AMERICAN JOURNAL OF BIOSCIENCE AND CLINICAL INTEGRITY
https://biojournals.us/index.php/AJBCI
ISSN: 2997-7347

Article

Comparison of Immunized and Non-Immunized Women Against the Rubella Virus in Relation to Toxoplasmosis in Kirkuk, Iraq

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Citation: Ali O. S. Comparison of Immunized and Non-Immunized Women Against the Rubella Virus in Relation to Toxoplasmosis in Kirkuk, Iraq. American Journal Of Bioscience And Clinical Integrity 2026, 3(5), 43-50.

Received: 05th Feb 2026
Revised: 15th Mar 2026
Accepted: 20th Apr 2026
Published: 22nd May 2026

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Abstract: Rubella and toxoplasmosis are significant teratogenic infections that may cause serious complications in pregnant women, particularly in regions with limited vaccination coverage and insufficient public health awareness. This study aimed to compare the incidence of rubella virus infection and toxoplasmosis among vaccinated and non-vaccinated women in Kirkuk, Iraq, through the assessment of their immunological status. A cross-sectional study was conducted involving 160 women, aged 15-45 years. Blood samples were collected from all participants, and enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of IgM and IgG antibodies against *Toxoplasma gondii* and rubella virus. Participants were classified according to vaccination status and pregnancy condition. The effect of immunization was confirmed by the significantly higher prevalence of Rubella IgG seropositivity among vaccinated women compared with unvaccinated women ($p < 0.00001$). In contrast, *Toxoplasma* IgM positivity was higher in the unvaccinated group (23.6 %), indicating recent exposure to infection. No significant correlation was observed between toxoplasmosis and rubella vaccination status. The findings highlight the importance of dual screening programs for pregnant women and emphasize the need of strengthen vaccination strategies. Public health interventions should address both viral and parasitic infections to reduce the risk of congenital complications.

Keywords: Rubella, Toxoplasmosis, Vaccination, ELISA, IgM, IgG.

Introduction

Rubella is an RNA virus that causes rubella. Paramyxovirus is a group. Primarily, it is transmitted within families. Roughly thirty percent to fifty percent. This implies that the rubella virus will be detrimental to the fetuses of the women who are exposed to it within the first three months of their pregnancies. The rubella virus can easily invade the placenta and the developing embryo over the period of pregnancy [1]. A woman that contracts the rubella virus during the first two or three months of the pregnancy may deliver a child with severe congenital defects such as blindness and hearing [2]. The most severe congenital viral disease, which is rubella, presents such symptoms as deafness, myopia, cataracts, glaucoma, intellectual impairment, congenital heart disease, and low birth weight [3]. In cases where a pregnant woman is infected with the rubella virus in the first trimester of her pregnancy, it may have an impact on her unborn baby. This disease is referred to as congenital rubella

syndrome (CRS). Although there has been a decline in the number of cases of CRS in the world today, the issue of rubella still poses a threat to the African world of health. To prevent CRS, the load of the disease must be measured and the outcomes of the rubella-control activities monitored [4].

The highest burden of the effect of rubella on the congenital abnormalities was experienced in the Southeast Asian and African regions in 2008 with more than 110,000 infants born with CRS (Reference). Nevertheless, analysts forecasted that worldwide, 190,100 CT cases will be experienced in the same regions per year [5]. As a result, the proper treatment of infected people will depend on the diagnosis at an early stage.

Rubella is a mild disease that is normally caused by the rubella virus yet it is dangerous during the season of pregnancy especially to the unvaccinated pregnant women. Congratulatory rubella infection in mothers, particularly in the first trimester is linked with critical teratogenic outcomes, such as miscarriage, stillbirth, and congenital rubella syndrome (CRS). CRS involves the presence of a triad of inborn malformations, sensorineural deafness, cataracts, and cardiac defects and can be accompanied by microcephaly, intellectual disability, and hepato-splenomegaly [6].

It is most likely to cause fetal harm at infection during the first 12 weeks of gestation and then reduces as pregnancy progresses [7]. Rubella and its complications have been shown to be highly prevented with vaccination programs especially those involving the measles-mumps-rubella (MMR) vaccine.

Preconception screening and timely immunization of women of reproductive age are essential to mitigate these risks and prevent CRS-related congenital deformities [8].

A serious public health concern for pregnant women and their fetuses, toxoplasmosis is brought on by the protozoan parasite *Toxoplasma gondii*. Although most immunocompetent persons hardly show symptoms, primary infection during pregnancy can have major effects on the mother and child. Pregnant women who contract congenital toxoplasmosis can breach the placental barrier. Early maternal infections cause more severe foetal infections. Miscarriages, stillbirths, hydrocephalus, cerebral calcifications, chorioretinitis, and developmental problems can all strike [9].

In addition, sick neonates might show eye infections, hearing problems, convulsions, cognitive difficulties, and developmental delays. *T. gondii* and other organisms can cause co-infections that aggravate medical conditions. Since both the rubella virus and *Toxoplasma gondii* are recognized as major members of the TORCH group of congenital infections, co-infection during pregnancy may substantially increase the risk of fetal malformations and adverse pregnancy outcomes [10], [11].

Therefore, the current study aimed to compare the seroprevalence of rubella and toxoplasmosis among vaccinated and unvaccinated women in Kirkuk, Iraq, to assess potential co-infections, and to evaluate the influence of vaccination and demographic factors on infection patterns.

Materials and Methods

Ethical Approval:

The consent was obtained from the Ibn-Nafees Medical Laboratory authority, and was officially validated in September 2024 under administrative issue No. 53. Written informed consent was obtained from all participants prior to enrollment in the study. Demographic and clinical information for each participant was recorded using a structured questionnaire.

Period and place:

The present study was conducted at Ibn-Nafees Medical Private Facility in Kirkuk, Iraq, from September 2024 to May 2025.

Study design and subjects

A cross-sectional study was carried out involving two groups of women: pregnant and non-pregnant participants. The participants were further classified according to their rubella vaccination status.

Patients and Blood samples collection

A total of 160 venous blood samples were collected from women aged 15–45 years, including pregnant participants and non-pregnant controls. The collected samples were incubated in a water bath for 15 minutes to facilitate clot formation and were subsequently centrifuged at 3,000 rpm for 5 minutes to obtain clear, non-hemolyzed serum. The sera were stored at −20°C until further analysis, following standard serum preparation procedures, including coagulation and centrifugation [12].

Laboratory tests:

Enzyme-linked immunosorbent assay (ELISA) kits were used to detect IgM and IgG antibodies against Toxoplasma gondii and Rubella virus in all samples from both study groups. The ELISA kits were purchased from the German Diagnostic Reagent Group (DRG), Germany.

For the detection of anti-Toxoplasma and anti-Rubella IgM antibodies, 49 μL of enzyme reagent was mixed with 1 μL of serum sample. The mixture was then diluted with 50 μL of sample diluent before being transferred into the ELISA wells, followed by the addition of 100 μL for further processing. Positive and negative controls were added in duplicate to wells A2, A3, A4, and A5 of the ELISA microplate, while well A1 was used as a blank control without any solution. The plate was incubated for 1 hour at 37°C and then washed five times with 350 μL of diluted washing solution. After drying, 100 μL of conjugate solution was added to all wells except A1. The plate was incubated for an additional 30 minutes, followed by five washing cycles and drying.

Subsequently, 100 μL of substrate solution was added to all wells, including A1, and the plate was kept in the dark for 15–20 minutes. Finally, 100 μL of stop solution was added to each well, as previously described by Salman [9].

The same procedure was applied for the detection of anti-Toxoplasma IgG and anti-Rubella IgG antibodies, except that the enzyme reagent was omitted. In this assay, 10 μL of serum sample was mixed with 100 μL of sample diluent before being added to the ELISA wells.

Statistical analysis

All experimental data were tabulated and carefully verified for accuracy and consistency prior to statistical analysis. Statistical analyses were performed using standard statistical formulas implemented in Microsoft Excel. Questionnaire data were also included and analyzed accordingly. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 presents the overall infection status among the study groups. The difference in the prevalence of toxoplasmosis and co-infection between the test and control groups was not statistically significant ($p > 0.05$). In contrast, Rubella infection demonstrated a highly significant difference between the groups ($p < 0.001$).

Table 1. Comparison of Toxoplasmosis and Rubella Seropositivity Between Test and Control Groups.

Infection Status	Test Group (%)	Control Group (%)	P-value
Toxoplasmosis Positive	43.39	39.13	P > 0.05
Toxoplasmosis Negative	62.86	60.87	
Rubella Positive	25.71	89.95	P < 0.001
Rubella Negative	74.29	10.05	
Both Positive	12.38	17.39	P > 0.05
Neither Positive	49.52	82.60	

Table 2 presents the seroprevalence of *Toxoplasma gondii* and Rubella antibodies among the test and control groups. Although the difference in toxoplasmosis infection rates between the two groups

was not statistically significant ($p > 0.05$), Rubella seropositivity was markedly higher in the control group, indicating a significant disparity in immunity levels ($p < 0.001$).

Table 2. *Toxoplasma gondii* and Rubella IgM and IgG antibodies in the test and control groups.

Infection	Antibody	Test Group (%)	Control Group (%)	P-value	Significance
Toxoplasma	IgM	23.59	4.3	< 0.001	Highly significant
Toxoplasma	IgG	19.82	43.5	0.002–0.005	Significant
Rubella	IgM	16.99	4.3	< 0.01	Significant
Rubella	IgG	17.93	87.0	< 0.00001	Highly significant

Table 3 illustrates no statistically significant association between rubella vaccination status and *Toxoplasma gondii* seropositivity ($p = 0.233$). However, the seroprevalence of toxoplasmosis was higher among vaccinated women (28.30%) compared with unvaccinated women (15.09%), although this difference was not statistically significant ($P > 0.05$).

Table 3. *Toxoplasma gondii* positive and negative rates among women regarding the mode of vaccination.

Vaccination Status	Total	Toxoplasma -ve (%)	Toxoplasma +ve (%)
Vaccinated	60 (56.61%)	30 (28.30%)	30 (28.30%)
Unvaccinated	46 (43.39%)	30 (28.30%)	16 (15.09%)

Table 4 and Figure 1 demonstrate the relationship between rubella vaccination status and *Toxoplasma gondii* seropositivity. No statistically significant association was observed, suggesting that rubella vaccination status does not influence exposure to *T. gondii*. Furthermore, the results revealed a higher prevalence of Toxoplasma IgM antibodies (17.88%) compared with Rubella IgM antibodies (3.31%), indicating a greater frequency of recent or acute Toxoplasma infection among the studied women ($P < 0.05$).

Table 4. Immune responses against *Toxoplasma gondii* and Rubella virus according to serological markers and clinical interpretation (IgM=acute, IgG=chronic, or immunization, IgM +IgG=subacute).

Pathogen	Total (%)	IgM+IgG (%)	IgG (%)	IgM (%)
Rubella	17.88	2.64	11.92	3.31
Toxoplasma gondii	33.77	3.31	12.58	17.88
Total	51.65	5.96	24.50	21.19

Table 5 demonstrates a statistically significant association between age and rubella seropositivity, particularly in the control group ($P < 0.05$), and partially in the test group. Among control participants, younger women, especially those aged 16–25 and 26–35 years, exhibited higher rubella antibody prevalence compared with older age groups. Although the youngest age group (16–25 years) narrowly failed to reach statistical significance ($P = 0.052$), the 26–35 and 36–45 age groups showed significant associations ($P = 0.039$ and $P = 0.014$, respectively), suggesting a decline in rubella immunity with increasing age.

Similarly, in the test group, rubella seropositivity was significantly associated with age within the 26–45-year range ($P < 0.05$), with the highest immunity observed in the 36–45 age group. The reduction in seropositivity observed in both groups (6.52% in the test group and 1.88% in the control group) did not demonstrate statistically significant differences ($P > 0.05$); however, it may indicate

relatively weaker immunity among older women. This observation may be explained by the absence or limited implementation of childhood vaccination programs in older generations. Overall, the findings suggest that rubella immunity is age-dependent, with younger women exhibiting higher seropositivity rates, particularly in the control group. This trend may reflect the positive impact of vaccination campaigns implemented in younger populations during previous decades.

Table 5. Frequencies of rubella sero-positive in test and control groups concerning women's ages.

Ages (Years)	Total Positive (%) Test group	P-value	Total Positive (%) Control group	P-value
16–25	21.73%	0.045	19.81%	0.052
26–35	30.43%	0.032	21.69%	0.039
36–45	32.60%	0.021	4.71%	0.014
45+	6.52%	0.310	1.88%	0.455

Logistic Regression Analysis of Rubella Seropositivity.

Investigation of the joint effect of age, group (pregnant vs. non-pregnant) and vaccination status on rubella seropositivity was done using a logistic regression analysis (Table 6). It was a statistically significant model ($p < 0.0001$) with a pseudo-R 2 of 0.089 meaning that these variables are collectively significant in explaining a significant amount of the variation in rubella immunity.

Table 6. Predictors of Seropositivity in Pregnant and Non-Pregnant Women.

Variable	Coefficient (β)	p-value	Interpretation
Age 26–35	+0.399	0.0236	Higher odds of seropositivity vs. 16–25
Age 36–45	0.000	1.0000	No significant difference from 16–25
Age 45+	–1.516	<0.0001 ★	Much lower odds of seropositivity
Group: Test (Pregnant)	+0.802	<0.0001 ★	Higher seropositivity vs. Control
Vaccinated	+0.685	<0.0001 ★	Higher seropositivity vs. Unvaccinated

Interpretation

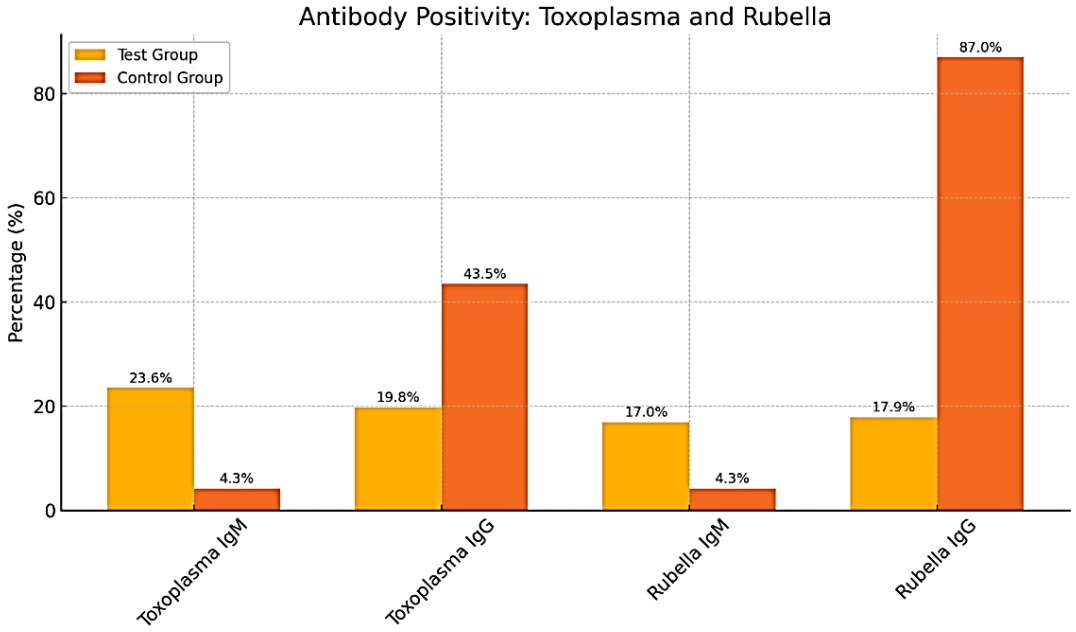

Figure 1. Histogram comparing both the test and control groups, illustrating IgM and IgG antibody levels for both Toxoplasma and Rubella.

Discussion

The findings of the present study demonstrated the prevalence of Toxoplasma gondii and rubella virus antibodies among vaccinated and unvaccinated women in Kirkuk, Iraq. These results provide important epidemiological information regarding the burden of TORCH infections in the region and highlight deficiencies in vaccination coverage and infectious disease control programs.

Rubella IgG seropositivity was significantly higher in the control group (87%) than in the test group (17.93%), suggesting a protective effect of previous vaccination or prior exposure to the virus. These findings are consistent with previous studies conducted in Kirkuk (13), which reported high levels of immunity against rubella among women previously exposed to the virus. Furthermore, Tahseen et al. (2022) reported an increase in rubella infections in Kirkuk since 2003, which was attributed to interruptions in vaccination programs caused by political instability and deficiencies in healthcare services (14).

Mass immunization campaigns using the Measles-Mumps-Rubella vaccine have resulted in high rubella seroprevalence among women of reproductive age in Iran, exceeding 90% in some reports (15). In contrast, studies from Jordan and Turkey identified vulnerable populations, particularly among women from rural or low socioeconomic backgrounds (15). At the international level, the World Health Organization reported in 2020 that sustained vaccination coverage above 80% could potentially lead to rubella elimination (16). In the present study, the test group showed a higher proportion of Toxoplasma IgM positivity (23.6%), indicating recent infection, whereas the control group demonstrated higher IgG positivity (43.5%), reflecting previous exposure and the possible development of protective immunity (17).

Motoi et al. (2020) reported a declining trend in toxoplasmosis among pregnant women in Romania, likely due to improvements in food safety measures (18). In Saudi Arabia, the seroprevalence of toxoplasmosis has been reported to range between 35–45%, particularly among younger women who lacked appropriate prenatal screening programs (19).

Previous studies from Iran have demonstrated toxoplasmosis seroprevalence rates ranging from 30% to 60%, depending on environmental and dietary exposure factors (16). Similarly, Robert-Gangneux and Dardé (2012) documented prevalence rates approaching 50% in certain European countries. The findings of the current study fall within these internationally reported ranges (20). Although co-infection between rubella and toxoplasmosis was observed in both groups, the association was not statistically significant. Nevertheless, concurrent infection during pregnancy represents a serious public health concern because of its teratogenic effects, particularly among immunocompromised individuals (21).

The absence of a significant correlation between rubella vaccination and toxoplasma seropositivity ($p = 0.233$) may be explained by the completely different modes of transmission of the two pathogens. However, the findings emphasize the importance of implementing dual screening strategies during pregnancy (22).

Age-related analysis revealed that older women in the control group exhibited higher immunity against rubella, whereas older women in the test group demonstrated lower immunity levels. Similar observations have been reported in studies conducted in Romania, Brazil, and Mexico, where age, previous immunization history, and cumulative exposure were considered important contributing factors (4, 23, 24).

Vaccination was identified as the strongest predictor of rubella immunity, with an odds ratio approaching two. Pregnant women also demonstrated a higher probability of immunity compared with non-pregnant controls. Age-related variation was evident, as women older than 45 years exhibited the lowest immunity levels, while women aged 26–35 years showed the highest immunity rates, possibly due to previous national vaccination campaigns. These findings underscore the importance of vaccination programs and age-targeted public health interventions for rubella prevention and control.

Conclusion

The study demonstrated significantly higher rubella immunity among vaccinated women compared with non-vaccinated women in Kirkuk, Iraq. Recent *Toxoplasma gondii* infection was more common among non-vaccinated women; however, no significant association was found between rubella vaccination and toxoplasmosis. These findings highlight the importance of rubella vaccination in enhancing immunity and preventing congenital complications. The study also emphasizes the value of routine TORCH screening and continued vaccination programs, particularly for women of reproductive age, to improve maternal and fetal health outcomes.

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