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Seroprevalence of Toxoplasma gondii and Association with ABO/Rh Blood Groups among Pregnant Women in Yafran, Libya

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A B S T R A C T

Background: Toxoplasma gondii is a widespread parasitic protozoan with significant public health implications, particularly for pregnant women due to the risk of congenital transmission. Data on its seroprevalence and associated risk factors, including potential links with ABO/Rh blood groups, are scarce in Libya. Aim: This study aimed to determine the seroprevalence of T. gondii (IgG and IgM) among pregnant women in Yafran, Libya, and to investigate its association with ABO/Rh blood groups and demographic factors.

Methods: A cross-sectional study was conducted from August to October 2025 at Yafran General Hospital. A total of 80 pregnant women were enrolled. Serum samples were tested for anti-T. gondii IgG and IgM antibodies using a commercial rapid immunochromatographic test (Cassette Test). ABO/Rh blood typing was performed using standard slide agglutination methods with monoclonal antisera. Data on demographic characteristics and potential risk factors were collected via a structured questionnaire.

Results: The overall seroprevalence of anti-T. gondii IgG was 26.25% (21/80), while IgM seropositivity was 1.25% (1/80), indicating a low rate of recent infection. Analysis by blood group showed the highest IgG seropositivity rates in women with O- (75%, 3/4) and B- (100%, 1/1) blood types, although sample sizes for these groups were small. The most common blood types, A+ and O+, showed seropositivity rates of 27.3% and 24.2%, respectively. No statistically significant associations were found between seropositivity and age, cat ownership, or history of miscarriage ($p > 0.05$).

Conclusion: This study reveals a moderate seroprevalence of toxoplasmosis among pregnant women in Yafran, Libya. The observed variations in seropositivity across different ABO/Rh blood groups warrant further investigation with larger sample sizes. The findings underscore the need for enhanced prenatal screening and health education programs focused on toxoplasmosis prevention in this region.

Keywords: Toxoplasma gondii, Seroprevalence, Pregnant Women, ABO Blood Group, Rh Factor, Libya

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1. Introduction

Toxoplasmosis is a disease caused by the protozoan Toxoplasma gondii which infects all warm-blooded animals including birds and mammals [1]. However, toxoplasmosis is an important health problem worldwide [2]. The seroprevalence of T. gondii varies widely across different geographical regions, influenced by factors such as climate, dietary habits, sanitation, and cultural practices [3]. Cats and other members of the Felidae family are the definitive hosts for T. gondii. On the other hand, practically all vertebrate, including humans, acts as an intermediary host. Usually, the infection is contracted by eating live tissue cysts in undercooked meat or by consuming sporulated oocysts released by cats to the environment and can be ingested through contaminated food or drink [4]. The disease is either asymptomatic or causes a mild sickness with flu-like symptoms in the majority of immunocompetent people. However, in immunocompromised people or pregnant women, especially in the early stages of gestation, it might cause serious illness [5].Congenital transmission can occur when an uninfected mother contracts the infection during pregnancy, although pregnant women are generally asymptomatic or have relatively minor symptoms, this infection can affect the developing fetus, leading to spontaneous abortion, stillbirth, or serious fetal damage [6].Accurate information regarding the existence and effects of this parasite is vital for healthcare systems, as is increasing our understanding of how to prevent and effectively manage the risks associated with it [7]. To detect toxoplasmosis, various methods have been used, such as serological, histological, molecular, or their combination [8]. In Libya, data on the epidemiology of toxoplasmosis, particularly among the vulnerable population of pregnant women, are limited and outdated. No prior study has investigated a potential link between ABO/Rh blood groups and toxoplasmosis in the Libyan context. Therefore, this study aimed to determine the current seroprevalence of T. gondii infection among pregnant women attending Yafran General Hospital and to explore its association with ABO/Rh blood groups and other demographic variables.

2. Methods

Study Design, Setting, and Period

A hospital-based cross-sectional study was conducted at the Obstetrics and Gynecology Department of Yafran General Hospital, Yafran, Libya. Data and sample collection occurred over three months, from August to October 2025. Pregnant women who were seriously ill or did not provide informed consent were excluded from this study.

Study Population and Sampling

A convenient sample of 80 pregnant women attending antenatal clinics was enrolled. Inclusion criteria comprised pregnant women of any gestational age who provided verbal informed consent to participate. Exclusion criteria included women with known immunosuppressive conditions or those who had received recent blood transfusions that could potentially interfere with serological results.

Data and Sample Collection

Following verbal informed consent, a structured questionnaire was administered through a face-to-face interview to collect demographic data (age, residence, occupation) and information on potential risk factors (e.g., cat ownership, history of miscarriage).

Approximately 10 mL of venous blood was drawn from each participant. The sample was divided: one aliquot was collected in a plain tube for serological analysis, and another was placed in an EDTA tube for blood group determination. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until further analysis.

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Laboratory Analysis

Serological Testing: Detection of anti-T. gondii IgG and IgM antibodies was performed using a commercial rapid diagnostic immunochromatographic test (Cassette Test) according to the manufacturer's instructions. Briefly, 10 µL of serum was mixed with the provided diluent and applied to the test cassette. Results were interpreted after 10 minutes; the appearance of a red line at both the control (C) and the IgG or IgM test lines indicated a positive result.

Blood Group Typing: ABO and Rh (D) blood typing was performed using the standard slide agglutination technique with commercially available monoclonal anti-A, anti-B, and anti-D antisera.

Ethical Considerations

Verbal informed consent was obtained from all participants. The study protocol was approved by the ethical committee of Yafran General Hospital and Almawakib alrayda institute, Yafran, Libya.

Statistical Analysis

Data were analyzed using statistical software (SPSS version 2021). Descriptive statistics were presented as frequencies and percentages for categorical variables. The Chi-square test (or Fisher's exact test where appropriate) was used to assess associations between T. gondii seropositivity (IgG) and independent variables (blood group, age group, cat ownership, miscarriage history). A p-value of < 0.05 was considered statistically significant.

3. Results

Demographic Characteristics of Participants

The study included 80 pregnant women with a mean age of 29.3 years. The majority of participants (51.25%, n=41) were in the 26-35 years age group. The most common ABO/Rh blood types were A+ and O+, each constituting 41.25% (n=33) of the sample. The full demographic profile is presented in Table 1 and Figure 1.

Table 1: Demographic Characteristics of the Study Participants (N=80)

Variable	Frequency (n)	Percentage (%)
Total Sample	80	100
Age Group (Years)		
17 – 25	25	31.25
26 – 35	41	51.25
36 – 45	14	17.50
ABO/Rh Blood Group		
A+	33	41.25
O+	33	41.25
B+	6	7.50
O-	4	5.00
AB+	1	1.25
B-	1	1.25

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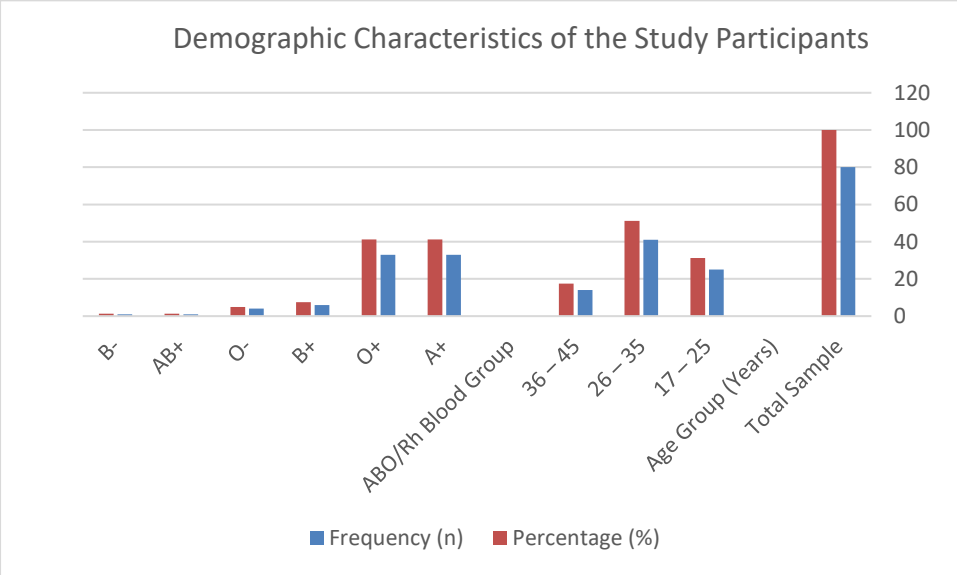

Figure 1: Demographic Characteristics of the Study Participants

Seroprevalence of Toxoplasma gondii Infection

The overall seroprevalence of anti-T. gondii IgG antibodies was 26.25% (21/80), indicating past infection. Only one participant (1.25%) tested positive for IgM antibodies, suggesting a rare incidence of recent/acute infection. Fifty-eight participants (72.5%) were seronegative for both antibodies (Table 2).

Table 2: Seroprevalence of T. gondii IgG and IgM among Pregnant Women

Serological Status	Frequency (n)	Percentage (%)
IgG Positive	21	26.25
IgM Positive	1	1.25
IgG & IgM Negative	58	72.50

Association with ABO/Rh Blood Groups

The distribution of IgG seropositivity across different blood groups is shown in Table 3. The highest proportions of seropositivity were observed in women with O- (75.0%, 3/4) and B- (100.0%, 1/1) blood types. However, the numbers in these categories were very small. Among the more common blood types, seropositivity was 27.3% (9/33) for A+ and 24.2% (8/33) for O+. Statistical analysis did not show a significant association between ABO/Rh blood groups and T. gondii seropositivity in this sample ($p > 0.05$).

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Table 3: Association between ABO/Rh Blood Groups and T. gondii IgG Seropositivity

Blood Group	Total N	IgG Positive n (%)	IgG Negative n (%)
A+	33	9 (27.3%)	24 (72.7%)
O+	33	8 (24.2%)	25 (75.8%)
B+	6	3 (50.0%)	3 (50.0%)
O-	4	3 (75.0%)	1 (25.0%)
B-	1	1 (100.0%)	0 (0.0%)
AB+	1	0 (0.0%)	1 (100.0%)

4. Discussion

The present study provides contemporary data on the seroepidemiology of Toxoplasma gondii infection among pregnant women in Yafran, western Libya. It also explores a potential association with ABO/Rh blood groups, a research question not previously investigated in the Libyan context. The finding of 26.25% IgG seroprevalence indicates a moderate level of exposure to the parasite within this community and contributes to the growing body of epidemiological data on toxoplasmosis across different Libyan regions.

Comparison of Seroprevalence with Other Libyan Studies

The seroprevalence rate observed in Yafran (26.25%) is notably lower than figures reported in several other Libyan investigations. Mahmoud et al. (2019) conducted a large-scale study at Aljalla Maternity and Gynaecology Hospital in Tripoli involving 500 women and reported a substantially higher seroprevalence of 50.8% (95% CI: 46.42%-55.18%) [9]. Similarly, Ajedi and Wheda (2024) investigated 73 women with a history of abortion in the Elmergib region and found anti-T. gondii IgG in 51.00% of cases, indicating high exposure among women with adverse pregnancy outcomes in that area [10].

In Alkhoms city, a study examining 361 pregnant women reported a seroprevalence of 39.3% using ELISA [11]. This intermediate figure falls between the Yafran and Tripoli/Elmergib rates. Gashout et al. (2016) documented prevalence rates in Benghazi ranging between 44.8% and 50% [12], while Setta and Yamani (2008) found a lower prevalence of 18.14% among non-pregnant women in Tripoli [13], suggesting that pregnancy status and study population characteristics significantly influence reported rates.

The Tarhoona study by Al-Majdoub (2015), which included 1,050 women (504 pregnant and 546 non-pregnant), reported that the seropositivity by LATEX test in infected pregnant women ranged from 21-26%, while ELISA-IgG testing showed an overall seropositivity of 50% (24% in pregnant women, 26% in non-pregnant women) [14]. These findings highlight the variability in seroprevalence depending on diagnostic methods and population characteristics.

The variation in seroprevalence across Libyan regions—ranging from 18.14% in Tripoli (non-pregnant women) to 51.00% in Elmergib (women with a history of abortion)—reflects the complex epidemiology of toxoplasmosis and the influence of multiple ecological, behavioral, and demographic factors. Mahmoud et al. (2019) noted that Tripoli's Mediterranean climate, characterized by warm, moist winters, favors the survival of T. gondii oocysts in the environment [9]. Yafran, located in the Nafusa Mountains, experiences different climatic conditions that may affect oocyst survival and transmission dynamics, potentially contributing to the lower seroprevalence observed in this study.

Risk Factors for Toxoplasma gondii Infection

Cat Ownership and Animal Contact: The Yafran study found no statistically significant association between cat ownership and seropositivity,

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which contrasts with findings from several other Libyan investigations. Mahmoud et al. (2019) reported a significantly higher seroprevalence of 78.63% (95% CI: 71.21%-86.06%) among women who kept cats, compared to 42.30% (95% CI: 37.35%-47.25%) among those who did not, with an odds ratio of 1.712 ($p < 0.0001$) [9]. Similarly, the Alkhoms study identified contact with cats as a significant risk factor for acute infection [11]. Alkhunfas (2008) and Magrhi et al. (2003) also corroborated these findings in their respective Libyan studies [15] [16]. However, it is noteworthy that some earlier studies, such as those by Kassem and Morsy (1991) in Benghazi [17], did not find significant associations with cat ownership, suggesting that the role of cats in transmission may vary based on environmental contamination levels and human behavioral practices.

Consumption of Undercooked Meat and Contaminated Food/Water: The consumption of undercooked meat, particularly lamb and mutton, is a well-established risk factor for T. gondii infection [3] [10]. Contaminated vegetables and water also serve as important routes of transmission. The Tarhoona study similarly identified contaminated vegetables and water as significant risk factors, with infection rates of 7.9%-16% in pregnant women and 13%-21% in non-pregnant women attributable to this route [21].

Rural Versus Urban Residence: Residence emerged as a significant risk factor in multiple Libyan studies. Mahmoud et al. (2019) reported a seroprevalence of 67.3% (95% CI: 61.35%-72.75%) among women living in villages compared to 33.2% (95% CI: 27.09%-39.02%) among urban residents, with an odds ratio of 1.482 ($p < 0.0001$) [9]. The authors attributed this difference to free-roaming cats in rural areas, farming activities with direct soil contact, and consumption of unwashed farm produce. The Alkhoms study similarly found significant differences between rural (35.0% seropositive) and urban (45.0% seropositive) residents [11], suggesting that geographical context significantly influences exposure risk.

Age as a Risk Factor: The relationship between age and seropositivity showed consistent patterns across Libyan studies. The Alkhoms study demonstrated increasing seroprevalence with age: 37.5% in the 20-25 years group, 38.3% in 25-30 years, 34.6% in 30-35 years, 40.9% in 35-40 years, and 47.4% in those over 40 years [11]. The authors noted that this association reflects cumulative exposure over time rather than age itself being a risk factor, as IgG antibodies persist for years after infection. The Yafran study found no statistically significant association with age, which may reflect the relatively small sample size and uneven age distribution (51.25% of participants concentrated in the 26-35 years age group). Ajedi and Wheda (2024) similarly found that patient age had no significant relation with T. gondii infection in their sample of aborted women [10], suggesting that in some populations, other risk factors may overshadow age-related cumulative exposure.

Abortion and Pregnancy Outcomes: The relationship between toxoplasmosis and abortion has been extensively studied in Libya. Ajedi and Wheda (2024) specifically focused on women with a history of abortion in the Elmergib region, finding 51.00% seropositivity for anti-T. gondii IgG [10]. This high rate among women with miscarriage history supports the conclusion that toxoplasmosis could be considered a potential risk factor for abortion in Libya. The authors noted that most cases of abortion occur in the acute phase of infection during early pregnancy, emphasizing the importance of preconception screening. The very low IgM seropositivity (1.25%) in the Yafran study suggests that acute infection during pregnancy is uncommon in this cohort, which is a positive public health indicator.

ABO/Rh Blood Groups and Toxoplasmosis Susceptibility

A unique contribution of the present study is its investigation of the association between ABO/Rh blood groups and T. gondii seropositivity—a research question not previously addressed in Libyan toxoplasmosis studies. The observed descriptive trends suggested higher seropositivity in women with O- (75.0%) and B- (100.0%) blood types, though sample sizes were too small for definitive conclusions. This novel line of inquiry draws on biological plausibility regarding blood group antigens serving as receptors or modulators for pathogen attachment. While

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previous international studies have yielded conflicting results on this association [22] [23], the present study represents an important first step in exploring host genetic factors in the Libyan context. Abdullah et al. (2024) in Duhok, Kurdistan Region, Iraq, and Mizuri and Mero (2020) in Zakho City have also explored such associations, though with varying results [22] [23]. The findings from Yafran warrant larger-scale investigations to adequately examine this relationship in the Libyan population.

Immunological and Hormonal Considerations

Although the Yafran study did not investigate hormonal parameters, the Tarhoona study by Al-Majdoub (2015) provided valuable insights into the systemic effects of toxoplasmosis [21]. That study found that T. gondii infection resulted in decreased levels of estrogen, progesterone, and prolactin in infected pregnant women compared to uninfected controls. Although infected pregnant women showed elevations in these hormones during pregnancy, the increases were less pronounced than in uninfected women, suggesting a possible toxic effect of the parasite on hormonal regulation. Additionally, infected pregnant women showed elevated percentages of neutrophils and lymphocytes, indicating immune system alterations that may have implications for pregnancy outcomes.

Methodological Considerations

The varying diagnostic methods employed across Libyan studies complicate direct comparisons. The Yafran study utilized a rapid diagnostic test (RDT), while Mahmoud et al. (2019) employed the Vidas automated enzyme-linked fluorescent immunoassay (ELFA) with >99% sensitivity and >98% specificity [9]. The Alkhoms and Ajedi and Wheda studies used ELISA [10,11], and the Tarhoona study employed both ELISA and Latex agglutination tests [14]. The Yafran study authors appropriately acknowledged that rapid diagnostic tests may have lower sensitivity and specificity compared to reference laboratory methods like ELISA, potentially affecting comparability with studies using more sensitive techniques.

Sample sizes also varied considerably across studies: Yafran (n=80), Mahmoud et al. (2019) (n=500) [9], Alkhoms (n=361) [11], Ajedi and Wheda (2024) (n=73) [10], and Tarhoona (n=1,050) [14]. The Tarhoona study's large sample size provides particularly robust estimates, though its focus on a single region limits generalizability. The Yafran study's smaller sample size, especially for subgroup analyses like rare blood groups, limits statistical power to detect significant associations.

Public Health Implications

The moderate to high seroprevalence rates documented across Libyan studies—26.25% in Yafran, 39.3% in Alkhoms, 50.8% in Tripoli, and 51.0% in Elmergib (women with a history of abortion)—collectively support the importance of implementing routine serological screening for toxoplasmosis in antenatal care programs. Mahmoud et al. (2019) emphasized that primary infection during gestation can lead to placental infection and vertical transmission, with serious consequences for the unborn child [9].

The consistent identification of modifiable risk factors across studies provides clear targets for health education. These include advising pregnant women to avoid handling cat litter, wear gloves when gardening, and wash hands thoroughly after contact with cats [9,11,16,17]; ensuring meat, particularly lamb and mutton, is thoroughly cooked [3,10]; thoroughly washing vegetables and fruits, especially when consumed raw [9,11,21]; improving water quality and treatment, particularly in areas relying on rain or well water [3]; and targeting interventions in rural farming communities with education about soil contact and farm hygiene [9].

The high seroprevalence among women with a history of abortion documented by Ajedi and Wheda (2024) [10] and the hormonal effects observed by Al-Majdoub (2015) [14] underscore the importance of preconception toxoplasmosis screening. Women of childbearing age should know their immune status before pregnancy, as maternal infection acquired before gestation poses little risk to the fetus, while acute

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infection during pregnancy carries significant risks [24].

Limitations

This study has several limitations. The use of a rapid diagnostic test (RDT), while practical for field studies, may have lower sensitivity and specificity compared to reference laboratory methods like ELISA or chemiluminescence. Positive IgM results, in particular, require confirmation with more specific tests to exclude false positives. The cross-sectional design cannot establish causality. The relatively small sample size, especially for subgroup analyses like rare blood groups, limits the statistical power to detect significant associations. Finally, the convenient sampling method may affect the generalizability of the findings to the broader population of pregnant women in Yafran or other Libyan regions.

5. Conclusion

This study confirms that Toxoplasma gondii infection is prevalent among pregnant women in Yafran, Libya, with a seroprevalence of 26.25% for anti-T. gondii IgG antibodies and 1.25% for IgM antibodies. This rate, while lower than figures reported in Tripoli (50.8%), Elmergib (51.0% among women with a history of abortion), and Alkhoms (39.3%), nevertheless indicates moderate exposure to the parasite and highlights the need for attention from public health authorities.

The investigation into ABO/Rh blood groups, although inconclusive due to limited sample size, represents a novel contribution to toxoplasmosis research in Libya. The observed descriptive trends suggesting higher seropositivity in women with O- and B- blood types warrant further investigation with larger, adequately powered samples to conclusively examine this relationship.

The absence of statistically significant associations with age, cat ownership, or history of miscarriage in this study may reflect the relatively small sample size rather than the absence of true epidemiological relationships, as multiple larger Libyan studies have consistently identified these as significant risk factors [9,10,11,16,17].

The very low IgM seropositivity (1.25%) suggests that acute infection during pregnancy is uncommon in this cohort, which is a positive public health indicator. However, the moderate IgG seroprevalence indicates that a substantial proportion of women have been exposed to the parasite and may be at risk of reactivation if immunocompromised.

The findings from this study, considered alongside the broader body of Libyan toxoplasmosis research, support the importance of implementing routine serological screening for toxoplasmosis in antenatal care programs, along with targeted health education campaigns on preventive measures. These should emphasize the well-established risk factors: avoiding contact with cat feces, consuming only well-cooked meat (particularly lamb and mutton), thoroughly washing vegetables and fruits, ensuring safe water sources, and practicing good hand hygiene, especially after gardening or soil contact.

Additionally, larger-scale multicenter studies across Libya are recommended to better determine national seroprevalence, explore potential host genetic factors such as blood groups, and identify region-specific risk factors to inform effective prevention strategies. Such studies should employ standardized diagnostic methods, include diverse populations from different geographical regions, and utilize sample sizes adequate for subgroup analyses.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally.

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