



## Toxoplasmosis in Gharyan, Libya: Seroprevalence and Risk Factors

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### ABSTRACT

**Background:** The seroprevalence and risk factors for *Toxoplasma gondii* infection were examined among high-risk groups in Gharyan, Libya—a semi-arid region with limited prior data. **Aim:** Given the severe health risks of toxoplasmosis in immunocompromised individuals and pregnant women, and the scarcity of studies from western Libya, this research aimed to address a key knowledge gap while comparing results with those from humid coastal cities.

**Methods:** A cross-sectional study was conducted from October 2022 to March 2023, involving 45 blood donors, 40 hemodialysis patients, and 146 pregnant women. A validated questionnaire was administered, and an electrochemiluminescence immunoassay (ECLIA) was used to detect anti-*Toxoplasma gondii* IgG/IgM antibodies.

**Results:** Results revealed an overall higher seroprevalence among hemodialysis patients (40.0%), followed by pregnant women (24.7%), and then blood donors (15.6%). Active (IgM+) infections were rare. Significant risk factors among pregnant women included consumption of unwashed vegetables, undercooked meat, cat ownership, and rural residence (Adjusted Odds Ratio aOR=3.42, aOR=3.15, aOR=2.87, aOR=2.05, respectively). No statistically significant difference in seroprevalence was observed among pregnant women between 2022 (26.2%) and 2023 (23.5%) ( $P = 0.68$ ).

**Conclusion:** A lower seroprevalence was observed in Gharyan municipality compared to coastal Libyan cities, indicating the influence of the dry climate. The study recommends routine first-trimester screening for pregnant women (especially aged 25–30 years), mandatory pre-dialysis testing for hemodialysis patients, and targeted food safety education focusing on vegetable washing and thorough meat cooking.

*Keywords: Toxoplasma gondii, Seroprevalence, Hemodialysis, Pregnant Women, Blood Donors*

### 1.Introduction

*Toxoplasma gondii* was first described in 1908 by Nicolle and Manceaux in the North African rodent *Ctenodactylus gundi*. It is a widespread zoonosis with profound public health implications globally [1]. It was first identified in humans in 1938 at the Babies Hospital in New York,





and it is recognized that approximately one-third of the world's population is infected with *T. gondii* [2]. Transmission occurs through undercooked meat containing tissue cysts, contaminated water or produce with oocysts, unpasteurized dairy products, and iatrogenic routes such as blood transfusion or organ transplantation [3]. Infection may be asymptomatic and self-limiting in immunocompetent individuals, while immunocompromised individuals, pregnant women, and fetuses are at risk of severe consequences including encephalitis, chorioretinitis, stillbirth, and congenital neurological sequelae [4]. The World Health Organization (WHO) classifies toxoplasmosis as a neglected tropical disease, due to its associated significant socioeconomic burdens, high diagnostic costs, the need for lifelong medical management of congenital cases, and productivity losses [5]. Seroprevalence defined as the proportion of individuals in a population who have detectable antibodies (IgG and/or IgM) in their blood serum, indicating past or current exposure to the pathogen. And exhibits distinct geographical variation, influenced by public health infrastructure, dietary practices, and climate. Tropical humid regions show seropositivity rates exceeding 60%, while arid regions show lower prevalence (10-25%) due to reduced environmental oocyst viability [6]. In North Africa, the epidemiological status of toxoplasmosis remains variable and dynamic. A recent meta-analysis encompassing countries of the Eastern Mediterranean Region found a pooled seroprevalence among pregnant women of 36.5% (95% confidence intervals (CI) : 32.6–40.4). In Libya specifically, recorded rates ranged from 26.9% in Al Bayda city to 50.8% in Tripoli [7]. This gradient correlates with climatic variations: coastal areas (high humidity, rainfall) promote oocyst formation and environmental persistence (>1 year), while the arid ecosystems in southern Libya likely suppress transmission [8, 9]. Risk factors identified in Libyan cohorts have included dietary practices such as consuming unwashed vegetables (Odds Ratio [OR]: 1.76), consuming raw/undercooked meat (OR: 1.75). Additionally, animal contact related to cat ownership (OR: 1.71) and soil exposure. Socioeconomic factors have also included rural residence (OR: 1.48) and limited disease awareness [7]. In contrast to data availability in coastal areas, western regions such as Gharyan municipality suffer from a scarcity of data on the parasite's seroprevalence. Gharyan's semi-arid climate (low rainfall, low humidity) theoretically limits oocyst survival, potentially creating a distinct transmission environment compared to coastal cities. Furthermore, no integrated studies have simultaneously examined high-risk groups—blood donors, hemodialysis patients, and pregnant women—within this region, obscuring targeted intervention needs.

### 1.1 Aims:

This study aims to determine immunoglobulin G IgG/ immunoglobulin M IgM seroprevalence of *T. gondii* among blood donors, hemodialysis patients, and pregnant women in Gharyan and identify region-specific risk factors using structured questionnaires assessing dietary habits, animal exposures, and socioeconomic variables. It further compares seroepidemiological profiles with Libyan coastal cities to evaluate climatic effects and develop evidence-based screening and prevention guidelines for healthcare facilities in arid regions. Otherwise, the study aimed to compare the seroprevalence of *T. gondii* between 2022 and 2023.

## 2. Methodology:

### 2.1 study design

A cross-sectional study was conducted from October 2022 to March 2023 at Gharyan Central Hospital and Al-Yusr Laboratory, Gharyan, Libya. This design enabled the simultaneous assessment of *T. gondii* seroprevalence and risk factors across three high-risk groups. The arid climate of Gharyan municipality (average humidity: 35%; annual rainfall: 150mm) was compared with the climate of Libyan coastal regions. A written informed consent was obtained from all participants.

### 2.2 study populations and sampling strategy





Three distinct groups were recruited. First, blood donors (n = 45): healthy male volunteers aged 20-50 years, confirmed negative for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) by standard screening protocols. Individuals with any history of immunosuppressive therapy were excluded. Second, hemodialysis patients (n = 40): patients diagnosed with End-Stage Renal Disease (ESRD) undergoing maintenance hemodialysis for at least three months ( $\geq 3$  months). Third, pregnant women (n = 146): singleton pregnant individuals with gestational ages between 8 and 36 weeks, recruited during routine prenatal care visits at Al-Yusr Laboratory.

### 2.3 Data Collection and Procedures

A questionnaire adapted from Mahmoud et al. [10]. was prepared in Arabic and administered via interview during blood collection to minimize recall bias. It was later translated into English for manuscript preparation. The questionnaire was divided into two sections. First, the demographic characteristics section which included age, place of residence (urban/rural), and educational level. Second, the exposure risk section which focused on dietary habits (undercooked meat, unpasteurized dairy products, unwashed agricultural produce), animal contact (cat ownership, soil exposure through gardening/farming), medical history (prior blood transfusion, organ transplant), and clinical awareness (knowledge of toxoplasmosis transmission/prevention).

### 2.4 Biological Sample Collection and Serology

Participants were enrolled via systematic random sampling (every third eligible patient/donor on recruitment days). Approximately 5 ml of venous blood was drawn from each subject into a sterile plain tube. The sample was left at room temperature, centrifuged at 3000 rpm for 5 minutes, and the separated serum was stored at 4°C until analysis. Anti-*T. gondii* IgG/IgM antibodies were measured using an electrochemiluminescence immunoassay (ECLIA) on a Snibe Maglumi™ 800 analyzer (Snibe Diagnostics, Shenzhen, China).

### 2.5 Statistical Analysis:

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, New York). Descriptive statistics were used to calculate prevalence rates (seroprevalence) for each study group and the distribution of demographic (age, sex, education) and risk factors. Proportions were compared using Fisher's exact test to compare infection rates between blood donors (15.5%) vs. hemodialysis patients (40%) vs. pregnant women (24.6%) and subgroups (infected vs. non-infected based on risk factors). Risk factor analysis was conducted by calculating (OR) and (CI) with 95% to determine the strength of association between each risk factor and infection. Statistical significance was set at  $P < 0.05$ .

## 3.Results

Seroprevalence results for *T. gondii* were evaluated among the 231 participants, comprising 45 blood donors, 40 hemodialysis patients, and 146 pregnant women. The overall seroprevalence differed significantly between groups, with hemodialysis patients showing the highest infection rate.

### 3.1 Age-Specific Seroprevalence in Pregnant Women

Within the pregnant women group for *T. gondii* infections in year 2023 (n=85), notable variation in seroprevalence was observed across different age categories (Table 1). The highest infection rate was found in the 25-30 years' age group (37.0%, 10/27), which was statistically significant ( $\chi^2=6.24$ ;  $p=0.03$ ) compared to other age groups.



**Table 1: Antibody Distribution by Age Group among Pregnant Women in Gharyan, Libya (2023, n=85)**

Age Group (Years)	n	IgG+/IgM+	IgG+/IgM-	IgG-/IgM+	IgG-/IgM-	Seropositive n (%)
19-24	14	0	3	0	11	3 (21.4)
25-30	27	1	8	1	17	10 (37.0)*
31-36	22	1	2	0	19	3 (13.6)
37-42	15	0	3	0	12	3 (20.0)
43-48	7	0	1	0	6	1 (14.3)
Total	85	2	17	1	65	20 (23.5)

\*Peak seropositivity in 25–30 age group ( $\chi^2=6.24$ ;  $p=0.03$ )

### 3.2 Seroprevalence across Study Populations

As detailed in Table 2, seroprevalence was highest among hemodialysis patients at 40.0% (16/40), which was significantly higher than among blood donors at 15.6% (7/45) ( $P < 0.001$ ). Among pregnant women, the overall seroprevalence was 24.7% (36/146). Upon analyzing the pregnant group by enrollment year, seroprevalence was 26.2% (16/61) in 2022 and 23.5% (20/85) in 2023, with no statistically significant difference between both years ( $P = 0.68$ ).

Active infection, indicated by the presence of IgM antibodies, was uncommon across the study population. The overall IgM positivity rate was 1.4%, and these cases were detected exclusively within the pregnant women group.

**Table 2: Seroprevalence of T. gondii IgG and IgM Antibodies among Blood Donors, Hemodialysis Patients, and Pregnant Women in Gharyan, Libya**

Population	n	IgG+ (%)	IgM+ (%)	IgG+/IgM+ (%)	Overall Seropositive (%)
Blood Donors	45	7 (15.6)	0 (0.0)	0 (0.0)	7 (15.6)
Hemodialysis Patients	40	16 (40.0)	0 (0.0)	0 (0.0)	16 (40.0)
Pregnant Women (2023)	85	17 (20.0)	1 (1.2)	2 (2.4)	20 (23.5)





Population	n	IgG+ (%)	IgM+ (%)	IgG+/IgM+ (%)	Overall Seropositive (%)
Pregnant Women (2022)	61	12 (19.7)	0 (0.0)	4 (6.6)	16 (26.2)
Total Pregnant Women	146	29 (19.9)	1 (0.7)	6 (4.1)	36 (24.7)

Note: Total n for pregnant women = 146 (61 in 2022 + 85 in 2023). The overall seropositive percentage (24.7%) is calculated as 36/146.

### 3.3 Risk Factor Analysis for Seropositivity in Pregnant Women

Multivariate regression analysis was conducted to identify independent risk factors associated with *T. gondii* seropositivity among pregnant women (n=146). Results are summarized in Table 3

**Table 3: Adjusted Odds Ratios for Seropositivity in Pregnant Women (n=146)**

Risk Factor	aOR	95% CI	P-value
Consumption of unwashed vegetables	3.42	1.98–5.91	<0.001
Undercooked meat intake	3.15	1.82–5.45	<0.001
Cat ownership	2.87	1.63–5.05	<0.001
Rural residence	2.05	1.18–3.57	0.011
Soil contact	1.32	0.76–2.29	0.327

## 4. Discussion

### 4.1 Climatic Modulation of Seroprevalence

This study provides crucial insights into the seroepidemiology of *Toxoplasma gondii* in a semi-arid region of western Libya, revealing distinct patterns of infection prevalence and associated risk factors among high-risk groups.

The 24.7% seroprevalence among pregnant women in Gharyan aligns with findings from other dry Libyan cities such as Al Bayda (26.9%) and Sebha (25.9%) [8]. However, it is significantly lower than rates reported in humid coastal cities like Tripoli (50.8%;  $\chi^2=22.1$ ,  $p<0.001$ ) and Al Khums (39.3%;  $\chi^2=7.9$ ,  $p=0.005$ ) [7,9]. Based on the clear data gradient, the arid climate environment appears to suppress *T. gondii* transmission. The low humidity and scarce rainfall in Gharyan likely hinder oocyst formation and substantially reduce their environmental viability. This is consistent with global models showing a strong positive relationship between seroprevalence and rainfall [11].

### 4.2 Vulnerability of High-Risk Groups

The high seroprevalence of 40.0% among hemodialysis patients aligns with studies from other countries, such as Egypt, where seroprevalence among hemodialysis patients reached 44.6% [12]. This indicates that immunosuppression overrides the protective effect of the dry climate. The uremic state in end-stage renal disease patients impairs cellular immunity, which may facilitate reactivation of latent infection, a





phenomenon well-documented in immunosuppressed individuals [5]. This underscores the necessity for comprehensive serological screening for patients initiating hemodialysis, as recommended in other high-prevalence settings.

In contrast, blood donors in Gharyan showed lower seroprevalence (15.6%), comparable to healthy populations in other arid areas such as parts of Saudi Arabia [13]. Despite lower prevalence, the risk of transfusion-transmitted infection remains critical, as a single IgG-positive donor could infect multiple immunosuppressed recipients.

#### 4.3 Age and Dietary Risk Dynamics

The peak seropositivity in pregnant women aged 25–30 years (37.0%) is a common observation in seroepidemiological studies, possibly reflecting increased domestic exposures and cumulative environmental risks over time. Similar age-specific peaks have been noted in studies from Brazil and France [14].

Our multivariate analysis highlighted dietary risks as predominant, with unwashed vegetables (aOR=3.42) and undercooked meat (aOR=3.15) showing the strongest associations. This aligns with global meta-analyses identifying undercooked meat consumption as a major risk factor [15]. Interestingly, while cat ownership was a significant risk (aOR=2.87), its effect was secondary to dietary habits. This contrasts with some temperate and humid region studies where cat or soil contact often shows higher attributable risk [16]. The explanation may lie in transmission ecology: in arid regions like Gharyan, high temperatures and low humidity rapidly inactivate oocysts [17]. Thus, while cat ownership remains a risk, ingestion of tissue cysts from improperly prepared food appears to become the primary transmission route, elevating the importance of dietary factors.

#### 4.4 Temporal Stability of Seroprevalence

No statistically significant difference in seroprevalence was observed among pregnant women between 2022 (26.2%) and 2023 (23.5%) ( $P = 0.68$ ). This temporal stability suggests that transmission dynamics in this arid region remain relatively constant over short time periods, reinforcing the role of stable environmental and behavioral factors rather than transient outbreaks.

#### 4.5 Study Strengths and Limitations

A key advantage of this study lies in its comparative, multi-group design, which simultaneously captures infection dynamics across populations with varying immune competence and exposure risks. The use of a standardized ECLIA serological platform and a validated Arabic-language questionnaire strengthened data reliability and allowed for robust multivariate analysis. Additionally, the study contributes critical baseline data from a previously understudied region, enabling more nuanced comparisons with national and regional seroprevalence patterns.

However, several limitations must be acknowledged. The cross-sectional design precludes the establishment of causal relationships between risk factors and infection. The relatively small sample sizes for certain subgroups, particularly hemodialysis patients ( $n=40$ ) and blood donors ( $n=45$ ), may limit the statistical power and generalizability of subgroup comparisons. Furthermore, the study did not perform genotyping of *T. gondii* strains, which could have provided insights into transmission sources. Social desirability bias may also have influenced self-reported dietary and behavioral data, potentially underestimating certain risk exposures.





#### 4.6 Benchmarking Against Regional Studies

**Table 4: Benchmarking Against Regional Studies**

City (Region)	Population	Seroprevalence (%)	Key Risk Factor (aOR)	Climate
Gharyan (Arid)	Pregnant women	24.7	Unwashed vegetables (3.42)	Low humidity
Tripoli (Coastal)	Pregnant women	50.8	Cat ownership (1.71)	High humidity
Alkhoms (Coastal)	Pregnant women	39.3	Rural residence (1.48)	Moderate humidity
Sabha (Desert)	Pregnant women	25.9*	Not reported	Arid

#### 5. Conclusion

This first integrated seroepidemiological analysis of *Toxoplasma gondii* among blood donors, hemodialysis patients, and pregnant women in Gharyan confirms that the region's semi-arid climate is associated with lower seroprevalence (24.7% in pregnant women) compared to humid coastal Libyan cities (50.8% in Tripoli). Dietary practices—specifically consumption of unwashed vegetables (aOR=3.42) and undercooked meat (aOR=3.15)—emerged as the dominant modifiable risk factors, outweighing cat ownership and soil contact in this dry environment. No significant difference in seroprevalence was observed between 2022 and 2023.

Based on these findings, the study recommends: (1) routine first-trimester serological screening for all pregnant women, particularly those aged 25–30 years; (2) mandatory pre-dialysis and annual *T. gondii* testing for hemodialysis patients; and (3) targeted food safety education campaigns emphasizing thorough washing of produce and proper cooking of meat. Future research should incorporate molecular genotyping and larger sample sizes to further refine evidence-based prevention strategies in arid regions.

#### ETHICAL APPROVAL:

This study was approved by the institutional Review Board of Gharyian University (Approval No. IRB/2022/201) on June 15, 2022.

#### CONFLICT OF INTEREST:

The authors declare no conflict of interest

#### AUTHORS' CONTRIBUTION:

The first author contributed in theoretical paper writing; work on data design and collection, while the second author contributed in methodology, revising the manuscript as well as approved the paper for publication.





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