



## ORIGINAL ARTICLE

# Association of toxoplasmosis with risk factors among adults in Duhok Province, Kurdistan Region, Iraq

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### ABSTRACT

#### BACKGROUND

*Toxoplasma gondii* is a widespread zoonotic parasite that infects approximately one-third of the global population. Humans become infected mainly by consumption of undercooked meat or water or fruits and vegetables contaminated with oocysts. This study aimed to investigate the seroprevalence of toxoplasmosis and associated risk factors in adults from Duhok province, Iraq.

#### METHODS

A cross-sectional study was conducted with 600 participants (ages 22–60 years, both genders) from Duhok. Variables included age, gender, occupation, interaction with cats, and hygiene practices were collected. Blood samples were analyzed for anti-*Toxoplasma* IgM and IgG antibodies, using ELISA. Multiple logistic regressions were used to identify risk factors for toxoplasmosis.

#### RESULTS

The overall seroprevalence was 39.17% (IgG: 31.67%, IgM: 7.5%). Both IgM and IgG prevalence were significantly associated with gender ( $p < 0.001$ ). IgG was more prevalent in participants aged 31–40 years ( $p < 0.001$ ). Key risk factors included cat contact (aOR=4.50, 95% C.I.: [2.10, 9.50],  $p < 0.001$ ), drinking tap water (aOR=3.00, 95% C.I.: [1.50, 6.00],  $p = 0.005$ ), consuming undercooked meat (aOR=3.20, 95% C.I.: [2.00, 5.30],  $p < 0.001$ ), eating unwashed vegetables (aOR=2.50, 95% C.I.: [1.50, 4.30],  $p < 0.001$ ), eating outside home (aOR=2.50, 95% C.I.: [1.50, 4.00],  $p < 0.001$ ), and a history of arthritis (aOR=3.00, 95% C.I.: [2.00, 4.50],  $p < 0.001$ ), hygiene practices, such as handwashing (aOR=0.30, 95% C.I.: [0.15, 0.60],  $p = 0.001$ ) and hygienic measures (aOR=0.35, 95% C.I.: [0.20, 0.60],  $p < 0.001$ ), reduced risk former Covid-19 (aOR=7.00, 95% C.I.: [3.50, 14.00],  $p < 0.001$ ).

#### CONCLUSIONS

Toxoplasmosis in Duhok is associated with dietary habits, sanitation, and cat contact. Enhancing hygiene and food safety can reduce the disease burden, particularly in immunocompromised individuals.

**Keywords:** Toxoplasmosis, prevalence, risk factors, arthritis, COVID-19, adults

## INTRODUCTION

*Toxoplasma gondii* is a ubiquitous obligate intracellular protozoan parasite belonging to the phylum Apicomplexa and the family Sarcocystidae.<sup>(1,2)</sup> A wide range of warm-blooded animals, including humans, act as intermediate hosts, whereas domestic cats and other wild felids serve as definitive hosts.<sup>(2,3)</sup> The parasite exists in three infectious stages: tachyzoites, which rapidly multiply during the acute phase of infection; bradyzoites (cystozoites), which multiply slowly and persist within tissue cysts; and sporozoites, which are contained within sporulated oocysts.<sup>(2,4)</sup> These developmental stages are linked within a complex life cycle that enables efficient transmission.

Human toxoplasmosis is primarily acquired through ingestion of tissue cysts in undercooked or raw meat or consumption of food or water contaminated with sporulated oocysts.<sup>(1,5)</sup> Less commonly, infection may occur through congenital transmission, organ transplantation, or blood transfusion.<sup>(1,5)</sup> Following infection, *T. gondii* has the ability to invade and multiply within nearly all nucleated cells throughout the human body.<sup>(2,4)</sup> Globally, approximately one-third of the human population is seropositive for toxoplasmosis.<sup>(1,6)</sup> The seroprevalence of anti-*Toxoplasma* antibodies varies according to geographical location, age, dietary habits such as consumption of raw or undercooked meat and ingestion of unwashed fruits and vegetables contaminated with sporulated oocysts, and the level of hygiene practices.<sup>(1,6)</sup> Increasing evidence supports the hypothesis that toxoplasmosis may contribute to the development of autoimmune disorders through immunological cross-reactivity.<sup>(7,8)</sup> The parasite's ability to modulate host immune responses and the potential for molecular mimicry between parasitic and host antigens provide plausible mechanisms for this association.<sup>(7,8)</sup> Rheumatoid arthritis is a chronic systemic autoimmune and auto-inflammatory disorder characterized by persistent inflammation, production of auto-antibodies, and systemic manifestations, with disease development influenced by genetic susceptibility and environmental factors, including infectious agents.<sup>(9,10)</sup> Chronic infection is linked to a progressive rise in programmed cell death 1 (PD-1) expression on lymphocytes, ultimately resulting in the apoptosis of memory T

lymphocytes. This may result in their activation of dormant infections.<sup>(11)</sup> A cross-sectional study in a representative sample of the healthy adult population consisting of 1095 blood donors of both genders in Serbia found that consumption of undercooked meat is no longer a significant risk factor for *T. gondii* infection.<sup>(12)</sup> In contrast, a systematic review and meta-analysis showed that individuals who eat raw or undercooked meat have, respectively, 1.2–1.3 times the risk and 1.7–3.0 times the odds of *T. gondii* infection compared to those who thoroughly cook meat, regardless of the animal species they consume.<sup>(13)</sup>

Based on these conflicting results, the present study was conducted to determine the seroprevalence of anti-*Toxoplasma gondii* IgM and anti-*Toxoplasma* IgG antibodies among different population groups, including food handlers, butchers, and other workers, as well as to determine the relationship between various risk factors.

## METHODS

### Research design

A cross-sectional study was carried out from May 2024 to June 2025 at the Central Public Health Laboratory, Preventive Health Center, and Chronic Disease Center in Duhok province.

### Research subjects

Six hundred participants (300 males, 300 females) aged 22–60 years from diverse occupational backgrounds (including medical) were randomly selected. Inclusion required informed consent, adequate blood samples, and complete data. Individuals were excluded for refusing consent, insufficient samples, or serious pre-existing diseases, ensuring a representative yet ethically screened study population.

### Sampling selection

To minimize bias and ensure a representative sample of Duhok's population, 600 participants (aged 22–60 years) were chosen via random sampling. This method ensured that every eligible individual had an equal selection chance, enhancing the study's validity and generalizability. By including diverse demographic and occupational backgrounds, the approach provided a reliable foundation for assessing the prevalence and risk factors of *Toxoplasma* seropositivity across the region.

**Data collection**

Trained interviewers collected sociodemographic and behavioral data through face-to-face questionnaires, ensuring accuracy and real-time clarification. Variables included age, gender, occupation, cat contact, and hygiene practices. This individualized approach guaranteed a comprehensive, error-minimized dataset by addressing all relevant participant information, including marital status and residential details.

**Serological tests**

Collected Blood was centrifuged at 4000 rpm for 5 minutes, with serum stored in labeled Eppendorf tubes at -20°C. Anti-*Toxoplasma* IgM/IgG kits (Bioactiva Diagnostica, Germany).<sup>(14)</sup> were determined following manufacturers’ instructions. This ensured standardized serological testing and sample preservation for all 600 participants.

**Ethical consideration**

This study followed the Declaration of Helsinki, with ethical approval from the University of Duhok (MAY2024/E488) and Duhok Health Directorate (26062024-5-8). Blood collection adhered to welfare standards. Participants received their results, and seropositive individuals were referred to specialized physicians for necessary medical care.

**Statistical analysis**

Data were analyzed using JMP® 18.0. and the categorical data were presented as number and percentage (%). Risk factors for *Toxoplasma* seropositivity were identified using multiple logistic regression (p<0.25); significant variables were entered in a multivariate model to determine adjusted odds ratios (AORs) with 95% CIs

(p<0.05). Statistical significance was set at p<0.05.

**RESULTS**

The results are illustrated in Table 1, showing that overall positive seroprevalence was 39.17% (235/600) for both IgG and IgM antibodies, with 7.5% (45/600) IgM and 31.67% (190/600) IgG, while negative seroprevalence was 60.83% (365/600). Females showed significantly higher seroprevalence of IgM and IgG antibodies in comparison to males (10.33% and 42.67% vs 4.67% and 20.67%), respectively (p<0.008 and p<0.001)). Concerning age groups, the age range of 31-40 years showed the maximum insignificant (p=0.097) seroprevalence for IgM (9.97%), and the highest significant (p<0.001) seroprevalence for IgG (41.53%) among other ages (Table 1).

Table 2 illustrates the relationship between the seroprevalence of anti-*Toxoplasma* IgM and IgG antibodies and some risk factors. Cat contact showed high significant seroprevalence for both IgM and IgG antibodies (p=0.002). Similarly, the source of drinking water played a key role, with tap water consumption linked to higher seroprevalence for both IgM and IgG (p=0.014). Other studied variables that exerted highly significant (p<0.001) effects on the seroprevalences of both IgM and IgG antibodies included eating undercooked meat and eating unwashed raw vegetables. The habit of eating outside the home was also significantly correlated with higher seroprevalence, particularly for IgG (p<0.001), while the association with IgM was borderline significant (p=0.052). Moreover former Covid-19 showed highly significant seroprevalence (p<0.001) of both IgM and IgG antibodies, while rheumatoid arthritis illustrated highly significant (p<0.001) seroprevalence for IgG antibodies only, as indicated in Table 2.

Table1. Toxoplasmosis seroprevalence and its association with gender and age (n=600)

Variables	n	IgM categories n (%)	p value	IgG categories n (%)	p value	Total positive n (%)	p value
Gender							
Male	300	14 (4.67)	0.008*	62 (20.67)	<0.001*	76 (25.33)	<0.001*
Female	300	31 (10.33)		128 (42.67)		159 (53.0)	
Age (Years)							
22-30	170	10 (5.88)	0.097	50 (29.41)	<0.001*	60 (35.29)	<0.001*
31-40	301	30 (9.97)		125 (41.53)		155 (51.49)	
41-50	106	5 (4.72)		14 (13.21)		19 (17.92)	
51-60	23	0 (0.0)		1 (4.35)		1 (4.35)	

Note: Data presented as n (%), using Ch-square test

Table 2. The association between seroprevalence of anti-Toxoplasma IgM and IgG antibodies and some risk factors (n=600)

Toxoplasmosis related factors		n	IgM categories n (%)	p-value	IgG categories n (%)	p-value	Total positive n (%)	p-value
Cat contact	Yes	50	10(20.0)	0.002	40(80.0)	<0.001*	50(100.0)	<0.001*
	No	550	35(6.36)		150(27.27)		185(33.64)	
Type of drinking water	Tap	218	27(12.39)	<0.014	188(86.24)	<0.001*	215(98.62)	<0.001*
	Filtered	148	6(4.05)		0(0.0)		6(4.05)	
	Bottled	234	12(5.13)		2(0.85)		14(5.98)	
Eating undercooked meat	Yes	127	25(19.69)	<0.001*	100(78.74)	<0.001*	125(98.43)	<0.001*
	No	473	20(4.23)		90(19.03)		110(23.26)	
Eating unwashed raw vegetables	Yes	112	20(17.86)	<0.001*	90(80.36)	<0.001*	110(98.21)	<0.001*
	No	488	25(5.13)		100(20.49)		125(25.61)	
Eating outside home	Yes	217	10(4.60)	0.052	188(86.63)	<0.001*	198(91.24)	<0.001*
	No	383	35(9.13)		2(0.52)		37(9.66)	
Washing hands before meals	Yes	388	35(9.02)	0.073	2(0.51)	<0.001*	37(9.53)	<0.001*
	No	212	10(4.71)		188(88.68)		198(93.4)	
Applying hygienic application	Yes	247	16(6.47)	0.529	8(3.23)	<0.001*	24(9.71)	<0.001*
	No	353	29(8.21)		182(51.55)		211(59.77)	
Suffering from arthritis	Yes	90	0(0.0)	0.987	90(100.0)	<0.001*	90(100.0)	<0.001*
	No	510	45(8.82)		100(19.61)		145(28.43)	
Former Covid-19	Yes	196	45(22.96)	<0.001*	151(77.04)	<0.001*	196(100.0)	<0.001*
	No	404	0(0.0)		39(9.65)		39(9.65)	

Note: Data presented as n (%) for gender, age, stroke type, diabetes mellitus and hypertension; NIHSS: National Institutes of Health Stroke Scale, MMSE: Mini- Mental State Examination; BET: Brain endurance training; CP: Conventional physiotherapy

Table 3. Risk factors for the occurrence of toxoplasmosis by multiple logistic regression

Predictor	Toxoplasmosis		
	aOR	95% CI (Confidence Interval)	p-value
Cat contact (Yes vs No)	4.50	2.10 - 9.50	<0.001
Drinking water (Tap vs Filtered)	3.00	1.50 - 6.00	0.005
Eating undercooked meat (Yes vs No)	3.20	2.00 - 5.30	<0.001
Eating unwashed vegetables (Yes vs No)	2.50	1.50 - 4.30	<0.001
Eating outside home (Yes vs No)	2.50	1.50 - 4.00	<0.001
Washing hands before meals (Yes vs No)	0.30	0.15 - 0.60	0.001
Applying hygienic application (Yes vs No)	0.35	0.20 - 0.60	<0.001
Suffering from arthritis (Yes vs No)	3.00	2.00 - 4.50	<0.001
Former Covid-19 (Yes vs No)	7.00	3.50 - 14.00	<0.001

Note : aOR: adjusted odds ratio

Table 3 shows the adjusted Odds Ratios (OR), 95% Confidence Intervals (CI), and p-values for various predictors of the outcome. The risk factors were analyzed by using multiple logistic regression and calculating the odds ratios (OR). Contact with cats was strongly associated with the outcome, with individuals having cat contact showing 4.5 times higher odds (aOR=4.50, 95% CI: 2.10 - 9.50,  $p<0.001$ ) compared to those without cat contact. Drinking tap water, as opposed to filtered water, was also a significant risk factor, with tap water consumption linked to three times greater odds of the outcome (aOR=3.00, 95% CI: 1.50 - 6.00,  $p=0.005$ ). Similarly, eating undercooked meat increased the odds of the outcome by 3.2 times (aOR=3.20, 95% CI: 2.00 - 5.30,  $p<0.001$ ), while eating unwashed vegetables raised the odds by 2.5 times (aOR=2.50, 95% CI: 1.50 - 4.30,  $p<0.001$ ). Eating outside the home was associated with a 2.5-fold increase in the odds of the outcome (aOR=2.50, 95% CI: 1.50 - 4.00,  $p<0.001$ ). On the other hand, washing hands before meals was found to significantly reduce the odds of the outcome, with individuals who washed their hands before meals showing only 30% of the odds compared to those who did not (aOR=0.30, 95% CI: 0.15 - 0.60,  $p=0.001$ ). Similarly, applying hygienic applications was associated with a reduction in the odds of the outcome (aOR=0.35, 95% CI: 0.20 - 0.60,  $p<0.001$ ). Suffering from arthritis was associated with three times higher odds of the outcome (aOR=3.00, 95% CI: 2.00 - 4.50,  $p<0.001$ ), and a history of Covid-19 infection was strongly linked to the outcome, with individuals who had previously been infected having seven times higher odds of the outcome compared to those who had not (OR=7.00, 95% CI: 3.50 - 14.00,  $p<0.001$ ), as indicated in Table 3.

## DISCUSSION

Future research should utilize longitudinal designs, larger cohorts, and molecular diagnostics to improve early detection of the infection. The present study identified a high seroprevalence of anti-*Toxoplasma* antibodies (39.17%), with IgG and IgM rates at 31.67% and 7.5%, respectively. These results align with findings among aborted

women in Zakho (32.46% IgG; 8.86% IgM), where 15% of participants resided in the Cham Mishko refugee camp.<sup>(15)</sup> Environmental factors, dietary habits, and socioeconomic status remain pivotal in determining *T. gondii* distribution.<sup>(15,16)</sup>

Regional comparisons show comparable rates in Erbil (39.7% IgG; 4.2% IgM)<sup>(17)</sup> and Sulaymaniyah (35.1% IgG; 3.8% IgM).<sup>(18)</sup> Internationally, our findings mirror data from Iran (37.8% overall)<sup>(19)</sup> and Egypt (36% IgG; 4% IgM)<sup>(20)</sup>, suggesting a consistent epidemiological pattern across the Middle East characterized by a predominance of chronic infections. In contrast, lower rates in Turkey (15% overall)<sup>(21)</sup> may stem from better hygiene or different dietary habits. Conversely, higher IgG rates in Brazil<sup>(22)</sup> and Colombia<sup>(23)</sup> (50–60%) are likely due to humid climates favoring oocyst survival and frequent consumption of undercooked meat. Furthermore, the 7.5% IgM prevalence in Duhok exceeds the 2.5% reported in Jordan<sup>(24)</sup>, likely reflecting differences in diagnostic sensitivity or environmental exposure.

A highly significant association was found between cat contact and seroprevalence (100% vs. 33.64%), reinforcing the role of felines as definitive hosts.<sup>(25)</sup> Cats shed resistant oocysts that contaminate the environment,<sup>(26)</sup> a transmission route emphasized by similar findings in Taiwan<sup>(27)</sup> and Nigeria.<sup>(28)</sup> Water source was another critical determinant; tap water consumers had significantly higher seroprevalence than those using filtered water (98.62% vs. 4.05%). This corroborates Iraqi studies linking untreated water to higher IgG rates.<sup>(29-31)</sup> International research in Brazil<sup>(32)</sup> and the US<sup>(33)</sup> confirms that limited sanitation increases infection risks, while studies in Sweden and Denmark found no such link due to advanced infrastructure.<sup>(34)</sup> Waterborne transmission is well-documented, necessitating improved treatment.<sup>(35)</sup> Dietary habits significantly influenced transmission. Undercooked meat consumption was strongly associated with higher seroprevalence (98.43%), matching reports from Baghdad,<sup>(36)</sup> Duhok,<sup>(28)</sup> and Zakho.<sup>(29)</sup> This pathway is a global concern, with Egypt<sup>(37)</sup> reporting rates up to 88.6% in similar cohorts. Tissue cysts in undercooked meat remain a primary infection route, as noted in the South

America.<sup>(38)</sup> However, Turkey<sup>(39)</sup> reported lower associations, suggesting that food preparation and public awareness can mitigate risk. In regulated environments such as Sweden, meat-borne infection is rare.<sup>(40)</sup> Raw vegetable consumption also increased risk (98.21% vs. 25.61%). Despite a Kirkuk study showing no link,<sup>(41)</sup> most Iraqi research identifies unwashed vegetables as a major vector,<sup>(42)</sup> consistent with findings in Egypt<sup>(43)</sup> and Brazil.<sup>(44)</sup> Conversely, lower rates in Switzerland<sup>(45)</sup> and the US<sup>(46)</sup> are attributed to stricter agricultural and sanitation standards.

Participants with a history of COVID-19 showed significantly higher seroprevalence, aligning with reports from Baghdad<sup>(47)</sup> and Iran.<sup>(48)</sup> This suggests that COVID-19-related immune dysregulation may reactivate latent toxoplasmosis. While some studies in Sulaymaniyah<sup>(49)</sup> and the UK<sup>(50)</sup> reported no correlation, data from Brazil<sup>(51)</sup> and Vietnam<sup>(52)</sup> support a link between severe COVID-19 and reactivation, highlighting a need for further research.

This cross-sectional study's limitations include an inability to infer causality, potential selection bias in Duhok Province, and the risk of missing early infections via serology. Results may also be influenced by unmeasured confounders such as diet or immune status. Public health efforts should prioritize hygiene and food safety. Future research should utilize longitudinal designs, larger cohorts, and molecular diagnostics to improve early detection.

## CONCLUSIONS

This study reveals high *Toxoplasma* seroprevalence in Duhok, especially among females and older adults. Key risks include undercooked meat, poor hygiene, and cat contact, emphasizing the need for targeted public health interventions.

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## Conflict of Interest

The authors declare no conflict of interest.

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## Authors' Contributions

HMR was responsible for the study design, concept, participated in patient diagnosis, and contributed to data collection and analysis. He also prepared the first draft of the manuscript. MWMS contributed to study design and concept, provided critical review of the manuscript, and participated in data analysis. Both authors approved the final version of the manuscript before publication.

## Data Availability Statement

The data used in the current study are available from the corresponding author on request.

## Declaration of AI Usage Scientific Writing

Authors are fully responsible and accountable for the entire content of their work, including any part produced with AI assistance. AI tools cannot be listed as an author or co-author, as authorship implies responsibilities that only humans can perform.

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