groups. Tukey's post-hoc tests were performed where necessary to identify specific group differences. Normality assumptions were assessed using the Shapiro-Wilk test, and variance homogeneity was evaluated with Levene's test. When these assumptions were not met, appropriate non-parametric alternatives were employed to ensure validity of the statistical findings.

Pearson correlation coefficients were calculated to assess linear relationships among numerical variables, particularly between age and antibody titers. Grouped bar plots were generated to illustrate gender distribution across TORCH serostatus categories. Boxplots and violin plots were used to depict age distributions according to serostatus. These graphical methods were employed to support statistical analysis and facilitate comparative evaluation of seroprevalence trends.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Al-Bayda Teaching Medical Center. Written informed consent was obtained from all participants or their legal guardians. The study adhered to the Declaration of Helsinki and relevant national ethical regulations. Participant confidentiality was rigorously maintained, with no personal identifiers included in the dataset or reports.

Results

Study Population Characteristics

A total of 61 participants were included in this study. The gender distribution showed a predominance of females (n = 46, 75.41%) compared to males (n = 15, 24.59%). This reflects the recruitment pattern, which predominantly included female patients attending routine laboratory testing or seeking evaluation for TORCH infections. The age of participants ranged from infancy (<1 year) to 51 years, with a mean age of 17.90 ± 15.91 years and a standard error (SE) of 2.04. The 95% confidence interval (CI) for age was 13.82–21.98 years, reflecting a diverse population spanning both pediatric and adult age groups. The wide age distribution enables analysis of age-related seroprevalence trends and stratification by serostatus.

Descriptive Statistics of Categorical Variables

The categorical variables, including gender and TORCH serostatus for TOXO, Rubella (RUB), and CMV, are summarized in (Table 1).

Table 1. Distribution of Gender and TORCH Serostatus (n = 61)

Variable	Category	n (%)
Gender	Female	46 (75.41 %)
	Male	15 (24.59 %)
TOXO IgG Serostatus	Negative	45 (73.77 %)
	Positive	16 (26.23 %)
TOXO IgM Serostatus	Negative	61 (100 %)
TOXO Serostatus	No Prior Exposure	45 (73.77 %)
	Past Infection	16 (26.23 %)
	Ongoing Infection	0 (0.00 %)
RUB IgG Serostatus	Negative	9 (14.75 %)
	Positive	52 (85.25 %)
RUB IgM Serostatus	Negative	24 (39.34 %)
	Positive	37 (60.66 %)
RUB Serostatus	Acute Infection	1 (1.64 %)
	No Prior Exposure	8 (13.11 %)
	Ongoing Infection	36 (59.02 %)
CMV IgG Serostatus	Negative	13 (21.31 %)
	Positive	48 (78.69 %)
CMV IgM Serostatus	Negative	23 (37.70 %)

	Positive	38 (62.30 %)
CMV Serostatus	Acute Infection	3 (4.92 %)
	No Prior Exposure	10 (16.39 %)
	Ongoing Infection	35 (57.38 %)
	Past Infection	13 (21.31 %)

Analysis of serological markers for *Toxoplasma gondii* (TOXO), Rubella virus (RUB), and Cytomegalovirus (CMV) among the study participants revealed distinct patterns of immunity and infection stages. TOXO IgG antibodies were detected in 26.23% of participants, indicating prior exposure to the pathogen. None of the participants were positive for TOXO IgM, suggesting that no acute or recent infections were present within the cohort. This serological profile indicates that while a quarter of the population had been exposed to *Toxoplasma* in the past, the risk of ongoing infection was minimal at the time of sampling. In contrast, Rubella IgG seropositivity was considerably higher, observed in 85.25% of participants, reflecting widespread prior immunity or exposure. Interestingly, RUB IgM positivity reached 60.66%, indicating that a significant proportion of the population had recent or ongoing Rubella infections. The coexistence of high IgG and IgM levels underscores the dynamic nature of Rubella immunity in this population, highlighting both established immunity and recent viral activity. For CMV, the serological results showed that 78.69% of participants were positive for CMV IgG, demonstrating a high prevalence of past exposure. At the same time, 62.30% were positive for CMV IgM, suggesting a notable proportion of individuals experiencing ongoing or recent infections. This combination of IgG and IgM positivity indicates that CMV remains highly circulating within the study population, posing potential implications for maternal and fetal health in seronegative or susceptible individuals. Further categorization of participants according to the combined IgG and IgM serostatus into “No Prior Exposure,” “Past Infection,” “Ongoing Infection,” and “Acute Infection” allows a more nuanced understanding of the infection landscape. This classification not only distinguishes between historical immunity and active infections but also provides a framework to identify at-risk groups who may require targeted preventive measures, vaccination, or closer clinical monitoring. Overall, these findings reflect a heterogeneous pattern of immunity and viral exposure, with TOXO showing low recent activity, while Rubella and CMV exhibit significant ongoing infections in the studied population.

Descriptive Statistics of Numerical Variables

The continuous variables, including age and antibody titers for TOXO, RUB, and CMV, are summarized in (Table 2).

Table 2. Descriptive Statistics of Age and Antibody Levels (n = 61)

Variable	Min	Max	Mean	SD	SE	95% CI
Age	0	51	17.90	15.91	2.04	(13.82-21.98)
TOXO IgG	0	21	1.40	3.08	0.39	(0.61-2.19)
TOXO IgM	0	0.69	0.17	0.14	0.02	(0.13-0.20)
RUB IgG	0	7.66	1.52	1.49	0.19	(1.13-1.90)
RUB IgM	0	0.88	0.31	0.24	0.03	(0.25-0.37)
CMV IgG	0	9.00	3.28	2.76	0.35	(2.57-3.99)
CMV IgM	0	1.30	0.36	0.27	0.03	(0.29-0.43)

The study population encompassed a broad age range from infancy to adulthood, allowing for stratified analyses across different age groups to assess patterns of exposure and immunity. This wide age distribution provides a comprehensive overview of seroprevalence trends in both pediatric and adult cohorts. *Toxoplasma gondii* (TOXO) serology showed that IgG titers were generally low to moderate, indicating previous exposure in a subset of participants. Conversely, TOXO IgM values were negligible, confirming the absence of acute or recent infections within the studied cohort. This pattern suggests that while some individuals had historical exposure to TOXO, the immediate risk of active infection is minimal. For Rubella virus (RUB), IgG levels were consistent with established prior immunity, reflecting either natural infection or vaccination history. In contrast, RUB IgM titers were elevated in over half of participants, indicating that a considerable proportion of the population had recent or ongoing Rubella infections. This dual presence of IgG and IgM antibodies highlights the coexistence of long-term immunity with active or recently acquired infections in this cohort. Cytomegalovirus (CMV) serology revealed high IgG titers, demonstrating that past exposure is common across the study population. Notably, IgM antibodies were also detected in a substantial proportion of participants, reflecting ongoing or recent CMV infections. This finding underscores the persistent circulation of CMV within the population and the potential clinical significance for susceptible groups, particularly in pediatric or immunocompromised individuals. Overall, the antibody titer profiles for TOXO, RUB, and CMV provide a comprehensive picture of immunity and infection dynamics, revealing low acute

TOXO activity, significant recent Rubella infections, and persistent CMV circulation. These results facilitate a nuanced understanding of population-level susceptibility and potential risk groups, guiding public health monitoring and preventive strategies.

Correlation Analysis of Age and TORCH Antibody Levels

A Pearson correlation analysis was conducted to assess the relationships between age and antibody titers for Toxoplasma gondii (TOXO), Rubella virus (RUB), and Cytomegalovirus (CMV). Both the correlation coefficients (r) and their corresponding p -values were examined to evaluate the strength and statistical significance of associations. (Figure 1) represents these correlations.

Age correlations

Age demonstrated weak positive correlations with CMV IgG ($r = 0.17$, $p = 0.185$) and CMV IgM ($r = 0.14$, $p = 0.282$). While these findings suggest a possible trend toward increased past exposure and ongoing infection with advancing age, the associations did not reach statistical significance. In contrast, age was not meaningfully correlated with either Toxoplasma or Rubella antibodies ($r \leq 0.08$, $p > 0.05$), indicating that seropositivity for these pathogens appeared largely independent of participant age. This pattern highlights the pathogen-specific nature of age-related serological trends within the studied population.

TOXO correlations

TOXO IgG and TOXO IgM demonstrated a moderate correlation ($r = 0.27$, $p = 0.038$), indicating a modest association between evidence of past exposure and residual IgM levels, even though no acute infections were identified within the cohort. In contrast, TOXO antibodies exhibited weak and statistically non-significant correlations with other TORCH markers ($r \leq 0.14$, $p > 0.05$), suggesting that serostatus for Rubella or CMV was largely independent of Toxoplasma antibody patterns. This finding highlights the pathogen-specific nature of serological responses and underscores the limited cross-association among TORCH infections in the studied population.

Rubella correlations

RUB IgG and RUB IgM exhibited a moderate positive correlation ($r = 0.43$, $p = 0.001$), which aligns with the expected immunological relationship between markers of prior immunity and indicators of recent infection. This finding reinforces the biological consistency of rubella serological responses. Additionally, RUB IgM demonstrated moderate to strong correlations with CMV IgG ($r = 0.41$, $p = 0.001$) and CMV IgM ($r = 0.51$, $p < 0.001$). These associations suggest that a subset of participants may have experienced concurrent or overlapping recent infections with both Rubella and CMV. Such co-occurrence highlights the potential epidemiological interplay between TORCH pathogens and underscores the importance of comprehensive serological screening in populations at risk.

CMV correlations

CMV IgG and CMV IgM demonstrated a moderate correlation ($r = 0.46$, $p < 0.001$), which is consistent with the expected overlap between markers of past exposure and indicators of ongoing infection. This relationship reflects the natural progression of CMV serology, where IgG persists long-term while IgM may reappear during reactivation events. Additionally, CMV IgM showed significant correlations with TOXO IgM ($r = 0.33$, $p = 0.009$) and RUB IgM ($r = 0.51$, $p < 0.001$). These findings suggest that a subset of participants may have experienced simultaneous or overlapping active infections involving CMV, Toxoplasma, and Rubella. Such co-occurrence underscores the epidemiological complexity of TORCH pathogens and highlights the importance of comprehensive screening strategies, particularly in populations where multiple infections may interact to influence clinical outcomes.

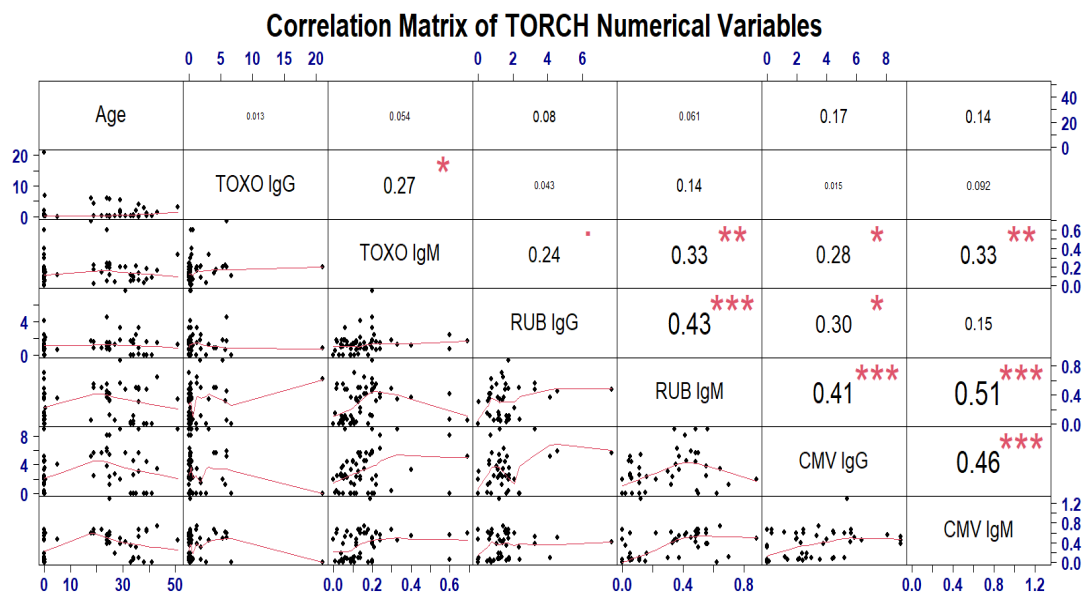


Figure 1. Pearson Correlation Matrix of Age and TORCH Antibody Levels

Pearson correlation coefficients (r) quantify the strength and direction of linear relationships among numerical variables, including participant age and antibody titers for TOXO, Rubella (RUB), and CMV. The matrix highlights patterns of association between past exposure (IgG) and recent infection (IgM) markers, as well as co-occurrence of active infections across the TORCH panel. Overall, the correlation patterns reveal that while age had minimal impact on serostatus, there was notable co-occurrence of recent infections, particularly between Rubella and CMV, and moderate associations between IgG and IgM for each pathogen. These findings underscore the dynamic nature of TORCH infections in the population, emphasizing the need for careful monitoring of individuals at risk for concurrent or sequential infections.

Gender Differences Across TORCH Serostatus

The relationship between participant gender and TORCH serostatus was evaluated using Fisher's exact tests, comparing males and females across the serostatus categories: No Prior Exposure, Past Infection, Ongoing Infection, and Acute Infection. For TOXO, no significant association with gender was found ($p = 0.738$, OR = 0.639, 95% CI: 0.100–2.947). Similarly, Rubella (RUB) serostatus showed no gender differences ($p = 1.000$), and CMV serostatus also did not differ significantly between males and females ($p = 0.413$). These results indicate that TORCH infection patterns are independent of participant gender in this cohort. (Figure 2) presents grouped bar plots illustrating the distribution of males and females across the serostatus categories for each TORCH infection. The plots visually confirm the statistical findings, showing comparable proportions of males and females in No Prior Exposure, Past Infection, Ongoing Infection, and Acute Infection groups for TOXO, RUB, and CMV. Although minor differences in absolute numbers are observable, they are not statistically meaningful, supporting the conclusion that gender does not significantly influence TORCH serostatus in this population.

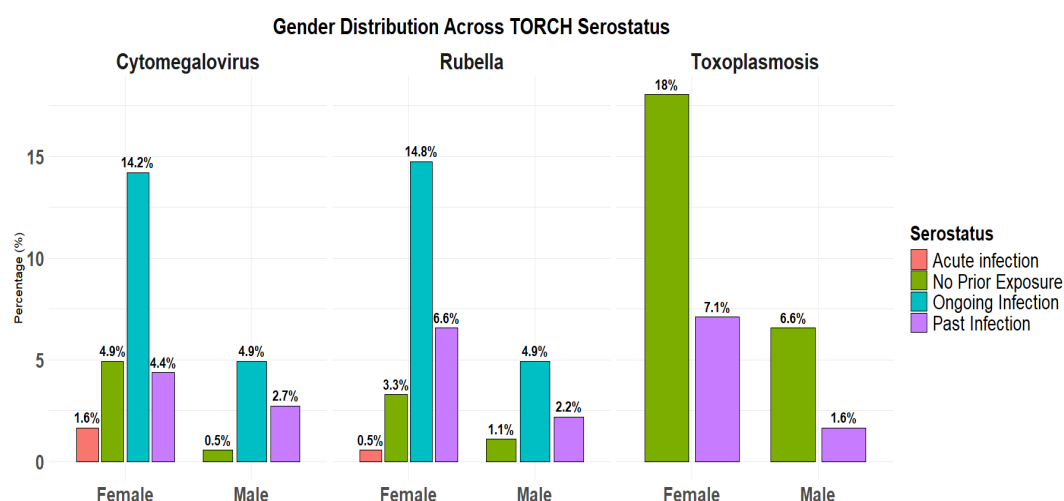


Figure 2. Gender Distribution Across TORCH Serostatus Categories.

Grouped bar plots display the proportion of male and female participants across No Prior Exposure, Past Infection, Ongoing Infection, and Acute Infection categories for TOXO, Rubella (RUB), and CMV. Statistical analysis using Fisher's exact tests showed no significant association between gender and serostatus for any TORCH infection.

Age Differences Across TORCH Serostatus

The relationship between participant age and TORCH serostatus was investigated to identify age-dependent seroprevalence trends. Boxplots and violin plots (Figure 3) provide a visual summary of age distributions across serostatus categories for TOXO, Rubella (RUB), and CMV. For *Toxoplasma gondii* (TOXO), normality testing using the Shapiro-Wilk test indicated that age in the "No Prior Exposure" group deviated from normality ($W = 0.792$, $p < 0.001$), whereas the "Past Infection" group did not ($W = 0.923$, $p = 0.189$). Levene's test confirmed homogeneity of variance ($F = 1.641$, $p = 0.205$), justifying the use of a two-sample t-test. The two-sample t-test revealed a significant difference in age between groups ($t = -2.456$, $df = 59$, $p = 0.017$), with participants in the "Past Infection" group (mean age = 25.96 years) being significantly older than those in the "No Prior Exposure" group (mean age = 15.03 years; 95% CI for difference: -19.83 to -2.02). Boxplots and violin plots (Figure 3a) demonstrate that older participants are more likely to have past TOXO infection, reflecting cumulative exposure over time. Younger individuals predominantly fall into the "No Prior Exposure" category, consistent with the epidemiology of *Toxoplasma*, where infection risk increases with age. For Rubella (RUB), age distributions across the four serostatus categories ("No Prior Exposure," "Acute Infection," "Ongoing Infection," "Past Infection") were examined using ANOVA. Results indicated no statistically significant differences ($F(3, 57) = 2.012$, $p = 0.122$), and Tukey post-hoc comparisons confirmed that all pairwise differences were non-significant (adjusted $p > 0.25$). Boxplots and violin plots (Figure 3b) illustrate that age distributions are relatively similar across Rubella serostatus categories. Unlike TOXO, Rubella immunity and recent infection do not show strong age dependence in this cohort, likely reflecting a combination of vaccination history and early-life natural exposure that is more evenly distributed across age groups. For Cytomegalovirus (CMV), Shapiro-Wilk tests indicated non-normal age distributions in several serostatus groups, including "No Prior Exposure" ($p < 0.001$), "Ongoing Infection" ($p = 0.0029$), and "Past Infection" ($p < 0.001$). Levene's test confirmed homogeneity of variance ($F = 0.772$, $p = 0.515$). ANOVA revealed significant differences in age across serostatus groups ($F(3, 57) = 2.919$, $p = 0.0417$).

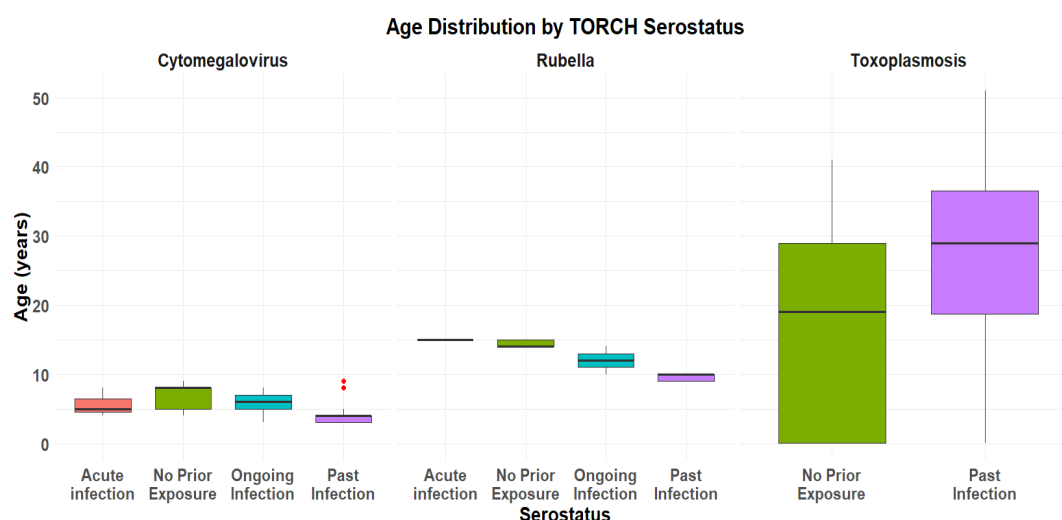


Figure 3. Age Distributions Across TORCH Serostatus Categories

Boxplots and violin plots depict participant age distributions according to serostatus categories for (a) TOXO, (b) Rubella (RUB), and (c) CMV. The visualizations highlight age-dependent seroprevalence trends, showing older participants are more likely to have past TOXO or CMV infections, whereas Rubella serostatus is relatively independent of age. Boxplots and violin plots (Figure 3c) show that mean age increases from the “No Prior Exposure” group to the “Ongoing” and “Past Infection” groups, suggesting that CMV exposure accumulates with age. These plots highlight age-dependent seroprevalence, reflecting both past infection history and the likelihood of ongoing infection in older participants. Overall, (Figure 3) collectively demonstrates age-related trends across TORCH infections. TOXO shows a clear increase in past infection prevalence with age, CMV exhibits age-dependent accumulation of exposure and ongoing infection, and Rubella shows relatively uniform age distribution across serostatus, likely due to vaccination and early-life exposure. These visualizations provide important epidemiological insight, emphasizing the role of age in shaping TORCH serostatus profiles in the study population.

Summary of Statistical Analysis

The statistical analysis of TORCH serology in the study population provides a comprehensive overview of exposure, immunity, and infection patterns. Descriptive statistics revealed the prevalence of past exposure, ongoing infections, and immunity levels across TOXO, Rubella (RUB), and CMV, highlighting heterogeneous serostatus profiles within the cohort. Toxoplasma exposure was relatively low, with past infection increasing with age, whereas Rubella and CMV demonstrated higher rates of both prior exposure and ongoing infection. Correlation analysis using Pearson coefficients identified linear relationships among antibody titers and age. While age showed weak correlations with CMV IgG and IgM, notable associations were observed between RUB IgM and CMV IgM ($r = 0.51$, $p < 0.001$), suggesting a co-occurrence of recent Rubella and CMV infections in certain participants. Moderate correlations between IgG and IgM antibodies within each pathogen further reflected the expected relationships between past exposure and ongoing or recent infection. Fisher’s exact tests were used to assess the relationship between gender and TORCH serostatus. Results indicated no statistically significant associations for any pathogen, suggesting that seroprevalence patterns are largely independent of participant sex. Comparisons across age groups using t-tests for TOXO and ANOVA for Rubella and CMV highlighted significant age-related differences in serostatus. For TOXO, participants with past infection were significantly older than those with no prior exposure, reflecting cumulative lifetime risk. For CMV, age increased progressively from the “No Prior Exposure” group to those with ongoing or past infections. Rubella serostatus showed a relatively uniform age distribution, consistent with early-life exposure or vaccination. Visualizations, including boxplots, violin plots, and grouped bar plots, effectively confirmed these trends, providing clear depictions of seroprevalence stratified by age, gender, and infection stage. Together, these analyses offer a nuanced understanding of the epidemiology of TORCH pathogens in the study population, highlighting age-dependent exposure patterns, co-occurring infections, and overall immunity profiles.

Discussion

The present study provides a comprehensive evaluation of TORCH infections, specifically *Toxoplasma gondii* (TOXO), Rubella virus (RUB), and Cytomegalovirus (CMV), in a diverse Libyan clinical population in 2025. The results reveal heterogeneous seroprevalence patterns, age-dependent exposure for certain pathogens,

and notable co-occurrences of recent infections, offering insights into the epidemiology of these pathogens in this setting.

***Toxoplasma gondii* Seroprevalence**

The TOXO IgG seroprevalence in this study was 26.23%, with no IgM positivity detected, indicating that past exposure is relatively low and acute infections are absent in this cohort. This finding aligns with previous Libyan studies reporting moderate *T. gondii* exposure among adults, with higher rates in older age groups [1, 2, 3]. The significant difference in mean age between participants with past *Toxoplasma* infection (mean 25.96 years) and those with no prior exposure (mean 15.03 years) further supports the hypothesis of cumulative lifetime exposure, consistent with the known epidemiology of *Toxoplasma*, which increases with age [4,5]. These results suggest that while historical exposure exists, the immediate risk of acute *Toxoplasma* infection is minimal in the general population studied, reinforcing the importance of preventive measures among younger, unexposed individuals. The moderate correlation observed between TOXO IgG and IgM ($r = 0.27$, $p = 0.038$) is notable despite the absence of acute infections, possibly reflecting residual low-level IgM in some participants or immunological variability in post-exposure antibody persistence [5,6]. Such patterns indicate that serological screening alone may occasionally detect low-level IgM without clinical infection, emphasizing the need for careful interpretation of TOXO serology, particularly in vulnerable populations such as pregnant women.

***Rubella Virus* Seroprevalence**

Rubella serology demonstrated a high IgG positivity (85.25%) coupled with substantial IgM positivity (60.66%), reflecting both widespread immunity and recent viral circulation. This dual pattern is consistent with the dynamic epidemiology of Rubella in populations with variable vaccination coverage or natural exposure histories [7,8]. The high IgG prevalence indicates effective immunological memory from prior exposure or vaccination, while the high IgM suggests active or recently acquired infections among a significant proportion of participants. Such findings underscore that even in populations with apparent immunity, Rubella remains epidemiologically active, necessitating continuous monitoring to prevent outbreaks and congenital infections [9,10]. Interestingly, the lack of age dependence for Rubella serostatus observed in this cohort, with ANOVA results showing no significant differences across age groups, likely reflects early-life exposure through vaccination or natural infection, as previously reported in population-level studies [11]. This uniformity suggests that Rubella immunity is established early and maintained throughout life, contrasting with TOXO and CMV, where exposure accumulates with age. The moderate positive correlation between RUB IgG and IgM ($r = 0.43$, $p = 0.001$) further highlights the coexistence of long-term immunity and ongoing infection in this cohort. Such serological patterns are clinically relevant for reproductive-aged individuals, emphasizing the need for targeted surveillance to prevent congenital rubella syndrome, which remains a global concern despite vaccination efforts [7,10].

The relatively high IgM seropositivity observed for Rubella and CMV in this study should be interpreted with caution. Unlike population-based serosurveys, the present study was conducted among individuals attending a clinical laboratory, which may disproportionately include participants with recent symptoms, suspected infections, or clinical indications for TORCH testing. Such a sampling framework can lead to an overestimation of IgM positivity when compared to community-based studies. Additionally, variability in vaccination coverage and infection control programs within the local Libyan context may contribute to ongoing viral circulation, particularly for Rubella.

Furthermore, the use of highly sensitive chemiluminescent immunoassays (CLIA) may detect persistent or low-level IgM responses, especially for CMV, where IgM antibodies can be present during viral reactivation or prolonged immune stimulation rather than primary infection. Therefore, IgM positivity in this context does not necessarily indicate acute primary infection but may reflect recent exposure, reactivation, or immunological persistence. These factors collectively provide a plausible explanation for the elevated IgM rates observed and highlight the importance of cautious interpretation of serological markers in clinical-based studies.

***Cytomegalovirus* Seroprevalence**

CMV serology revealed high IgG (78.69%) and IgM (62.30%) positivity, indicating both widespread prior exposure and active/recent infections in the population. These results are consistent with global epidemiological trends, where CMV is highly prevalent and remains circulating even in healthy populations [12,13]. The observed age-dependent increase in CMV serostatus, with older participants more likely to have past or ongoing infection, reflects the cumulative nature of CMV exposure and supports previous findings that CMV seroprevalence increases with age and socioeconomic/environmental factors [14,15]. Moderate correlations between CMV IgG and IgM ($r = 0.46$, $p < 0.001$), as well as between CMV IgM and Rubella IgM ($r = 0.51$, $p < 0.001$), indicate overlapping recent infections in a subset of participants. Such co-occurrence

may reflect shared transmission pathways, host susceptibility, or immunological interactions, consistent with the complex interplay of TORCH pathogens noted in previous studies [16,12]. From a clinical perspective, this co-circulation underscores the importance of monitoring CMV in conjunction with other TORCH infections, particularly among immunocompromised or pregnant individuals.

Age and Gender Patterns

Age emerged as a significant determinant for TOXO and CMV serostatus but not for Rubella. The increase in past TOXO and CMV infections with age aligns with the cumulative exposure hypothesis, whereas the uniform distribution of Rubella serostatus across ages reflects early acquisition via vaccination or natural infection [4,11]. This differential age pattern emphasizes that public health strategies must consider pathogen-specific epidemiology when designing preventive interventions. Gender did not significantly influence TORCH serostatus in this cohort, consistent with prior studies suggesting that exposure risk is largely independent of sex for TOXO, RUB, and CMV in general populations [16,4]. This finding implies that prevention and monitoring strategies should target the population as a whole rather than focusing on gender-specific risk groups.

Co-infection and Correlation Insights

The correlation analysis revealed notable associations between Rubella and CMV IgM positivity, as well as moderate correlations within IgG and IgM pairs for each pathogen. These patterns highlight the potential for simultaneous or sequential infections and suggest that TORCH pathogens do not act in isolation within the host population [16,12]. Recognizing these interactions is crucial for clinical management, particularly in reproductive health settings, where co-infections can compound risks for adverse pregnancy outcomes. The weak correlations between age and antibody titers, except for CMV, indicate that while cumulative exposure is important for certain pathogens, recent infection dynamics may be driven by other factors such as seasonal variation, contact patterns, or vaccination gaps [4,17]. These observations reinforce the value of integrated serological monitoring to detect active infections regardless of age.

Public Health Implications

The heterogeneous serostatus patterns observed in this study underscore the need for tailored public health strategies in Libya. TOXO, with low acute activity but age-dependent past exposure, warrants educational campaigns for younger populations to reduce primary infection risk, particularly among women of reproductive age [6,5]. Rubella, with high IgG and significant IgM positivity, highlights the importance of continuous immunization programs and surveillance to prevent congenital rubella syndrome [9,7]. CMV, with both widespread immunity and active infections, emphasizes the need for monitoring in high-risk groups, such as pregnant women and immunocompromised individuals [12,13]. Moreover, the observed co-occurrence of recent Rubella and CMV infections suggests that simultaneous monitoring of multiple TORCH pathogens may be more informative than single-pathogen screening, allowing early identification of at-risk individuals and timely interventions [16]. Such integrated surveillance is particularly relevant in regions with diverse exposure patterns and mixed vaccination coverage.

Limitations

While the study provides valuable insights, several limitations must be considered. The sample size was relatively small, and recruitment was biased toward females attending clinical services, which may limit generalizability. Additionally, TOXO IgM negativity could reflect the low sensitivity of serological assays for detecting acute infection in low-prevalence populations. Future studies with larger, population-based samples and molecular confirmation of active infections could enhance understanding of TORCH dynamics in Libya. In addition, the interpretation of IgM seropositivity should be approached cautiously, as serological assays alone cannot always distinguish between primary infection, reactivation, or persistent antibody responses without molecular confirmation.

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