

**Original Article**

# Comparisons of *Toxoplasma gondii* Prevalence in Rural and Urban Areas of Al-Najaf Province of Iraq Using Serological Methods

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## Abstract

*Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite that multiplies within the host cell and causes the disease toxoplasmosis. *T. gondii* is distributed worldwide and is capable of infecting almost all warm-blooded animals. The current study was conducted in several urban and rural regions in AL-Najaf province of Iraq from September 2020 to April 2021. In total, 190 blood samples were collected and screened for *T. gondii* IgG and IgM antibodies using Rapid Diagnostic immunochromatographic test and the enzyme-linked immunosorbent assay (ELISA). These two tests were performed on 5 ml of blood samples. The results of the ICT test showed that 80 (42.1%) samples were positive for IgG; however, no IgM positive sample was recorded. The results of ELISA revealed that 27 (33.7%) and 4 (5%) samples were positive for *T. gondii* IgG and IgM antibodies, respectively. The estimated incidence of toxoplasmosis increased significantly in the 21-30 years age group and females ( $P < 0.05$ ), compared to other groups. The wide prevalence of toxoplasmosis was observed in Iraq, especially in Najaf province, which was reflected in the results of the study after taking random samples from different places with no symptoms of the disease. Therefore, all members of the community should undergo periodic examinations to diagnose possible infection through the most accurate tests.

**Keywords:** IgG, IgM, Seroprevalence, *Toxoplasma gondii*

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## 1. Introduction

Toxoplasmosis is a zoonotic disease caused by coccidian protozoa, *Toxoplasma gondii*, with global distribution in a wide range of vertebrates (1).

Humans become infected by ingesting tissue cysts in meat or oocyst from contaminated soil, water, food, or directly from faeces of cats and other feline species (2).

Toxoplasmosis is an important tropical disease with a global distribution that is estimated to infect one-third of the world's human population (3). In addition to the transmission through food, the congenital infection may also cause the disease. The

parasite's life cycle is complete as it passes from warm-blooded intermediate hosts. Felidae are the only known host in which the parasite reproduces sexually and produces oocysts that are shed in the feces of domestic cats (4).

*T. gondii* infection can be detected using several laboratory tests, including serological test, Polymerase Chain Reaction (PCR) techniques, and histological demonstration of parasites in tissue and body fluid and animal inoculation by isolation of organisms (5). General clinical signs of toxoplasmosis include fever, anorexia, or dyspnea, and more specific signs with neural, respiratory, cutaneous, or ocular involvement.

The diagnosis can be complicated due to the wide range of clinical signs. In many cases, following the initial infection, *T. gondii* remains under the control of the immune system in a dormant status and converts to active form only in case of immunosuppression. Serological screening is one way to control the disease, especially in developing countries. (6). Several serological tests have been developed to detect antibodies or antigens with different sensitivities and characteristics (7). The use of an inexpensive, easy-to-use test facilitates screening which is essential for zoonosis prevention, allows proper management of the disease, and diminishes the health burden caused by it (8). The development of immunochromatographic tests (ICTs) that can properly detect toxoplasmosis has gained worldwide attention in recent years. In many countries, researchers have tested different types of ICTs, as a potential alternative screening tool to previous diagnostic methods that require special settings with promising results (9, 10).

Regarding the environmental conditions and the lack of information about the prevalence of *T. gondii* infection and its human risk factors in Iraq, this study aimed to determine the seroprevalence of *T. gondii* infection in urban and rural regions of AL-Najaf province in Iraq.

## 2. Materials and Methods

### 2.1. Sample Collection

In total, 190 blood samples were collected randomly from different zones in Iraq, including Al-Zahraa Teaching Hospital for Maternity and Children; Faculty of Science, Kufa University, and several urban and rural regions in AL-Najaf province. All samples were screened for *T. gondii* IgG and IgM antibodies using the Rapid Diagnostic immunochromatographic test to confirm *T. gondii* infection.

### 2.2. Serological Test for *T. gondii* Antibodies

About 5 mL blood samples were collected from each

individual, and the serological test was carried out for the presence of *T. gondii* IgG and IgM antibodies by the immunochromatography assay and the ELISA technique, according to the manufacturer's instructions. The ICT was conducted using ICT, On SiteToxoIgG/IgM Combo Rapid Test (CTK-Biotech, San Diego, CA, USA, catalog No. R0234C). It is a lateral flow immunoassay for the simultaneous detection and differentiation of IgM and IgG antibodies to *T. gondii* in human serum, plasma, or whole blood. This method is intended as a screening test and provides an initial test result to aid diagnose *T. gondii* infection. The ELISA was using Toxoplasma IgM ELISA Kit (Nova Tec Immunodiagnostica GmbH, Germany, catalog No. 0460, and ToxoIgG (Nova Tec Immunodiagnostica GmbH, Germany, catalog No. 0483) were used to determine parasite antibodies in blood samples. These tests were performed according to the manufacturer's instructions.

### 2.3. Statistical Analysis

In the present study, the data were analyzed using SPSS software (Version 20). The categorical and quantitative variables were presented as percentages and mean±standard deviation (SD). Multivariate binomial logistic regression models were applied to account for the impact of age and gender on toxoplasmosis seroprevalence.

## 3. Results

### 3.1. Immunochromatographic Test

Toxoplasmosis was primarily identified by ICT, and all of the 190 blood samples underwent rapid diagnosis by ICT. The result of ICT showed that 80 (42.1%) and 110 (57.9%) samples were positive and negative for IgG antibodies, respectively. There was a significant difference between positive and negative results ( $P<0.05$ ). Moreover, no positive result was recorded for IgM antibodies in ICT, and therefore all 190 (100%) samples were negative (Table 1)

**Table 1.** Results of the Immunochromatographic test for *Toxoplasma gondii* IgG and IgM antibodies

ICT anti- <i>T. gondii</i> antibodies	Test results (n=190)		P-value (Sig.)
	No. of samples	%	
gG	Positive	80*	42.1%
	Negative	110*	57.9%
gM	Positive	0	0
	Negative	190*	100%

**3.2. Enzyme-Linked Immune Sorbent Assay (ELISA) for Anti-*T.gondii* Antibodies IgG and IgM**

The ELISA is a highly sensitive and inexpensive technique and is mainly used for the routine screening of toxoplasmosis infection since it allows quantitative and semi-quantitative measurements of antibodies. In addition, this method can be used primarily to evaluate the effectiveness of recombinant routines for serodiagnosis (11). The results revealed that 33.7% of samples were positive for IgG *T. gondii* antibodies and 66.3% were seronegative. Moreover, 5% of samples were positive for IgM for *T. gondii* antibodies, and IgM antibodies were not detected in 95% of the samples (Table 2).

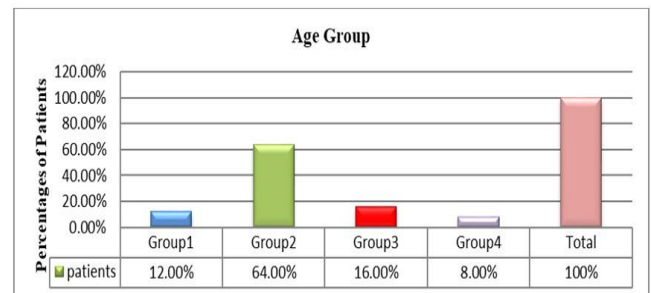
**Table 2.** Results of ELISA for *Toxoplasma gondii* IgG and IgM antibodies

ELISA anti- <i>T. gondii</i> antibodies	Test results (n=80)		P-value (Sig.)
	No. of samples	%	
IgG	Positive	27*	33.7%
	Negative	53*	66.3%
IgM	Positive	4*	5%
	Negative	76*	95%

**3.3. Age and Gender-Dependent Seroprevalence**

Tests were conducted to detect toxoplasmosis in the age groups from 11 to 50 years and determine the greatest percentage of infection (by exposure to the

factors causing most infection, including drinking contaminated water, eating undercooked meat, and other factors) among the age groups. The age distribution of toxoplasmosis was assessed in four age groups. The highest prevalence (64%) was observed in the 21-30 years age group, and females constituted the highest percentage (49%), while the percentage of infected males within this age group was 15%. Moreover, the lowest prevalence of toxoplasmosis (8%) was observed in the 41-50 years age group, in which females constituted all cases infected with toxoplasmosis, while no positive sample was recorded for males in this age group (Figure 1).



**Figure 1.** Age distribution of patients with toxoplasmosis (Group1: 11-20 years, Group 2: 21-30 years, Group3: 31-40 years, Group 4: 41-50 years).

**4. Discussion**

The current study estimated the prevalence of *T. gondii* infection in AL-Najaf province of Iraq. A total of 190 samples were collected and analyzed with Rapid Diagnostic immunochromatographic test for the anti-Toxoplasma IgG and IgM antibodies to confirm *T. gondii* infection. It was determined that 80 (42.1%) blood samples were positive for anti- *T. gondii* antibodies IgG; however, no sample was positive for anti- *T. gondii* antibodies IgM. The ICT method is a rapid qualitative diagnostic test based on immunochromatography technology. This ICT method is easy to use and interpret and does not require special equipment for application in the field (12-14). The development of ICT for the detection of toxoplasmosis has gained attention among researchers worldwide in

the last few years. In many countries, researchers have tested different types of ICT as a potential alternative screening tool to the most commonly used diagnostic methods (8-10, 15).

According to the findings of the present study, ICT detected *T. gondii* IgG antibodies (42.1%), indicating that ICT can be used to detect *T. gondii* antibodies especially chronic infections, which was consistent with the results of the study performed by Hassanein (16). The *T. gondii* ICT test properly diagnosed 21 positive samples with low IgG titers that couldn't be detected by other techniques (8). The result of this study was consistent with the study conducted by Wassef and Abdel-Malek (17) in Cairo which reported a high prevalence of IgG (46.6%). In Pakistan, the results of the study conducted by Sadiqui, Shah (18) revealed that 24.8% of patients were positive for IgG antibodies by ICT. Moreover, another study by Mohammed and Al-Janabi (19) in Sudan reported that the prevalence of IgG was 22.4% using *Toxoplasma* ICT test. In the present study, none of the blood samples were positive for anti-*T. gondii* antibodies IgM. This result can be explained by the fact that IgM is the first antibody that usually appears one week after infection, and the decline takes nine months to come until it is not detected (20). The results of the study conducted by Gomez, Budvytyte (21) on the performance of ICT revealed that the performance of rapid ICT was good to moderate in detecting IgG toxoplasma antibodies; however, the diagnostic accuracy of this method in detecting IgM antibodies was lower, compared to ELISA, especially in detecting IgM antibodies.

Another study (3) compared ELISA and rapid ICT tests in terms of toxoplasmosis diagnosis in Nigeria. The results suggested that the ICT could not detect any IgM, while IgM antibodies (3.8%) were detected using the ELISA method. Eventually, they recommended that although ELISA has been the gold standard for diagnosing toxoplasmosis, ICT is recommended as a preliminary screening tool for diagnosing toxoplasmosis in remote areas with limited facilities due to the fact that this method is faster and less expensive and has a high

specificity and good diagnostic efficiency in detecting IgG. Positive rates of IgM antibodies have been detected in some studies using ICT. Nadayang, Fajrunni (22) found that 6.1% and 93.9% of patients were found to be positive and negative for IgM antibodies using the ICT method, respectively. The results of the study by Vaz, Thomaz-Soccol (23) administered in Brazil revealed that 3.26% of samples were positive for *T. gondii* IgM antibodies. In the study carried out by Tolistiawaty, Rosmini (20), 0.7% of samples were showed seropositivity for IgM *T. gondii* antibodies. Out of 80 serum samples, specific IgG and IgM antibodies to *T. gondii* were positive in 27 (33.7%) and 4 (5%) cases, and negative in 53 (66.3%) and 76 (95%) cases, respectively. Moreover, there was a significant difference between positive and negative results ( $P<0.05$ ).

Diagnosis of toxoplasmosis through ELISA aimed to detect different antigen or antibody classes. The toxoplasma serological profile is very important to differentiate between past and early infection. The presence of IgG antibodies does not imply the timing of infection, and IgM is detectable about one week after infection. In addition, IgA antibodies may persist for several months and be produced earlier than IgM; therefore, these can be considered as markers of acute infection. The IgE is a greater indication of current infection (14, 24, 25). The positive ELISA test for *T. gondii* IgG titers indicates the chronic infection, whereas high IgM titers indicate recent or acute infection. Another study conducted by Hadi and Alomashi (26) in Al Najaf, Iraq, reported that IgG antibody was positive among 47.1% of those with toxoplasmosis. The result of this study was in line with the study performed by Harith and Ban (27) in Baghdad province of Iraq, which reported a high prevalence of IgG (31.70%). Another two studies carried out in Baghdad showed that IgG antibodies were positive in 42.5% and 40.65% of cases, respectively.

Darweesh, Hussein (28) reported that IgG-ELISA was positive in 38% of total cases. In Dhi Qar Province, Iraq, Ali, Mahdi (29) reported that the rate of seroprevalence for IgG antibodies was 36.45% among toxoplasmosis patients. Abdullah and Mahmood (30) found that prevalence of IgG

antibodies to *T. gondii* was 34.8% in Erbil city of Iraq. These results were in line with those reported in a study performed by Mohammed, Salih Mero (31) in Duhok province of Iraq which indicated that 35.61% of patients were seropositive for IgG. These findings were comparable to those obtained by Mousavi-Hasanzadeh, Sarmadian (32), indicating that 33.5% of patients had IgG Anti-Toxoplasma antibodies. Based on the results obtained by Al-Sray, Sarhan (33), 17% of patients were found to be positive for ELISA IgG antibodies in Wasit Province, Iraq. The study conducted by Bakre (34) in Erbil also reported that IgG antibodies in sera were positive in 15.3% of cases. Al-Kadassy, Baraheem (35) reported that 11.3% of toxoplasmosis patients in Yemen were positive for *T. gondii*-specific IgG antibodies. In Iran, Moshfe, Arefkhah (36) found that only 16.30% of cases were seropositive for IgG ELISA antibodies.

For ELISA IgM, the prevalence of positive IgM was 4 (5%), according to the findings of the present study. This may be due to the rather short lifespan of IgM (less than 14 days). IgM antibodies are first to be produced in response to toxoplasmosis, and IgM antibody production rises for a short period of time and declines afterward. The amount of specific IgM may decrease to below the detection level less than 3 months after infection (37, 38). However, IgG antibody appears two weeks after infection among toxoplasmosis patients and peaks in three months. IgG antibody then remains at a plateau level for six months, and after one year begins to decrease gradually to lower levels in toxoplasmosis patients until the end of the infected patient's life. This occurs due to the persistence of latent cysts in immune-privileged organs (sites in the body where foreign tissue grafts can survive for an extended and often indefinite period of time) (39).

The obtained results in this study were consistent with those obtained by Hadi and Alomashi (26) in Al Najaf province of Iraq, reporting that none of the patients in their study population were positive for IgM Anti-*T. gondii* antibodies. Muhsin, Mohsin (40) in Baghdad province of

Iraq found that the rate of IgM antibody among toxoplasmosis patients was 7.5%. The prevalence of IgM antibodies to *T. gondii* was 4 in the study conducted by Mohammed and Al-Janabi (19) in Babylon, Iraq. Al-Sray, Sarhan (33), reported that the IgM-ELISA test was positive in 0.8% of total cases in Wasit province of Iraq. Another study conducted by Bakre (34) in Erbil showed that test results were positive in 5.3% of patients using the *Toxoplasma* IgM-ELISA test kit. Furthermore, this result was compatible with other studies, such as that conducted by Tabbara and Saleh (41) in Bahrain which showed that the *Toxoplasma* seropositivity rate was 5% for IgM. However, the study performed by Tayeb, Salman (42) in Kirkuk province of Iraq revealed that the *Toxoplasma* IgM-ELISA test was positive in 22.87% of cases. The study performed by Al-Kadassy, Baraheem (35) in Yemen reported that 14.4% of patients were positive for IgM Anti-*Toxoplasma* antibodies. Moreover, the prevalence of anti-*toxoplasma* antibodies and their relationship with age showed that most infections occurred at the age range of 21-30 years in females.

Toxoplasmosis infection rates increase with age; however, there is a large difference in infection rates among different countries and regions, according to health standards, dietary habits, and economic and social levels. A decrease in the prevalence of infection has been observed in some countries due to improved hygiene and farming systems (43).

Due to the prevalence of the disease in the region, more studies, including *T. gondii* surveys carried out in animals, food, and water should be performed to identify toxoplasmosis risk factors in AL-Najaf province of Iraq and control the disease.

#### Authors' Contribution

Study concept and design: K. A. A. J.

Acquisition of data: R. A. N.

Analysis and interpretation of data: K. A. A. J.

Drafting of the manuscript: R. A. N.

Critical revision of the manuscript for important intellectual content: K. A. A. J.

Statistical analysis: R. A. N.

Administrative, technical, and material support: K. A. A. J. and R. A. N.

### Ethics

The present study was approved by the Ethics Committee of the University of Kufa, Kufa, Iraq.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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