



Seroprevalence of toxoplasmosis among couples who intend marriage in Diyala Province

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Article Info

Article history:

Received 24, 12, 2024

Revised 09, 02, 2025

Accepted 03, 4, 2025

Published 30, 07, 2026

Keywords:

Seroprevalence

Toxoplasmosis

IgG

IgM

ABSTRACT

Toxoplasmosis is a zoonotic disease caused by the parasite *Toxoplasma gondii*. It is a parasite of cosmopolitan distribution present in warm-blooded animals, including humans. The aim of the current study is to determine the extent of toxoplasmosis among couples and to find out the relationship between the toxoplasmosis and some socioeconomic characteristics. The current study has been conducted in Diyala Province from March to August /2024. Blood samples were collected randomly from couples who attended Al-Zahraa Hospital and Baqubah Teaching Hospital. ELISA test was conducted for all samples (376) to detect the infection and determine the presence of toxoplasma IgG and IgM specific antibodies. The results revealed the percentage of presence of toxoplasma IgG males and females were higher than the toxoplasma IgM. The results also illustrated no significant differences in toxoplasma infection between males and females. The results also indicated that the highest prevalence of IgG and IgM was observed in the 20-29 years' age group, while there was no infection observed in the ≥ 40 years' age group. The level of infection with *Toxoplasma gondii* in rural was higher than in urban. Also the results showed that the infection rate was higher in people with a high degree of education than other educational levels. There are still more immunological and socioeconomic factors that need to be assessed in further studies in order to better to understand the seroprevalence of toxoplasmosis in any community.

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1. INTRODUCTION

Toxoplasma gondii (*T. gondii*) is the etiological agent of toxoplasmosis [1]. It is an intracellular protozoan parasite that can infect both people and animals [2]. Approximately 25% of the global population is believed to be at risk of acquiring *T. gondii*, a widely dispersed parasite [3]. Toxoplasmosis is a prevalent parasitic infection in Iraq [4]. *T. gondii* possesses three infectious stages: the rapidly dividing tachyzoite, the slowly developing bradyzoite within tissue cysts, and the environmental sporozoite encased in an oocyst [5]. *T. gondii* infection typically does not elicit clinical manifestations in healthy individuals [6]. Serological surveys reveal that around 80% of initial infections are asymptomatic, attributed to the efficacy of the immune system [7]. Tissue parasitism during the proliferative phase may transpire asymptotically [8]. This may result in a temporary sickness marked by lymphadenopathy, fever, tiredness, arthralgia, dermatosis, malaise, headache, and myalgia [9]. Latent toxoplasmosis is characterized by the persistent presence of cysts and elevated levels of anti-*T. gondii* antibodies in immunocompetent individuals are deemed asymptomatic and benign [10].

The global infection rate ranges from less than 10% to about 90% depending on the diagnosis of anti-parasite antibodies (Anti-Toxoplasma antibodies) in the serum [11]. Toxoplasmosis can have more severe complications and consequences such as birth defects, eye infections, or fatal toxoplasmic encephalitis in immunocompromised individuals [12].

The disease is transmitted to humans through the consumption of raw or undercooked meat, blood transfusions, organ transplants, or the absorption of oocysts introduced into the environment by cats or congenitally during pregnancy [13]. The infection is passed to the fetus of a mother newly infected with *Toxoplasma* during pregnancy, with congenital infection being the most severe form [14]. Congenital toxoplasmosis is a significant infectious etiology of abortion and pregnancy problems globally [15]. Toxoplasmosis is affected by various factors, including environmental conditions, nutritional and cultural practices, and cleanliness [16]. Congenital toxoplasmosis can result in diverse presentations depending on the maternal gestational age, with severe clinical symptoms being more prevalent in women whose infections occurred during early gestation [17]. Conversely, the risk of vertical transmission escalates with gestational age, resulting in the highest transmission rates during the last stages of gestation [18].

Humoral immunity plays an important role in acquired resistance against toxoplasmosis, as immunity develops as the infection progresses, and this leads to the formation of immunoglobulin antibodies [19]. The first antibody formed is IgA, which appears for a short period, and begins to appear when the parasite enters the intestinal lining [20]. It is one of the most important antibodies found in the intestinal lining [19]. It is unstable at the beginning of infection with toxoplasmosis and is considered an indicator of acute infection [21]. If the parasite spreads to the rest of the body, immune globulin begins to appear about 1-2 weeks after infection, and after about 4-8 weeks of infection, IgM begins [22], it decreases and remains for 6 months while IgG appears after the fourth week of infection in people with complete immunity, IgG levels remain high for several months. The current study aimed to investigate the prevalence of *T. gondii* among couples who intended to marry using ELISA Technique. Both IgG and IgM have been measured. Also the demographic characters have been registered.

2. METHOD (10 PT)

2.1. Study Design

The current study was conducted in Diyala Province from March to August /2024 from both genders (males and females) and from Urban and Rural whose ages ranged between 14 to ≥ 40 years. 376 Blood samples were collected randomly from couples who attended Al-Zahraa Hospital and Baqubah Teaching Hospital.

2.2. Blood samples and Immunological tests

Five ml of venous blood was drawn using a 5 ml medical syringe equipped with a 23 mm plier. The blood samples were placed in clean plastic plan tubes devoid of any substance, then left for 15-30 minutes (allow the blood to clot by leaving it undisturbed at room temperature), after which they were placed in a centrifuge for 5 minutes/3000 rpm to separate the blood serum and kept frozen (-20C) until laboratory tests were performed. ELISA test was conducted for all samples (376) to detect the infection and determine the percentage of IgG and IgM specific antibodies by Bioassay Technology Laboratory kit of ELISA from China.

2.3. Statistical analysis

The statistical analysis of this study was done using SPSS program and the results were assessed by t-test and $p < 0.05$ value was considered significant.

3. RESULTS AND DISCUSSION

3.1. Results for Comparative study of IgG and IgM among gender

The sera were analyzed using the ELISA technique, revealing that out of 376 individuals, 12% tested positive for *Toxoplasma* IgG antibodies, comprising 11% of males and 12.9% of females. A substantial difference ($P < 0.001$) existed between the positive and negative toxoplasma IgG antibodies (Table 1). The prevalence of positive *Toxoplasma* IgM participants was lower than that of IgG, at 5.3% (4.7% for men and 6% for females), with a statistically significant difference at $P < 0.001$ between positive and negative *Toxoplasma* IgM antibodies (Table 1). Nonetheless, the present findings indicate a considerable difference between the positive *Toxoplasma* IgG and IgM antibodies (Table 1).

Table 1. Comparative study of total positive and negative IgG and IgM among study participants

Parameters			Groups		Total	P value
			Males (190)	Females (186)	(376)	
Toxoplasma IgG	Positive	no	21	24	45	< 0.001**
		%	11%	12.9%	12%	
	Negative	no	169	162	331	
		%	89%	87.1%	88%	
Toxoplasma IgM	Positive	no	9	11	20	< 0.001**
		%	4.7%	6%	5.3%	
	Negative	no	181	175	356	
		%	95.3%	94%	94.7%	

The study result indicated that, the presence of both IgG and IgM (Tables 1 and 2) in the serum of the study participants and for both genders (males and females). Additionally, the current results revealed that *Toxoplasma* IgG in males and females were higher (12%) than the *Toxoplasma* IgM (5.3%).

Table 2. Comparative study of total positive IgG and IgM among study participants in both gender

Parameter	Male	Female	P value	Total	P value
Positive IgG	21 (11%)	24 (12.9%)	> 0.05	45 (12%)	< 0.01
Positive IgM	9 (4.7%)	11 (6%)	> 0.05	20 (5.3%)	

Our results come in consistent with many other results investigated this point. A study conducted by [23] showed from a total of 180 blood samples that the IgG antibodies for females were (27.6 %), while for males (90.5 %), in the case of IgM, females were (4.8%) and for males was (3.4%). Another study conducted by [20] also showed a higher percentage of IgG compared to IgM, that the prevalence of positive IgM was (5%) lower than IgG which is close to our result. This may be attributed to the relatively brief lifespan of IgM (under 14 days). IgM antibodies are the initial response to toxoplasmosis, with their production increasing temporarily before subsequently declining. Specific IgM levels may decline to undetectable levels within three months' post-infection [24].

A study conducted by [25], which collected serum samples from 691 pregnant women at a University Hospital in Grenoble, France, showed that IgG antibodies arise two weeks' post-infection in patients with toxoplasmosis, peaking at three months. A study done by [26] demonstrated that, among 176 participants (blood samples) in Austin, Texas, IgG antibodies remain at a plateau for six months, subsequently declining gradually or one year in patients with toxoplasmosis until the end of the patient's life. This transpires because of the endurance of dormant cysts in immune-privileged organs, which are locations in the body where foreign tissue grafts can endure for prolonged and frequently endless durations.

Our study revealed a higher *Toxoplasma* Sero-prevalence compared to previous studies. That [27] collected 180 blood samples from women who had infection with bad obstetric History in Al-Anbar Province /Iraq, the study showed a low percentage of IgM (2%). Also, another study done by [28] conducted on 137 patients (males and females) revealed the toxoplasmosis infection in Najaf and Misan governorates in IRAQ was 2 %.

Although we understand that different results in various studies may be due to many reasons which include geographical conditions, sample size social and cultural habits, source of infection (oocyst or tissue cyst), differences in genetic susceptibility, timing of the infection, transmission route, age difference and different strains of *Toxoplasma* [29], we concern about the relatively high percentage of Toxoplasmosis infections (Table 2) compared to previous studies.

3.2. Results of toxoplasmosis according to age groups

Examinations were performed to identify toxoplasmosis in participants categorized by age group. The age distribution of toxoplasmosis was evaluated across four age categories (Table 3). The highest incidence of both IgG and IgM Toxoplasmosis antibodies was noted in the 20-29 age group (9%), followed by the under 20

age group at 4.7%. The 30-39 age group recorded a 3.7% incidence, but the > 40 years' age group reported no current or past infections of toxoplasmosis (Table 3).

Table 3. Distribution of toxoplasmosis according to age groups by ELISA

parameters			Groups			
			Total (376)	IgG +	IgM+	total IgG + IgM
Age groups	< 20	N	76	9	8	17
		%	20.2%	2.5%	2.2	4.7%
	20-29	N	228	24	10	34
		%	60.6%	6.4%	2.7	9%
	30-39	N	55	12	2	14
		%	14.6%	4%	0.5	3.7%
	≥ 40	N	17	0	0	0
		%	4.5%	0%	0%	0%

A study done by [30] who collected 190 blood samples from different regions in the AL-Najaf province of Iraq showed that the highest infection rate (64%) with *T. gondii* parasite was within the age group 18-26 years, while the lowest infection rate (8%) was within the age group 37-47 years. Although, the categories of the age groups are different between this study and our study, the concluding results in these studies are close.

In the study conducted by researchers [31] in Erbil, they found from a total of 300 blood samples that the highest prevalence infection rate of seropositivity for IgM and IgG was in the age group 21-30 which is matching to our results.

Contrary to the findings of the present investigation, [32] analyzed 262 serum samples in Western Romania and determined that the seroprevalence of the parasite exhibits an age-related rise, with a lower infection rate observed in younger women. This association may have arisen from prolonged exposure to risk factors linked to infection, including interaction with animals that harbor the parasite, such as cats. Infection rates of toxoplasmosis rise with age; however, significant disparities exist among various countries and regions, influenced by health standards, dietary practices, and socioeconomic factors [33].

3.3. Results for residence Dependent Seroprevalence

The current results shown in Table 4 revealed that the distribution of participant according to urban and rural is as follows: in the case of IgG+, the percentage of urban was 4.7% while in rural was 7.8%. In the case of IgM+, the percentage of urban individuals was 1.9% while in rural was 3.6% with significant difference at $P < 0.001$.

Table 4. Distribution of toxoplasmosis according to residency

Parameters			Groups			
			Total (376)	IgG+	IgM+	IgG+IgM
Residence	Urban	N	146	17	7	24
		%	38.8%	4.7%	1.9%	6.66%
	Rural	N	230	28	13	41
		%	61.2%	7.8%	3.6%	11.4%

The current findings (Table 4) indicate that the infection rates in rural areas (for both males and females) surpassed those in urban areas, a conclusion supported by several studies. A study by [30] found that the prevalence of toxoplasmosis infection was greater in rural individuals than in urban individuals. Another study [34] conducted a study involving 100 blood samples, revealing that the infection rates of parasitic infections in urban and rural areas of Samarra city, Iraq, were highest among men and women, with acute infection rates of 14.3% in urban areas and 18.5% in rural areas. The elevated infection rates in rural locations relative to urban areas in these studies can be attributed to the typically inadequate supply of clean water in

urban regions, lower hygiene standards, and more interaction with animals, because many animals are intermediate hosts for the parasite, in addition to cats as the final and intermediate host for this parasite.

. Nevertheless, this is not universally applicable. A study done by [35] demonstrated that, from a total of 1,140 blood samples collected from urban and rural populations, the infection rate in the rural area (63.1%) was lower than that in the urban area (81.3%), indicating that the risk of infection was 2.7 times greater in urban settings. The heightened frequency in rural regions has frequently been evidenced in Iran, attributable to poverty, overpopulation, and insufficient water delivery systems [36].

3.4. Results for Education Dependent Toxoplasmosis Seroprevalence

The current results shown in Table 5 revealed that the distribution of participants according to educational level is as follows: in the case of IgG+, the high percentage was at the college level 5.85% while less was in Illiteracy 7.8%. In the case of IgM+, the high percentage was in the Secondary level 1.9% while the lower percentage was in Illiteracy 0.5%. In the case of IgG+IgM, the high percentage was 7.4% at the college level while in Illiteracy was less 1.3% with a significant difference at $P < 0.001$.

Table 5. Distribution of toxoplasmosis associated with level of education

Parameters			Groups			
			Total (376)	IgG	IgM	IgG + IgM
Education	Illiteracy	N	18	3	2	5
		%	4.8%	0.8%	0.5%	1.3%
	Primary	N	107	7	5	12
		%	28.5%	1.9%	1.3%	3.2
	Secondary	N	107	13	7	20
		%	28.5%	3.5%	1.9%	5.3%
	College	N	144	22	6	28
		%	38.2%	5.85%	1.6%	7.4%

According to Table 5, the largest infection percentage was found among individuals with a bachelor's degree or higher, which likely indicates that this demographic comprises the majority of the participants. This indicates that the likelihood of contracting toxoplasmosis is not directly proportional to one's degree of education.

Research typically focuses on the correlation between toxoplasmosis infection and low levels of education. Researchers in Menoufia Governorate, Egypt, found that out of 120 blood samples taken from both sexes, illiterate women knew very little, if anything, about toxoplasma infection. This could be due to a combination of factors, including a lack of education about health issues and a general lack of interest in the disease and its consequences. Findings from a study of 120 women's blood and serum samples taken at Mosul's Al Salam Educational Hospital corroborated those of the prior study [37, 38], which also indicated that women in that city had a relatively low rate of schooling.

3.5. Results for Cats breeding Dependent Seroprevalence

The current results shown in Table 6 revealed that the distribution of precipitants according to cat breeding is as follows: in the case of cat breeding people high percentage was 24% in IgG positive, also IgM positive precipitants had a high percentage of 45% in non-cat breeding precipitants with significant difference at $P < 0.001$.

Table 6. Distribution of males and females according to cats breeding associated with toxoplasmosis

Parameters			Groups			
			Total (376)	IgG+	IgM+	IgG+IgM
Cats breeding	Yes	N	40	10	5	15
		%	10.6%	24%	12.5%	37.5%
	No	N	336	45	40	250
		%	89.4%	13.4%	11.9	74.4%

A study done by [39] analyzed 150 blood samples from cat breeders to assess the prevalence of acute toxoplasmosis in domestic cats exhibiting compatible clinical signs and residing in households with women of childbearing age. The findings indicated a higher infection rate of Toxoplasmosis in females compared to males.

While interaction or cohabitation with cats does not pose a risk for toxoplasmosis, seronegative cats that contract the parasite may excrete oocysts for a duration of 1 to 3 weeks [40]. Toxoplasmosis, induced by *Toxoplasma gondii*, is a protozoan zoonosis that poses significant health risks for pregnant women and immunocompromised individuals. Felids, encompassing domestic cats, are the definitive hosts of *T. gondii*. They release oocysts that, in the environment, become infectious to many animals, including humans, which serve as intermediate hosts [41].

4. CONCLUSION

Toxoplasma gondii is a widespread parasite found throughout the world, infecting many individuals annually. In our study, we detected Toxoplasmosis antibodies, both IgG and IgM, in both males and females. The percentage of IgG was higher than that of IgM, indicating that these participants had been infected with *Toxoplasma gondii* and had recovered from it, often with mild or no symptoms. Additionally, our findings revealed that the highest infection rate was among individuals aged 20 to 29 years. Infection rates were also higher in rural areas compared to urban ones, and higher education levels were correlated with increased rates of infection. Therefore, there is an urgent need to utilize various audio and print media to raise awareness about the risks of this infection. It is essential to inform the public about the dangers of direct contact with cats and other animals that may carry the parasite. To reduce the risk of infection, cat breeders should maintain strict hygiene practices in their facilities. This includes regular cleaning of cages, prompt removal of cat feces, and sanitizing the areas around the cages to prevent the spread of infective oocysts.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to all couples who participated in this study and provided informed consent for interviews and blood sample collection. We also thank the staff and administration of Al-Zahraa Teaching Hospital and Baqubah Teaching Hospital for their cooperation and support in facilitating sample and data collection.

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