Detection of Toxoplasma gondii antibody in epileptic patients in Mosul city

Saeed H. Hussein, MSc* Hiba M. Ahmed, MSC** Nawar Y. Ahmad, FIBMS*** Aya AH. Al-Noman, MBchB***

ABSTRACT

Epilepsy is a relatively common neurological disease characterised by involvement of certain parasites forming cysts in brain tissue, including Toxoplasma gondii. Objectives: To assess the prevalence of Toxoplasma gondii antibodies among patients with epilepsy and healthy controls and evaluate its potential association with epilepsy. A case-control study included 90 newly diagnosed patients with unprovoked convulsive epilepsy of unknown aetiology and 90 healthy individuals without neurological disorders. Serum samples were tested for anti-Toxoplasma gondii IgM and IgG antibodies using commercially available ELISA kits. Among epilepsy patients, 13 (14.4%) were IgM-positive, and 42 (46.6%) were IgG-positive, compared with 9 (10%) IgM-positive and 36 (40%) IgG-positive controls (p>0.05), while contact with cats was significantly associated with epilepsy (p=0.038). The rate of exposure to Toxoplasma gondii is slightly higher among epilepsy patients, with a relationship between cat contact and epilepsy reflecting the potential role of environmental exposure. However, seropositivity alone does not appear to be a determining factor in epilepsy development.

Keywords: Epilepsy, IgM antibodies, IgG antibodies, Seropositivity, Toxoplasma gondii.

INTRODUCTION

Toxoplasma gondii is a common parasite that is an intracellular inhabitant and results in an infectious disease known as toxoplasmosis, which can mainly infect humans and many warm-blooded animals[1,2]. Approximately one-third of the general population has been exposed to this parasite, with rates varying based on the region of exposure and lifestyle of the exposed population[3]. Infection can occur by eating undercooked meat with contaminated cysts, consumption of food or water contaminated with oocysts from cats, or through mother-to-fetus transmission during pregnancy[4,5]. Cats are the ultimate hosts and release oocysts into the environment, which can persist for long periods[4]. In healthy individuals, infection is usually mild or symptomless, but the parasite can form cysts in tissues like the brain and muscles[4]. These latent infections may alter brain function and perhaps cause neurological diseases, including seizure disorders[6].

Second only to stroke and Alzheimer's disease, epilepsy is a common neurological disorder, impacting about 1% of the general population[7,8]. In approximately two-thirds of patients, the causative agent is unknown and is known as cryptogenic epilepsy[7,9]. Epilepsy is a recurrent seizure affecting approximately 50million people worldwide. Central nervous system infections, including toxoplasmosis, are associated with triggering seizures[10]. Experimental preclinical studies demonstrated that chronic T. gondii infection can cause brain inflammation and modulate neurotransmitter mechanisms, which may increase seizure risk[11,12]. However, epidemiological research on the relationship between toxoplasmosis and epilepsy is lacking; some studies report higher rates of T. gondii antibodies in epilepsy patients, while others report non-significant differences[5,13]. These differences may be due to sample size, population characteristics, or the methods used to detect infection. Therefore, more research is needed to better understand the relationship between toxoplasmosis and epilepsy. The current study was designed to assess the seroprevalence of Toxoplasma gondii antibodies among patients with epilepsy.

PATIENTS AND METHODS

Study design and patients: In this case-control study, a total of 180 participants were enrolled, with 90 epileptic patients and 90 healthy control subjects. The study was conducted from January 2024 to September 2025 at Al Salam Teaching Hospital and Ibn-Sina Teaching Hospital in Mosul City (Iraq). The epileptic patients were diagnosed by specialists and already on stable therapy, with an age range from 15 to 60 years. Exclusion criteria included head trauma, medical history of meningitis, and intracranial space-occupying lesions. The healthy control participants had no history of any neurological diseases and were well matched with the epileptic patients group regarding age and sex. The healthy control subjects were recruited from relatives attending with their patients to the hospital, and they were individually assessed by a specialist and matched with the patients' group.

Blood sampling: Five millilitres of venous blood were withdrawn and allowed to clot at room temperature. Samples were centrifuged, serum separated, aliquoted and stored under -20°C to be ready for laboratory testing.

Detection of anti-Toxoplasma gondii antibodies: Serum samples were analysed for the presence of anti-*Toxoplasma gondii* immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies using enzyme-linked immunosorbent assay (ELISA) kits for IgM (Catalogue No. EIA3520) and IgG (Catalogue No. EIA3519) provided by DRG Instruments GmbH (Germany). The procedure steps were conducted as per the manufacturer's instructions. Both IgG and IgM follow the same principle, starting with dilution of serum samples and IgG-RF- or IgM-RF-Sorbent containing hyperimmune anti-human IgG or IgM antibodies and then loaded into a solid plastic phase 96-well plate precoated with detergent-treated *Toxoplasma gondii* antigen (Deelen strain). 30 to 60 min incubation period, the contents were discarded, and the plates were washed with washing buffer for 4 times to remove

* Department of Microbiology, College of Medicine
University of Mosul, Mosul, Iraq.
E-mail: saeedhameed@uomosul.edu.iq
** Ibn Al-Ather Teaching Hospital, Nineveh Health Directorate
Ninevah, Iraq.
*** Department of Paediatrics, College of Medicine
University of Mosul, Mosul, Iraq.

unbound residues, and then tapped gently on tissue. Next, the wells were loaded with horseradish peroxidase-conjugated anti-human IgM (for IgM kit) or anti-human IgG (for IgG kit) antibodies. After a 2-hour incubation period, the conjugates bind to their specific captured IgM or IgG antibodies; this will form enzyme-linked immune complexes. The contents were discarded, and plates were washed with washing buffer for 4 times to remove unbound conjugates, and then tapped gently on tissue. Next, we added tetramethylbenzidine (TMB) substrate solution; the enzyme catalyses a reaction that produces a blue colour, which converts to yellow with the addition of stop solution. The formed colour was quantified at optical density 450 nm, extrapolated to the standard solution provided in the kits.

The ELISA kit sensitivity was 0.20 DU/mL, and specificity was comparable to 100% for the DRG IgM and IgM ELISA. To ensure quality control of the tested experiment, a negative control sample was incorporated as a calibrator, alongside the standard; moreover, a substrate blank well was kept at an OD of less than 0.100.

Statistical analysis: The data were analysed using SPSS software version 26 (IBM Corp, USA). Data of seropositivity scores for IgM and IgG antibodies between epileptic patients and control subjects were statistically analysed using the IgM: Index > 1.0 → positive (recent infection) and IgG: >11 IU/mL or S/CO >1.1 → positive (past exposure). **Sample size** was calculated using a postulated odds ratio (e.g., OR = 2.0), estimated exposure prevalence in controls, alpha = 0.05, and power = 80%.

RESULTS

A total of 180 participants were enrolled in this study, 90 epilepsy patients and 90 apparently healthy controls. Non-significant (p>0.05) differences existed between subgroups regarding demographic parameters (age, sex, residency, or education level), allowing for valid comparisons of *T. gondii* seropositivity between the two populations. The majority (48.9%) of epilepsy patients were aged 20–40 years, nearly one-third were those >40 years, and 20% represented those <20 years, with a non-significant difference between the two groups (p=0.57). More males (56.7% and 53.3%) were enrolled than females (43.3% and 46.7%) in epilepsy versus control, respectively (p=0.64). Regarding residency, the majority of participants resided in rural areas (64.4% among epilepsy patients and 57.8% among controls) (p=0.39). Comparable educational level existed between participants of epilepsy and controls (p=0.46), with secondary education level being the commonest in both groups (Table 1).

Serological analysis revealed that anti-Toxoplasma gondii IgM antibodies were detected in 14.4% of epilepsy patients compared to 10% of controls, with a non-significant difference between groups (p-value = 0.35), and the odds ratio (OR) was 1.52, suggesting a higher likelihood of recent infection among epilepsy patients. Similarly, IgG seropositivity, which reflects past exposure, was observed in 46.6% of cases and 40% of controls, with a non-significant difference between groups (p-value = 0.38), and the odds ratio was 1.31, indicating a slight increase in exposure among epilepsy patients (Table 2).

In contrast, an association was identified between cat contact and epilepsy. A higher proportion of epilepsy patients reported contact with cats (38.8%) versus controls (24.4%). The calculated odds ratio was 1.97, indicating nearly twice the odds of exposure among epilepsy patients (p=0.038) (Table 3 and Figure 1).

Table 1. Demographic characteristics of epilepsy patients and controls

Variable, n(%)		Epilepsy (n=90)	Control (n=90)	*p* value
Age, years	<20	18 (20%)	22 (24.4%)	0.57
	20-40	44 (48.9%)	37 (41.2%)	
	>40	28 (31.1%)	31 (34.4%)	
Sex	Male	51 (56.7%)	48 (53.3%)	0.64
	Female	39 (43.3%)	42 (46.7%)	
Residency	Urban	32 (35.6%)	38 (42.2%)	0.39
	Rural	58 (64.4%)	52 (57.8%)	
Education	Illiterate	13 (14.4%)	9 (10%)	0.46
	Primary	26 (28.9%)	21 (23.3%)	
	Secondary	33 (36.7%)	36 (40%)	
	University	18 (20%)	24 (26.7%)	

The data are presented as frequency and percentage; p-value greater than 0.05 using Chi-Square was considered non-significant.

Table 2. Prevalence of Anti-Toxoplasma gondii IgM and IgG

Ig status		Epilepsy	Control	OR (95%CI)	*p* value
IgM Status	Positive	13 (14.4%)	9 (10%)	1.52 (0.60-3.80)	0.35
	Negative	77 (85.6%)	81 (90%)		
IgG Status	Positive	42 (46.6%)	36 (40%)	1.31 (0.75-2.29)	0.38
	Negative	48 (53.4%)	54 (60%)		

The data are presented as frequency and percentage; p-value greater than 0.05 using Chi-Square was considered non-significant.

Table 3. Cat Contact among patients and controls

Contact	Epilepsy	Control	OR (95% CI)	*p* value
Yes	35 (38.8%)	22 (24.4%)	1.97 (1.04-3.73)	0.038
No	55 (61.2%)	68 (75.6%)		

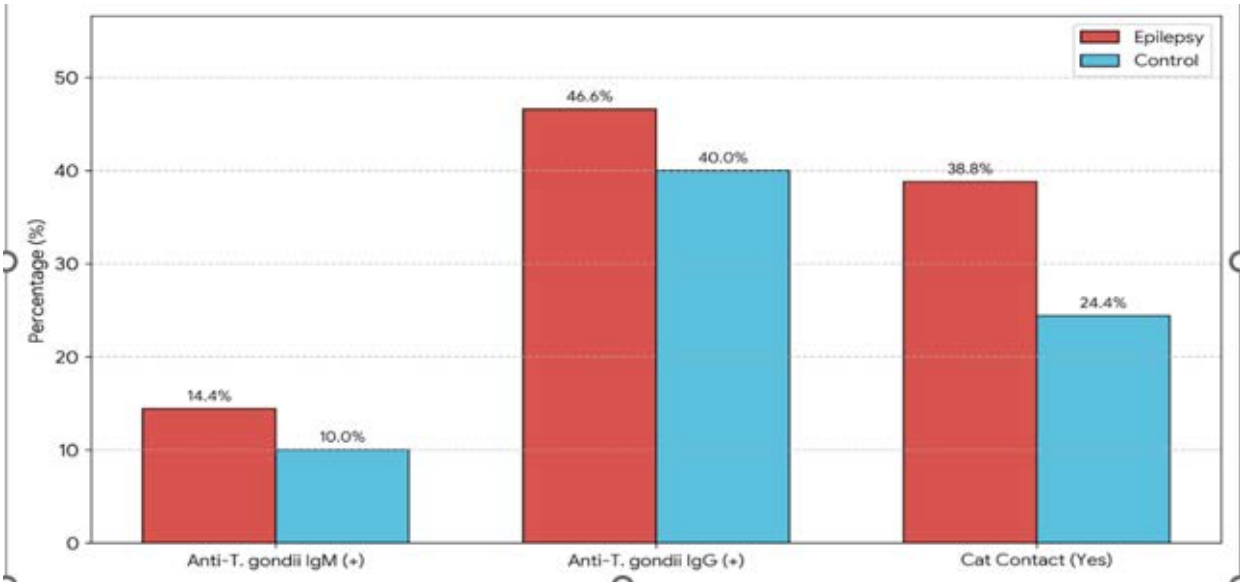

Figure 1. Comparative Analysis of *T. gondii* Antibody Levels Between Epilepsy Patients and Controls and Cat Contact (Bar chart testing IgG and IgM seropositivity in epileptic patients (n=90) and controls (n=90) with cat contact). We can see that although statistical significance was not reached, the median level of the antibody response in epileptic patients was higher than in controls.

DISCUSSION

In this study, we evaluated the seroprevalence of anti-Toxoplasma gondii IgM and IgG antibodies in epilepsy patients and healthy controls. The results demonstrated that epilepsy patients revealed slightly higher seropositivity rates for measured immunoglobulin compared to controls. The demographic characteristics of epilepsy and healthy groups were comparable. Most participants were between 20 and 40 years old, which is congruent with global data showing epilepsy is most common in individuals aged 15–49 years (Cai et al., 2026; Fiest et al., 2017). Males were slightly more predominant, consistent with previous reports of a mild male predominance in epilepsy[14].

Serological analysis showed that 14.4% of epilepsy patients were IgM-positive compared to 10% of controls. This indicates a slightly higher rate of recent infection among cases, with no significant correlation (OR=1.52, p=0.35). IgG antibodies, indicating past exposure, were found in 46.6% of patients and 40% of controls, with an odds ratio of 1.31 (p=0.38). These results suggest only a modest increase in exposure among epilepsy patients and are consistent with earlier studies conducted by Babaie et al. (2017) and Aydemir et al. (2024), who found a non-significant relationship between T. gondii infection and epilepsy[13,16].

A relatively higher rate of epilepsy patients reported cat contact compared to healthy individuals, with an odds ratio of 1.97, reflecting nearly twice the odds of exposure among epilepsy patients; this finding is reasonable because cats are the ultimate hosts of T. gondii and play a key role in environmental contamination through oocyst shedding[4,17]. The results of the present study align with a systematic review and meta-analysis of six studies by Ngoungou et al. (2015), who have demonstrated that toxoplasmosis continues to be considered a risk factor for epilepsy. Conversely, Aydemir et al. (2024) reported that toxoplasmosis did not increase epilepsy risk in Turkey, with lower IgG seropositivity for toxoplasmosis in cryptogenic epilepsy patients compared to controls[13].

In the present study, IgG seropositivity in 40% of controls is relatively higher than percentages in Mexico (6.1%) (18) and Turkey (15.5–28.7%)[13], but this aligns with rates of toxoplasmosis in the Middle East. In an Iraqi study testing T. gondii antibodies in mothers and neonates, seropositivity was 44% in mothers, with percentages reaching 70% among those with stillbirths (Hissain et al., 2020).

However, despite this association, seropositivity itself was not significantly linked to epilepsy, indicating that environmental exposure alone may not be sufficient to trigger seizures. Discrepant results about the link between T. gondii and epilepsy were reported in earlier studies; some studies found a higher seroprevalence in epilepsy patients[11,12], whereas others reported no association[5,13,16]. Alternatively, a recent study using monozygotic twins for epilepsy suggested that T. gondii seropositivity may increase epilepsy risk while controlling for genetic and environmental factors[20]. Moreover, a recent meta-analysis study demonstrated that latent toxoplasmosis may potentially increase the risk of epilepsy, with odds ratios ranging from 1.3 to 2.6[8,21]. Self-reported cat contact was connected with epilepsy in varied analyses (OR = 1.97, 95% CI 1.04-3.73). Cat contact was an unreliable determinant for T. gondii seropositivity in this study (kappa = 0.21).

Several limitations of this study should be addressed, including the relatively small sample size, which may compromise the statistical power and limit generalizability. Serological tests alone are not completely reliable for differentiating the latent from active infection, and the cross-sectional design of the study does not allow identification of causative agents. The epilepsy cases were not categorised by etiological cause, and hence no detection was done of T. gondii association with epilepsy subtypes. Further studies with larger sample size, molecular detection, and longitudinal follow-up design are recommended.

CONCLUSION

The study detected a non-significant association between seropositivity and epilepsy despite the fact that exposure was higher in epilepsy, represented by a significantly higher contact rate, necessitating a larger and longitudinal study to clarify the relationship. The higher rate of seroprevalence in the Iraqi population (40% in controls) perhaps encourages larger, well-designed prospective studies that classify by epilepsy subtypes, infection season, and confounders. Molecular detection techniques

to detect parasitic DNA in cerebrospinal fluid or brain tissue could identify whether the association of T. gondii infection alter seizure threshold or frequency or prognosis based on established therapy.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 06 June 2026

REFERENCE

1. Matlahova YS, Gorokhova VR, Penkina ER, et al. Perinatal Outcomes in Primary Toxoplasmosis during Pregnancy: Treatment Efficacy and Analysis of Neonatal Complications. J Biochem Technol. 2026;17(1):57.
2. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012 Apr;25(2):264–96.
3. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: Global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39(12):1385–94.
4. Hill D, Dubey JP. Toxoplasma gondii: transmission, diagnosis and prevention. Clin Microbiol Infect. 2002;8(10):634–40.
5. Vilela VLR, Feitosa TF. Recent Advances in Toxoplasma gondii Infection and Toxoplasmosis. Vol. 9, Tropical medicine and infectious disease. Switzerland; 2024.
6. Parlog A, Schlüter D, Dunay IR. Toxoplasma gondii-induced neuronal alterations. Parasite Immunol. 2015;37(3):159–70.
7. Luna J, Cicero CE, Rateau G, et al. Updated evidence of the association between toxocariasis and epilepsy: Systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(7):1–19.
8. Rostami A, Riahi SM, Gamble HR, et al. Global prevalence of latent toxoplasmosis in pregnant women: a systematic review and meta-analysis. Clin Microbiol Infect. 2020;26(6):673–83.
9. Sadeghi M, Riahi SM, Mohammadi M, et al. An updated meta-analysis of the association between Toxoplasma gondii infection and risk of epilepsy. Trans R Soc Trop Med Hyg. 2019;113(8):453–62.
10. Ngoungou EB, Bhalla D, Nzoghe A, et al. Toxoplasmosis and Epilepsy — Systematic Review and Meta-Analysis. PLoS Negl Trop Dis. 2015;9(2):1–10.
11. Baker TL, Wright DK, Thergarajan P, et al. A pre-existing chronic Toxoplasma gondii infection promotes epileptogenesis and neuropathology in a mouse model of mesial temporal lobe epilepsy. Brain Behav Immun. 2025;128:440–55.
12. Stommel EW, Seguin R, Thadani VM, et al. Cryptogenic epilepsy: an infectious etiology? Epilepsia. 2001 Mar;42(3):436–8.
13. Aydemir S, Afshar MT, Afshar ÖST, et al. Evaluation of Toxoplasma gondii in the Etiology of Cryptogenic Epilepsy: A Case-control Study. Arch Epilepsy. 2024;30(2):39–42.
14. Fiest KM, Sauro KM, Wiebe S, et al. Prevalence and incidence of epilepsy. Neurology. 2017;88(3):296–303.
15. Cai D, Li X, Yuan Q, et al. Long-Term Trends and Projections of the Global Epilepsy Burden: Insights From the Global Burden of Disease Study 2021. Heal Care Sci. n/a(n/a).
16. Babaie J, Sayyah M, Gharagozli K, et al. Seroepidemiological study of Toxoplasma gondii infection in a population of Iranian epileptic patients. EXCLI J. 2017;16:256–64.
17. Bisetegn H, Debash H, Ebrahim H, et al. Global seroprevalence of Toxoplasma gondii infection among patients with mental and neurological disorders: A systematic review and meta-analysis. Heal Sci Reports. 2023;6(6):e1319.
18. Alvarado-Esquivel C, Rico-Almochantaf Y del R, Hernández-Tinoco J, et al. Toxoplasma gondii exposure and epilepsy: A matched case-control study in a public hospital in northern Mexico. SAGE Open Med. 2018;6.
19. Hissain RM, Waely A, Najla IM S, et al. Seroepidemiology of toxoplasmosis in Iraqi mothers and their neonates. Kurdistan J Appl Res. 2020;5(4):1–8.
20. Dang YL, Esnault K, Leroi MJ, et al. Toxoplasma gondii seropositivity as a risk factor for epilepsy: A discordant monozygotic twin study. Epilepsy Behav. 2025;170:110492.
21. Henke K, Ntovas S, Xourgia E, et al. Who Let the Dogs Out? Unmasking the Neglected: A Semi-Systematic Review on the Enduring Impact of Toxocariasis, a Prevalent Zoonotic Infection. Int J Environ Res Public Health. 2023;20(21).