



Screening for Toxoplasma, Rubella and Cytomegalovirus Antibodies in Antenatal Mother's: Insights from a Hospital -Based Study in South India.

Dr. R. Maheswari^{1*}, Dr. S. Kartheeswari², Dr. Vishnu Priya³

¹Assistant Professor, Department of Microbiology, Government Medical College, Kallakurichi, Tamilnadu, India.

²Tutor, Department of Microbiology, Government Thiruvavur Medical College, Thiruvavur District, Tamilnadu, India.

³Assistant Professor, Department of Microbiology, Srinivasan Medical College and Hospital, Samayapuram, Trichy District, India.



Correspondence:

Dr R. Maheswari.

Assistant Professor, Department of Microbiology, Government Medical College, Kallakurichi, Tamilnadu, India.

Email:

drmahes1976@gmail.com

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Abstract

Background: Toxoplasma gondii, Rubella virus, and Cytomegalovirus (CMV) are major pathogens responsible for congenital infections and adverse pregnancy outcomes. Serological screening of antenatal mothers helps identify susceptible women and supports preventive interventions against congenital transmission. **Objective:** To determine the seroprevalence of Toxoplasma gondii, Rubella virus, and Cytomegalovirus antibodies among antenatal mothers attending tertiary and secondary care hospitals in South India, and to analyze associations with demographic and obstetric factors. **Materials and Methods:** A cross-sectional hospital-based study was conducted among 193 antenatal mothers. Venous blood samples were collected, and sera were tested for IgG and IgM antibodies against Toxoplasma gondii, Rubella virus, and CMV using commercial ELISA kits. Data were statistically analyzed using SPSS (version 20), and associations with age, gravida, and obstetric history were examined using chi-square tests. **Results:** Overall seropositivity (IgG or IgM) was 41.38% for Toxoplasma gondii, 80.82% for Rubella virus, and 37.82% for CMV. IgG antibodies were more prevalent than IgM across all pathogens, indicating predominant past exposure. Rubella IgG was detected in 76.68% of mothers, while 23.32% remained susceptible to primary infection. No statistically significant association was observed between age or obstetric history and seropositivity ($p > 0.05$). **Conclusion:** High Rubella immunity among antenatal mothers reflects effective vaccine coverage; however, moderate seroprevalence for Toxoplasma gondii and CMV indicates continued risk of maternal and fetal infection. Regular antenatal screening, targeted counseling on hygiene practices, and postpartum Rubella vaccination for non-immune women are essential preventive strategies.

Keywords: Toxoplasma gondii, Rubella virus, Cytomegalovirus, Antenatal screening, Seroprevalence.



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INTRODUCTION

Maternal infections remain a critical cause of perinatal morbidity and mortality worldwide. Among these, *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus (CMV) collectively form a triad of congenital infections that exert profound effects on pregnancy outcomes. These agents are grouped under the TORCH complex, an acronym first proposed by Andre J. Nahmias in 1971 to include *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, and Herpes simplex virus types 1 and 2. Infections with these pathogens, often asymptomatic or mild in the mother, can have catastrophic consequences for the fetus, including spontaneous abortion, stillbirth, congenital malformations, and long-term neurological sequelae. Early detection through antenatal screening and serological assessment remains the cornerstone of prevention and management in reducing the burden of congenital infections in developing countries such as India. Vygi vska LA et al. 2020^[1]

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite. Humans acquire the infection through ingestion of oocysts from cat feces, consumption of undercooked meat, unpasteurized milk, or contaminated water. Transplacental transmission can occur during maternal primary infection, especially during the first trimester, leading to fetal death or severe congenital toxoplasmosis characterized by the classical triad of hydrocephalus, intracranial calcification, and chorioretinitis. Although the risk of transmission increases with gestational age, the severity of fetal disease decreases in later trimesters. The global prevalence of toxoplasmosis varies from 10% to 80%, influenced by dietary habits, climate, and hygiene practices. In India, seroprevalence ranges between 22% and 60%, with regional variations depending on socio-cultural and environmental factors. Preventive strategies include public health education on safe food handling, avoidance of cat contact, and proper sanitation practices. Manjunathachar HV et al. 2020^[2]

Rubella, caused by Rubella virus (family *Togaviridae*, genus *Rubivirus*), is a contagious disease transmitted via respiratory droplets. Although infection in children is often mild, maternal infection during early pregnancy can lead to congenital

Rubella syndrome (CRS), resulting in cardiac, ophthalmic, and auditory defects, as well as intellectual disabilities. The risk of fetal infection is highest (up to 90%) during the first trimester and decreases markedly thereafter. Global elimination of Rubella has been achieved in some developed countries through mass immunization programs, yet it remains endemic in many parts of the world, including India. The introduction of the Measles-Mumps-Rubella (MMR) vaccine into India's Universal Immunization Programme is a pivotal step, but gaps persist in vaccine coverage and awareness. Routine serological screening for Rubella IgG and IgM antibodies among antenatal women helps identify susceptible individuals who can benefit from postpartum immunization. Frimpong C et al. 2017^[3]

Cytomegalovirus (CMV), a ubiquitous herpesvirus, is another major cause of congenital infections. Primary maternal CMV infection, often asymptomatic, carries a 30-40% risk of transplacental transmission. Congenital CMV infection is the leading non-genetic cause of sensorineural hearing loss and neurodevelopmental impairment in infants. In India, CMV seroprevalence among women of reproductive age is high (80-95%), yet primary infections still occur, particularly in lower-socioeconomic populations. Because there is no licensed CMV vaccine, preventive measures depend on behavioral modifications, early diagnosis using ELISA-based detection of specific IgM and IgG antibodies, and neonatal surveillance in suspected cases.

The public health significance of screening for TORCH infections, especially *Toxoplasma gondii*, Rubella, and CMV, lies in their preventability. Studies in India have shown that up to one-third of recurrent pregnancy losses, intrauterine deaths, or congenital malformations may be attributed to these infections. Despite this, routine TORCH screening is often neglected in many antenatal programs due to cost constraints and lack of awareness. The availability of enzyme-linked immunosorbent assay (ELISA) kits for simultaneous detection of IgM and IgG antibodies has facilitated large-scale screening and helped identify acute (IgM-positive) and past (IgG-positive) infections. Hajipour N et al. 2025^[4]

In developing countries, socio-demographic factors such as low education, poor sanitation, rural residence, and lack of immunization play pivotal roles in increasing susceptibility to TORCH infections. A study in South India reported TORCH IgG seropositivity for *Toxoplasma gondii*, Rubella, and CMV as 28%, 84%, and 92%, respectively, while IgM positivity rates were 6%, 3%, and 4%, respectively. These figures highlight the persistent endemicity of these pathogens and the need for universal screening among antenatal mothers. Ambey R et al. 2025^[5]

AIM

To determine the seroprevalence of *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus antibodies among antenatal mothers attending a tertiary and secondary care hospital in South India.

OBJECTIVES

1. To estimate the prevalence of IgG and IgM antibodies against *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus among antenatal women.
2. To analyze the association of seropositivity with demographic and obstetric factors such as age, gravida, trimester, and obstetric history.
3. To identify women susceptible to primary infection and recommend preventive and immunization strategies to reduce adverse pregnancy outcomes.

MATERIALS AND METHODS

Source of Data: Data were obtained from antenatal mothers attending the Obstetrics and Gynecology outpatient and inpatient departments of Dhanalakshmi Srinivasan Medical College and Hospital and the Government Headquarters Hospital, Perambalur, Tamil Nadu. All participants provided informed written consent, and ethical clearance was obtained from the Institutional Ethics Committee.

Study Design: A descriptive cross-sectional hospital-based study was conducted to determine seropositivity of *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus antibodies in antenatal women.

Study Location: The study was conducted in Perambalur district, Tamil Nadu - a predominantly rural region with a population of approximately 5.65 lakh and a literacy rate of 74%. The district is centrally located within Tamil Nadu and characterized by moderate climatic conditions favorable for parasitic persistence.

Study Duration: The study was conducted over 12 months, from January 2018 to December 2018.

Sample Size: A total of 193 antenatal mothers were included in the study.

Inclusion Criteria:

- Pregnant women aged 18-40 years attending antenatal clinics in all three trimesters.
- Willing participants who provided informed consent.

- Women without prior known immunization against Rubella or CMV.

Exclusion Criteria:

- Women unwilling to participate or those who planned to terminate pregnancy.
- Patients with molar pregnancy or known systemic diseases such as diabetes, hypertension, or coronary artery disease.

Procedure and Methodology: Under aseptic precautions, venous blood (approximately 3-5 mL) was collected using sterile syringes. Samples were transported to the microbiology laboratory within 20 minutes, centrifuged to separate serum, and stored at -20°C until analysis. Each serum sample was tested for specific IgM and IgG antibodies to *Toxoplasma gondii*, Rubella virus, and CMV using commercial ELISA kits (RD-Ratio Diagnostics). The principle of the assay was antigen-antibody reaction with peroxidase-linked conjugate and substrate chromogen (TMB). Absorbance was read at 450 nm using a microplate reader. Results were interpreted as positive, negative, or equivocal based on optical density ratios:

Positive: Ratio > 1.1

Negative: Ratio < 0.9

Doubtful: $\pm 10\%$ of the cut-off value.

Doubtful results were repeated; persistently equivocal samples were retested after two weeks.

Sample Processing: The ELISA reagents included antigen-coated microtitration wells, wash concentrate, sample diluent, enzyme conjugate, TMB substrate, and stopping solution. Controls (positive, negative, and cut-off) were processed alongside patient sera to ensure assay validity. Internal quality control was maintained as per kit instructions. The entire assay was performed at 37°C with timed incubation and washing cycles to minimize cross-reactivity.

Data Collection: Demographic data (age, residence, parity), clinical information (trimester, obstetric history, vaccination status, risk factors such as previous abortions or stillbirths), and laboratory findings were recorded in a structured proforma. All data were coded and entered into an electronic database for analysis.

Statistical Methods: Data were analyzed using SPSS software (version 20). Descriptive statistics such as frequencies, percentages, mean $\pm$ SD were used for categorical and continuous variables. Association between seropositivity and risk factors was tested using the chi-square test or Fisher's exact test as appropriate. A p-value < 0.05 was considered statistically significant. Graphical representations such as bar and pie charts illustrated the distribution of IgM and IgG seropositivity across different variables.

RESULTS

Table 1: Overall Seroprevalence of Toxoplasma gondii, Rubella Virus, and Cytomegalovirus Antibodies among Antenatal Mothers

Marker	Positive n/N (%)	95 % CI
Toxoplasma gondii (IgG or IgM)	78 / 193 (41.38 %)	34.6 - 48.5 %
Rubella virus (IgG or IgM)	156 / 193 (80.82 %)	74.6 - 85.7 %
Cytomegalovirus (IgG or IgM)	73 / 193 (37.82 %)	31.2 - 45.0 %

Table 1 presents the overall seroprevalence of *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus antibodies among 193 antenatal mothers. The results show that Rubella virus seropositivity (IgG or IgM) was the highest at 80.82% (95% CI: 74.6-85.7%), indicating widespread immunity in the study population, likely due to prior infection or vaccination. *Toxoplasma gondii* antibodies were detected in 41.38% (95% CI: 34.6-48.5%) of mothers, suggesting moderate endemicity and possible ongoing exposure in the community. In contrast, Cytomegalovirus seropositivity was 37.82% (95% CI: 31.2-45.0%), reflecting frequent subclinical infection or reactivation.

Table 2: Prevalence of IgG and IgM Antibodies against Toxoplasma gondii, Rubella Virus, and Cytomegalovirus

Pathogen	Antibody class	Positive n/N (%)	95 % CI
Toxoplasma gondii	IgG	28 / 193 (14.50 %)	10.3 - 20.0 %
	IgM	11 / 193 (5.69 %)	3.2 - 9.9 %
Rubella virus	IgG	148 / 193 (76.68 %)	70.1 - 82.1 %
	IgM	8 / 193 (4.14 %)	2.1 - 7.9 %
Cytomegalovirus	IgG	59 / 193 (30.57 %)	24.5 - 37.3 %
	IgM	14 / 193 (7.25 %)	4.4 - 11.6 %

Table 2 details the prevalence of IgG and IgM antibodies against the three pathogens. The presence of IgG antibodies reflects past exposure or immunity, while IgM positivity indicates recent or active infection. For *Toxoplasma gondii*, IgG positivity was 14.50% (95% CI: 10.3-20.0%), whereas IgM was detected in 5.69% (95% CI: 3.2-9.9%), suggesting a

smaller subset of recent infections. In the case of Rubella virus, IgG seropositivity was high at 76.68% (95% CI: 70.1-82.1%), supporting effective immunization coverage, while IgM positivity was low at 4.14% (95% CI: 2.1-7.9%), indicating limited recent transmission. Cytomegalovirus demonstrated IgG positivity in 30.57% (95% CI: 24.5-37.3%) and IgM positivity in 7.25% (95% CI: 4.4-11.6%), pointing to frequent exposure and occasional reactivation or new infections.

Table 3: Association of Seropositivity with Demographic and Obstetric Factors

Factor	Levels	Marker (seropositive)	Total positive n/N (%)	χ^2 (df=2)	95 % CI (overall proportion)	P value
Age group	< 25, 26-30, > 30	Toxoplasma IgM	11 / 193 (5.69 %)	0.99	3.2 - 9.9 %	0.6095
	< 25, 26-30, > 30	Rubella IgG	148 / 193 (76.68 %)	1.44	70.1 - 82.1 %	0.4867
	< 25, 26-30, > 30	Rubella IgM	8 / 193 (4.14 %)	0.17	2.1 - 7.9 %	0.9178
	< 25, 26-30, > 30	CMV IgG	59 / 193 (30.57 %)	5.82	24.5 - 37.3 %	0.0545
	< 25, 26-30, > 30	CMV IgM	14 / 193 (7.25 %)	0.40	4.4 - 11.6 %	0.8183
Obstetric history	Bad obstetric history (BOH)	Any TORC IgM	18 / 62 (29.03 %)	3.21	18.8 - 41.7 %	0.2004
	Abortion	Any TORC IgM	18 / 96 (18.75 %)	-	12.1 - 27.6 %	-
	Stillbirth	Any TORC IgM	6 / 22 (27.27 %)	-	13.1 - 48.0 %	-

Table 3 explores the association of seropositivity with demographic and obstetric factors. Across all age groups (<25, 26-30, >30 years), no statistically significant difference was observed in seropositivity rates for *Toxoplasma gondii* IgM ($p = 0.6095$), Rubella IgG ($p = 0.4867$), Rubella IgM ($p = 0.9178$), and CMV IgM ($p = 0.8183$). However, CMV IgG showed a near-significant association with age ($\chi^2 = 5.82$, $p = 0.0545$), suggesting that cumulative exposure may increase with maternal age. Regarding obstetric factors, IgM positivity for any TORC infection was higher in women with bad obstetric history (BOH) (29.03%), compared to those with abortions (18.75%) and stillbirths (27.27%), though these differences were statistically non-significant ($\chi^2 = 3.21$, $p = 0.2004$). This trend indicates a potential link between prior adverse pregnancy outcomes and TORC infections, warranting routine screening and counseling for at-risk mothers.

Table 4: Women Susceptible to Primary Infection (IgG-Negative) and Recommended Preventive Action

Susceptible group	n/N (%)	95 % CI	Recommendation indicator
Toxoplasma (IgG negative)	165 / 193 (85.49 %)	79.6 - 89.9 %	Health education on food and pet hygiene
Rubella (IgG negative)	45 / 193 (23.32 %)	17.9 - 29.9 %	Eligible for post-partum MMR vaccination
Cytomegalovirus (IgG negative)	134 / 193 (69.43 %)	62.4 - 75.7 %	Counseling on hand hygiene and avoidance of saliva/urine contact

Table 4 highlights the proportion of antenatal women susceptible to primary infection, defined as IgG-negative cases. The majority of mothers were susceptible to Toxoplasma infection (85.49%, 95% CI: 79.6-89.9%), indicating limited immunity and ongoing exposure risk, emphasizing the need for health education on food hygiene and avoidance of contact with cat feces. Rubella susceptibility was observed in 23.32% (95% CI: 17.9-29.9%), identifying a target group for postpartum MMR vaccination to prevent congenital Rubella syndrome in future pregnancies. Cytomegalovirus susceptibility was noted in 69.43% (95% CI: 62.4-75.7%), suggesting the need for counseling on hand hygiene, avoiding contact with saliva or urine of young children, and routine antenatal screening in high-risk cases.

DISCUSSION

Table 1 - Overall Seroprevalence In this hospital-based South Indian antenatal cohort ($n = 193$), the overall seropositivity (IgG + IgM) was highest for Rubella virus (80.8%), followed by *Toxoplasma gondii* (41.4%) and Cytomegalovirus (CMV, 37.8%). The high Rubella seroprevalence is consistent with the findings of *Shanmugasundaram D et al.* 2021^[6], who reported immunity rates between 72% and 88% among Indian women of childbearing age, attributed to increasing MMR vaccine coverage and natural exposure. The *T. gondii* seroprevalence (41.4%) lies toward the upper end of Indian ranges (10-45%) reported by *Murhekar M et al.* 2020^[7], influenced by dietary practices, cat exposure, and sanitation levels. Conversely, the CMV rate (37.8%) was lower than the 70-90% IgG prevalence documented in multiple Indian studies (*Frimpong C et al.* 2017^[3]; *Vygivska LA et al.* 2020^[11]). This disparity likely reflects that the present study's "overall" category aggregates IgM and IgG, whereas prior reports often quantified IgG alone (past exposure). The 95 % confidence intervals of our estimates (e.g., Rubella 74.6-85.7 %) overlap with published ranges, reinforcing their representativeness within Indian settings.

Table 2 - IgG/IgM Distribution by Pathogen Rubella IgG positivity (76.7%) in this study aligns with prior antenatal figures of ~70-90 % (Shanmugasundaram D et al. 2021^[6]; Frimpong C et al. 2017^[3]), confirming robust population immunity, whereas IgM (4.1 %) was minimal, reflecting limited recent transmission (Ambey R et al. 2025^[5]). *Toxoplasma gondii* IgG (14.5 %) and IgM (5.7 %) are similar to Hajipour N et al. 2025^[4], Manjunathachar HV et al. 2020^[2], who observed IgG rates between 10-25 % with occasional IgM positivity in high-risk or symptomatic women. The relatively low CMV IgG prevalence (30.6 %) contrasts with the 60-90 % range documented by Singh L et al. 2015^[8] and Shanmugasundaram D et al. 2021^[6], underscoring regional heterogeneity and the influence of urban-rural composition, parity, and exposure to young children. CMV IgM positivity (7.3 %) corresponds with expected low rates of primary or reactivated infection. Collectively, the observed antibody pattern-high Rubella IgG with low IgM, modest *Toxoplasma* exposure, and moderate CMV immunity-mirrors the prevailing epidemiologic landscape across Indian antenatal populations.

Table 3 - Association with Age and Obstetric Factors No statistically significant age-based differences were found for *Toxoplasma* IgM, Rubella IgG/IgM, or CMV IgM, while CMV IgG exhibited a near-significant age trend ($\chi^2 = 5.82$; $p = 0.0545$), implying cumulative exposure with increasing age. Similar age-neutral or weak associations have been reported by Murhekar M et al. 2020^[7], Singh L et al. 2015^[8], Mocanu AG et al. 2021^[9], and Frimpong C et al. 2017^[3], after adjusting for behavioral factors and parity. The absence of an age gradient for Rubella is consistent with universal exposure or immunization across cohorts (Mojarad N et al. 2024^[10]). Among obstetric groups, women with bad obstetric history (BOH) showed higher IgM positivity (29.0 %) compared with those with abortions (18.8 %) or stillbirths (27.3 %), though without statistical significance. Prior Indian studies Singh L et al. 2015^[8]; Mocanu AG et al. 2021^[9] also link TORCH seropositivity-particularly *Toxoplasma* and Rubella-with recurrent pregnancy loss but caution that IgM alone cannot establish causality. Our findings therefore align directionally with earlier evidence, underscoring the need for confirmatory IgG avidity or molecular tests to refine the interpretation of positive IgM results.

Table 4 - Susceptibility to Primary Infection and Preventive Measures High IgG-negativity for *Toxoplasma* (85.5 %) and CMV (69.4 %) underscores the importance of primary-prevention counseling, including safe-food handling and cat-contact avoidance for *Toxoplasma*, and meticulous hand hygiene and saliva/urine avoidance for CMV (Kaushik A et al. 2018^[11]; Murhekar M et al. 2020^[7]; Putera I et al. 2022^[12]). Rubella susceptibility of 23.3 % indicates the need for postpartum MMR vaccination, in line with national guidelines and prior reviews emphasizing immunity gap closure (Shanmugasundaram D et al. 2021^[6]; Mojarad N et al. 2024^[10]; Xie M et al. 2023^[13]). These preventive priorities are consistent with WHO and Indian public-health recommendations advocating targeted education, behavioral risk reduction, and postpartum vaccination strategies to curb congenital infections.

CONCLUSION

The present hospital-based study involving 193 antenatal mothers from South India provides valuable insight into the sero-epidemiological profile of *Toxoplasma gondii*, Rubella virus, and Cytomegalovirus infections during pregnancy. The findings demonstrated a high Rubella seroprevalence (80.82%), indicating substantial immunity due to prior infection or vaccination, whereas *Toxoplasma gondii* (41.38%) and Cytomegalovirus (37.82%) seropositivity reflected persistent endemicity and potential risk of vertical transmission. The prevalence of IgM antibodies was low across pathogens, suggesting limited current or recent infections but continuous exposure risk. No statistically significant association was found between maternal age or obstetric history and seropositivity, although women with bad obstetric history showed higher IgM positivity trends. The study emphasizes the importance of routine antenatal screening for TORCH infections, timely health education regarding food and hygiene practices, and postpartum immunization for Rubella-susceptible women to minimize congenital infections. Strengthening antenatal surveillance and integrating preventive strategies into maternal health programs can substantially reduce fetal and neonatal morbidity associated with these infections.

LIMITATIONS OF STUDY

1. The study was hospital-based and conducted in a single geographic region, which may limit generalizability to the broader population.
2. Only serological assays (ELISA) were used; confirmatory tests such as IgG avidity or molecular diagnostics (PCR) were not performed to differentiate between recent and past infections.
3. Cross-sectional design limits the ability to establish causality or temporal relationship between infection and adverse pregnancy outcomes.
4. The study did not include long-term neonatal follow-up to correlate maternal serostatus with congenital infection or infant morbidity.
5. Possible selection bias existed as only antenatal mothers attending hospital services were included, potentially underrepresenting asymptomatic or rural populations.

6. Socio-demographic and environmental factors influencing infection transmission, such as dietary habits, contact with animals, and vaccination history, were not analyzed in depth.

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