CENTRAL ASIAN JOURNAL OF
MEDICAL AND NATURAL SCIENCES
https://cajmns.centralasianstudies.org/index.php/CAJMNS
Volume: 06 Issue: 04 | October 2025 ISSN: 2660-4159

Article

Serological and Molecular Phylogeny of *Toxoplasma gondii* in Asymptomatic Pregnant Women, and Association to *MTHFR-C677T* gene Polymorphism in Wasit Province, Iraq

Baraa Abdulsalam Hraija*[1], Ghadah Mohammed Abdulridha Abdair[2]

1. Department of Microbiology, College of Medicine, University of Wasit, Wasit, Iraq
2. Department of Anatomy and Biology, College of Medicine, Wasit University, Kut, Iraq
*Correspondence: babdulsalam@uowasit.edu.iq

Citation: Hraija, B. A., Abdair, G. M. A. Serological and Molecular Phylogeny of *Toxoplasma gondii* in Asymptomatic Pregnant Women, and Association to *MTHFR-C677T* gene Polymorphism in Wasit Province, Iraq. Central Asian Journal of Medical and Natural Science 2025, 6(4), 2131-2144

Received: 30th Jul 2025
Revised: 07th Aug 2025
Accepted: 21st Aug 2025
Published: 5th Sept 2025

Copyright: © 2025 by the authors. Submitted for open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/)

Abstract: *Toxoplasma gondii* is an obligate intracellular parasite infects a wide range of warm-blooded animals as well as humans causing typically asymptomatic infection with severe life-threatening complications in patients. Serological and molecular surveying of human toxoplasmosis in asymptomatic pregnant women, sequencing and phylogenetic analysis of study *T. gondii* isolates and identification the associations of *MTHFR-C677T* gene polymorphism to positive infections. Overall 190 asymptomatic pregnant women admitted to the private gynecological clinics in Wasit province (Iraq) during March-April (2025) were selected randomly, and subjected to direct collection of venous blood that examined serologically by qualitative ELISA and molecularly using the conventional PCR. Phylogenetic analysis was done throughout the MEGA-11 Software; whereas, the quantitative and qualitative molecular analyses were served for MTHFR-C677T C>T rs1801133 genotyping. Seroprevalence of *T. gondii* infection in asymptomatic women was 37.89% with significant incidence of mild infection compared to moderate and severe infections. Molecular PCR demonstrated that 11.05% of study women have a positive reactivity to human toxoplasmosis. Phylogenetically, 17 of 21 study isolates were having a marked identity to local isolate obtained from cats; while, 4 of 21 study isolates were identical for another local isolate obtained from birds. For *MTHFR-C677T* gene polymorphism, Hardy Weinberg equilibrium demonstrated that the frequency of dominant wild type homozygous CC and mutant homozygous TT was increased significantly in infected women more than expected. However, genotypic frequency of mutant homozygous TT and heterozygous CT was higher in infected women than non-infected women; whereas for allele frequency, the mutant T allele was higher in infected than non-infected women. This study indicates serologically a marked high seroprevalence of anti-*T. gondii* IgG antibodies, particularly mild phase; and molecularly, an existence of mutant acute human toxoplasmosis. Phylogenetically, the study isolates have a significant identity to another Iraqi isolates identified in cat and bird suggesting the role of these hosts in transmission of infection and the isolate might circulate between different hosts including human. Genetic and allele frequencies of *MTHFR-C766T* gene demonstrate a significant trend to *T. gondii* infection.

Keywords: Toxoplasmosis, Intracellular Parasite, Sequence, Methylenetetrahydrofolate Reductase C677T

1. Introduction

Toxoplasma gondii is an obligate, single-celled, eukaryotic coccidian parasite which classified as one type in the genus, *Toxoplasma* under the family of Sarcocystidae that

belongs to Apicomplexa phylum (Razooqi *et al.*, 2022). In 1908, *T. gondii* was described initially by Nicolle and Manceaux in the North African rodent (*Ctenodactylus gundi*) at the Pasteur Institute in Tunisia and independently by Splendore in rabbit in Brazil (Atif *et al.*, 2024). The parasite appears potentially capable of infecting a majority of nucleated cells in warm-blooded animals causing a disease known as toxoplasmosis that infected approximately two billion individuals worldwide, with a higher seroprevalence pattern in several tropical and subtropical areas (Adem and Ame, 2023; Lozano *et al.*, 2024). The widespread distribution of *T. godnii* is largely attributed to its complex lifecycle that involves both sexual reproduction within the feline definitive hosts and asexual proliferation in intermediate host, highlighting its remarkable adaptability and survival strategies across diverse ecological niches (Dixit *et al.*, 2024; Ramírez-Flores and Mondragón-Flores, 2025). This unique intracellular lifestyle necessitate finely tuned balance of host manipulation and metabolic reprogramming, enabling the parasite to evade host immune responses and sustain its prolific replication (Hakimi, 2022). Crucial to these processes in the intricate signaling network within the parasite, particularly the triumvirate of cyclic GMP (cGMP), calcium, and phosphatidic acid which orchestrate microneme exocytosis, a pivotal event for invasion and egress (Bullen *et al.*, 2019). This sophisticated regulatory system, involving phosphodiestrases and cyclic nucleotide signaling which modulates parasitic motility and host cell invasion (Ye *et al.*, 2022).

MTHFR-C677T polymorphism, a common genetic variant, has garnered a significant attention due to its potential influence on host susceptibility and disease progressions in various infectious diseases including those caused by parasitic pathogens (Araszkiewicz *et al.*, 2025). This single nucleotide polymorphism in the MTHFR gene affects folate metabolism, which is fundamental for DNA synthesis and methylation, thereby potentially modulating immune responses and parasite replication within the host (Li *et al.*, 2025). The C677T polymorphism, specifically, leads to a thermolabile variant of the MTHFR reductase enzyme, resulting in reduced enzymatic activity and altered folate metabolism (Hiraoka and Kagawa, 2017). This reduction in activity can lead to elevated homocysteine levels and reduced folate levels, which have been implicated in various pathological conditions (Kim *et al.*, 2018).

Toxoplasma gondii represents one of the parasitic pathogens, which relies heavily on host folate metabolism for its survival and proliferation within infected cells (Sanchez and Besteiro, 2021). Consequently, variations in the MTHFR gene, such as C677T polymorphism, could theoretically impact the availability of main metabolites for *T. gondii* growth, thereby influencing infection dynamics and clinical outcomes (Berrett, 2018; Maltsev and Hurzhii, 2022).

In Iraq, several molecular studies have been conducted to detect the prevalence rate of human toxoplasmosis in aborted women (Al-Hadraawy and Hadi, 2017; Darweesh *et al.*, 2018), spontaneously-aborted or suffering from reproductive disorders (Al-Sray *et al.*, 2019), and pregnant women (Farhan *et al.*, 2024). Hence, the present study aims to molecular surveying of human toxoplasmosis in asymptomatic individuals in Wasit province (Iraq), sequencing of study *T. gondii* isolates with phylogenetic analysis to indicate their close-relationship with the global isolates/strains, and estimation the associations of infection to MTHFR-C677T gene polymorphism.

2. Materials and Methods

Ethical approval

This study was licensed by the Scientific Committee in the College of Medicine (University of Wasit).

Samples

Overall 190 asymptomatic pregnant women admitted to the private gynecological clinics in Wasit province (Iraq) during March-April (2025) were selected randomly to the

present study. Each woman was subjected to direct collection 5ml of venous blood that divided equally into labeled an EDTA-anticoagulant plastic tube and free-anticoagulant glass-gel tube. At laboratory, the glass-gel tubes were centrifuged, and the obtained sera were kept into 1.5ml plastic Eppendorf tubes. Samples of whole blood and sera were kept frozen (-20°C) until be tested.

Serology

Qualitative enzyme-linked immunosorbent assay (ELISA) was applied to detect anti-*T. gondii* IgG antibodies in study population. Following the manufacturer instructions (SunLong Biotech Company, China), the kits' contents of Toxo-IgG ELISA Kit (Cat. No: SL2054Hu) and serum samples were prepared, processed, and the ODs were measured at an absorbance of 450nm by the ELISA reader. Finally, effectiveness of positive and negative controls was determined at 1.508 and 0.0705, respectively; while critical value (cut off) was determined at 0.2205. The positive samples were identified at ≥ cut off.

Molecular testing

Conventional PCR

The manufacturer's instructions for the gSYNC™ DNA extraction kit (Genaid, Taiwan) were followed to extract DNA from the EDTA-blood samples. To preparation the MasterMix tubes at a final volume of 25 μL, one set of primers (Table 1) in addition to GoTaq® Green Master Mix Kit (Promega, USA) were utilized. Then, the MasterMix tubes were transferred to the Thermal Cycler system, subjected to modified conditions (Table 2), and subjected to electrophoresis in agarose-gel (1.5%) stained with Ethidium bromide at 100 V and 80 Am for 90 minutes. The positive samples were visualized under the UV illumination at a product size of 599 bp.

Quantitative and qualitative analyses of MTHFR-C677T gene polymorphism

Tetra-primer ARMS-PCR method was applied to analysis *MTHFR-C677T* gene polymorphism in positively and negatively infected individuals. Quantitatively, Real-Time PCR was done as described by Saxena *et al.* (2020). Briefly, the MasterMix tubes were prepared using two sets of primers (Table 1) at a final volume of 20 μL and transferred to the Miniopticon Real-Time PCR (Bio-Rad, USA) for DNA amplification under the modified condictions (Table 2). Further, the MasterMix tubes were transferred to the Thermal Cycler system and subjected to electrophoresis in agarose-gel (1.5%) stained with Ethidium bromide at 100 V and 80 Am for 90 minutes, and PCR products were visualized under the UV illumination.

Table 1. Primers utilized for identification *T. gondii* and *MTHFR-C677T* gene.

Tool	Primer	Product size	Reference
Conventional PCR	F:5'-GTGCACCTGAGCTTGTCTCT-3'	599 bp	This study
	R:5'-GGACGAATCCCGAACCTTGT-3'		(ID: AF158095.1)
Gene polymorphism	MTHFR677 F1-C:5'-AGGAGAAGGTGT CTGCGGGCGT-3'	101 bp	Mir *et al.* (2022)
	MTHFR677 R1-T:5'-AAGAAAAGCTGC GTGATGATGAAATAGG -3'	177 bp	
	MTHFR677FO:5'-AAGCATATCAGTCA TGAGCCCAGCC-3'	224 bp	
	MTHFR677RO:5'-GGGAAGAACTCAG CGAACTCAGCAC-3'		

Table 2. PCR thermal cycler conditions for conventional and MTHFR-C677T C>T rs1801133 genotyping.

Tool	Step	Temperature / Time	Cycle No.
Qualitative PCR	Initial denaturation	95°C / 5 min.	1
	Denaturation	95°C / 30 sec.	35
	Annealing	62°C / 30 sec.	
	Extension	72°C / 30 sec.	
	Final extension	72°C / 5 min.	1
Quantitative PCR	Initial denaturation	95°C / 5 min.	1
	Denaturation	95°C / 20 sec.	40
	Annealing / Extension Detection	60°C / 30 sec.	

Phylogenetic analysis

All positive DNA samples for *T. gondii* infection were sequenced, and the received data were submitted in NCBI-GenBank database and subjected to multiple sequence alignment (MSA) by the MEGA-11 Software and NCBI-Viewer, as well as to the phylogenetic tree analysis and homology sequence identity by the MEGA-11 Software to indicate the close-relationship between the local and global *T. gondii* isolates / strains.

Statistical analysis

The *t*-test and One-Way ANOVA in the GraphPad Prism Software were applied in this study to calculate significant differences between the obtained values at p<0.05 (Al-Graibawi *et al*., 2021). Hardy Weinberg equation was followed to calculate the distribution of CC, CT and TT in MTHFR-C677T genotypes.

3. Results

The The findings of qualitative ELISA detected that 37.89% (72/190) women were seropositive to anti-*T.gondii*-IgG antibodies, while 62.11% (118/190) of study women were seronegative to human toxoplasmosis (Figure 1). The seropositive samples were additionally identified the significant increases (p<0.0327; 95%CI: 35.59 to 102.3) in mild infection [63.89% (46/72)] when compared with those of moderate [26.39% (19/72)] and severe [9.72% (7/72)] infections (Figure 2). Also, values (mean ± standard error) of positive ODs were distributed respectively as 0.268 ± 0.006, 0.389 ± 0.007, and 0.529 ± 0.014 for mild, moderate, severe infections (p<0.0141; 95%CI: 0.0709 to 0.7198), (Figure 3).

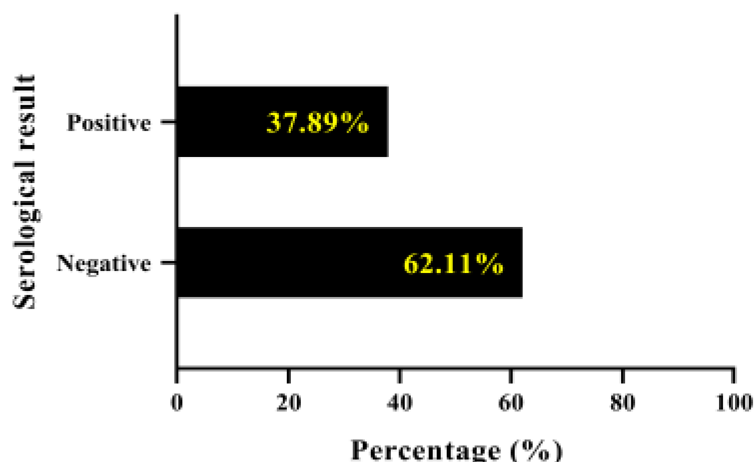

Figure 1. Total seropositive women to anti-*T.gondii*-IgG antibodies using ELISA.

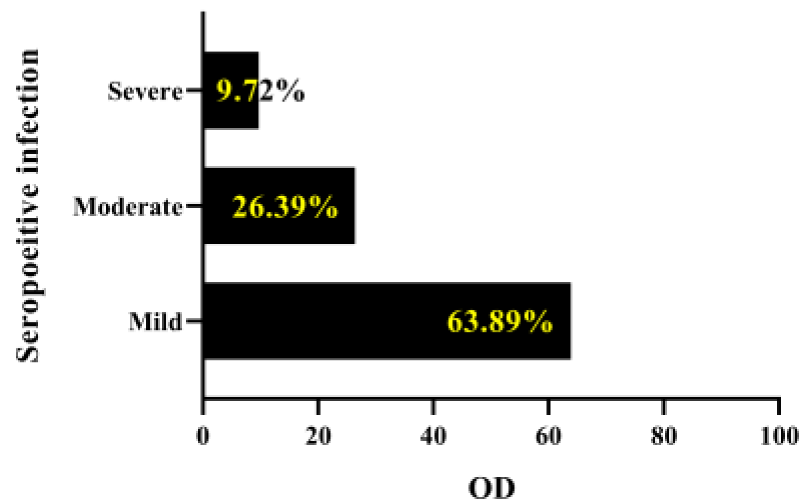

Figure 2. Seroprevalence of positives according to severity of infection.

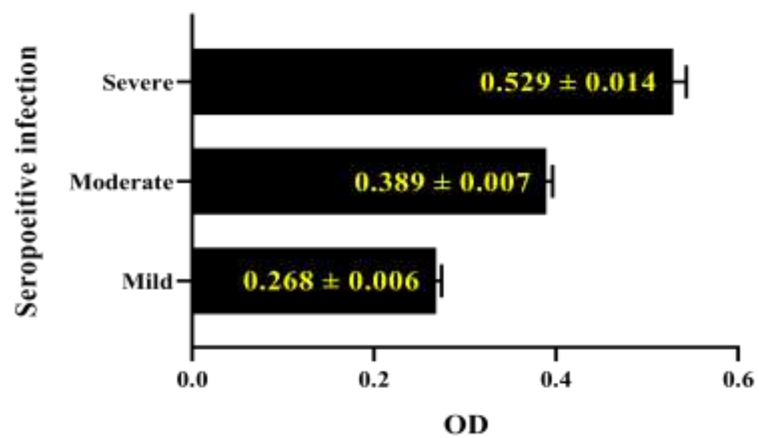

Figure 3. Distribution of seropositive ODs according to severity of infection.

Molecularly, 11.05% (21/190) of study women were shown a positive reactivity to human toxoplasmosis using the conventional PCR assay; whereas, [88.95% (169/190)] of them were being negatives (Figures 4, 5).

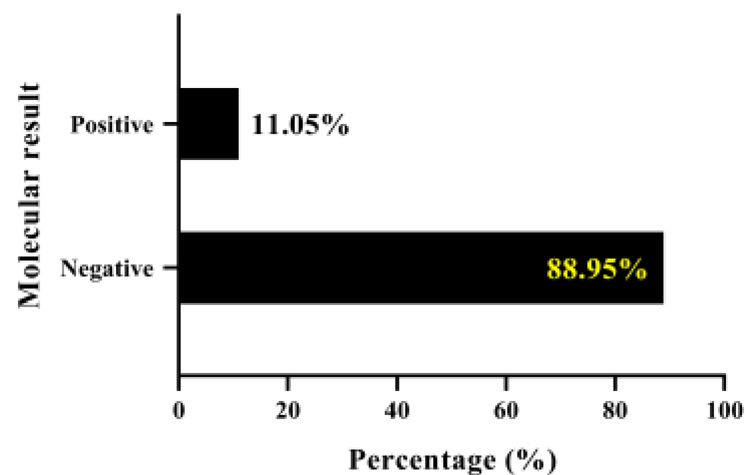

Figure 4. Total molecular results for positive women to *T.gondii* using PCR.

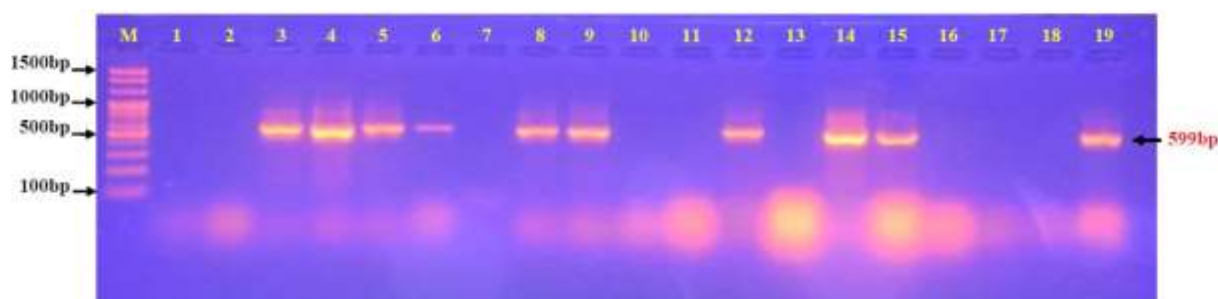

Figure 5. Electrophoresis of PCR products in agarose-gel. Line (M): Ladder marker (100-1500 bp); Lanes (1, 2, 7, 10, 11, 13, 16, 17, and 18): Negative samples; Lanes (3, 4, 5, 6, 8, 9, 12, 14, 15, and 19): Positive samples at a product size of approximately 599 bp.

Phylogenetically, sequencing analysis of all positive study *T. gondii* isolates with the NCBI-BLAST *T. gondii* isolates / strains reported that 17 of these isolates were having a marked identity to the Iraqi *T. gondii* isolate (GenBank ID: LC749846.1); while, four of study *T. gondii* isolates were identical to another Iraqi *T. gondii* isolate (GenBank ID: MH636821.1) at a level of similarity (*) ranged 99.55-99.97%, and a range of mutation/changes at 0.001-0.050% (Table 3, Figures 6, 7).

Table 3. Homology sequence identity (%) of study and global NCBI-GenBank *T.gondii* isolates.

Local isolate		NCBI-BLAST isolate				Identity (%)
Name	Access. No.	Isolate	Source	Country	Access. No.	
Iraq-Hasbraa 1	PV931630.1	RK1	Cat	Iraq	LC749846.1	99.82
Iraq-Hasbraa 2	PV931631.1	IQ-TB1	Bird	Iraq	MH636821.1	99.85
Iraq-Hasbraa 3	PV931632.1	RK1	Cat	Iraq	LC749846.1	99.82
Iraq-Hasbraa 4	PV931633.1	RK1	Cat	Iraq	LC749846.1	99.82
Iraq-Hasbraa 5	PV931634.1	RK1	Cat	Iraq	LC749846.1	99.90
Iraq-Hasbraa 6	PV931635.1	RK1	Cat	Iraq	LC749846.1	99.97
Iraq-Hasbraa 7	PV931636.1	RK1	Cat	Iraq	LC749846.1	99.82
Iraq-Hasbraa 8	PV931637.1	RK1	Cat	Iraq	LC749846.1	99.88
Iraq-Hasbraa 9	PV931638.1	IQ-TB1	Bird	Iraq	MH636821.1	99.90
Iraq-Hasbraa 10	PV931639.1	RK1	Cat	Iraq	LC749846.1	99.95
Iraq-Hasbraa 11	PV931640.1	RK1	Cat	Iraq	LC749846.1	99.11
Iraq-Hasbraa 12	PV931641.1	IQ-TB1	Bird	Iraq	MH636821.1	99.88
Iraq-Hasbraa 13	PV931642.1	RK1	Cat	Iraq	LC749846.1	99.97
Iraq-Hasbraa 14	PV931643.1	RK1	Cat	Iraq	LC749846.1	99.86
Iraq-Hasbraa 15	PV931644.1	IQ-TB1	Bird	Iraq	MH636821.1	99.86
Iraq-Hasbraa 16	PV931645.1	RK1	Cat	Iraq	LC749846.1	99.82
Iraq-Hasbraa 17	PV931646.1	RK1	Cat	Iraq	LC749846.1	99.86
Iraq-Hasbraa 18	PV931647.1	RK1	Cat	Iraq	LC749846.1	99.92

Iraq-Hasbraa 19	PV931648.1	RK1	Cat	Iraq	LC749846.1	99
Iraq-Hasbraa 20	PV931649.1	RK1	Cat	Iraq	LC749846.1	99.56
Iraq-Hasbraa 21	PV931650.1	RK1	Cat	Iraq	LC749846.1	99.55

Species/Abbrv	
1. Toxoplasma gondii isolate Iraq-Hasbraa1/Iraq (PV931630.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
2. Toxoplasma gondii isolate Iraq-Hasbraa2/Iraq (PV931631.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
3. Toxoplasma gondii isolate Iraq-Hasbraa3/Iraq (PV931632.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
4. Toxoplasma gondii isolate Iraq-Hasbraa4/Iraq (PV931633.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
5. Toxoplasma gondii isolate Iraq-Hasbraa5/Iraq (PV931634.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
6. Toxoplasma gondii isolate Iraq-Hasbraa6/Iraq (PV931635.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
7. Toxoplasma gondii isolate Iraq-Hasbraa7/Iraq (PV931636.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
8. Toxoplasma gondii isolate Iraq-Hasbraa8/Iraq (PV931637.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
9. Toxoplasma gondii isolate Iraq-Hasbraa9/Iraq (PV931638.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
10. Toxoplasma gondii isolate Iraq-Hasbraa10/Iraq (PV931639.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
11. Toxoplasma gondii isolate Iraq-Hasbraa11/Iraq (PV931640.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
12. Toxoplasma gondii isolate Iraq-Hasbraa12/Iraq (PV931641.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
13. Toxoplasma gondii isolate Iraq-Hasbraa13/Iraq (PV931642.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
14. Toxoplasma gondii isolate Iraq-Hasbraa14/Iraq (PV931643.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
15. Toxoplasma gondii isolate Iraq-Hasbraa15/Iraq (PV931644.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
16. Toxoplasma gondii isolate Iraq-Hasbraa16/Iraq (PV931645.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
17. Toxoplasma gondii isolate Iraq-Hasbraa17/Iraq (PV931646.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
18. Toxoplasma gondii isolate Iraq-Hasbraa18/Iraq (PV931647.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
19. Toxoplasma gondii isolate Iraq-Hasbraa19/Iraq (PV931648.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
20. Toxoplasma gondii isolate Iraq-Hasbraa20/Iraq (PV931649.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
21. Toxoplasma gondii isolate Iraq-Hasbraa21/Iraq (PV931650.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
22. Toxoplasma gondii isolate CT-1/USA (U17347.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
23. Toxoplasma gondii isolate Tg10/China (KX008033.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
24. Toxoplasma gondii strain RK1/Iraq (LC749846.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
25. Toxoplasma gondii isolate Group3/Pakistan (OM510428.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
26. Toxoplasma gondii isolate Iraq-Camel 4/Iraq (PQ891929.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
27. Toxoplasma gondii isolate W-N4/Australia (KM875436.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
28. Toxoplasma gondii isolate IQ-TB1/Iraq (MH636821.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
29. Toxoplasma gondii isolate /USA (AY663792.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT
30. Toxoplasma gondii isolate Iraq-Ghirban 20/Iraq (PV596110.1)	TGCCTCTTCCCCTGGAAGGGCAGTGTTTATTAGAT

Species/Abbrv	
1. Toxoplasma gondii isolate Iraq-Hasbraa1/Iraq (PV931630.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
2. Toxoplasma gondii isolate Iraq-Hasbraa2/Iraq (PV931631.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
3. Toxoplasma gondii isolate Iraq-Hasbraa3/Iraq (PV931632.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
4. Toxoplasma gondii isolate Iraq-Hasbraa4/Iraq (PV931633.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
5. Toxoplasma gondii isolate Iraq-Hasbraa5/Iraq (PV931634.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
6. Toxoplasma gondii isolate Iraq-Hasbraa6/Iraq (PV931635.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
7. Toxoplasma gondii isolate Iraq-Hasbraa7/Iraq (PV931636.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
8. Toxoplasma gondii isolate Iraq-Hasbraa8/Iraq (PV931637.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
9. Toxoplasma gondii isolate Iraq-Hasbraa9/Iraq (PV931638.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
10. Toxoplasma gondii isolate Iraq-Hasbraa10/Iraq (PV931639.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
11. Toxoplasma gondii isolate Iraq-Hasbraa11/Iraq (PV931640.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
12. Toxoplasma gondii isolate Iraq-Hasbraa12/Iraq (PV931641.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
13. Toxoplasma gondii isolate Iraq-Hasbraa13/Iraq (PV931642.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
14. Toxoplasma gondii isolate Iraq-Hasbraa14/Iraq (PV931643.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
15. Toxoplasma gondii isolate Iraq-Hasbraa15/Iraq (PV931644.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
16. Toxoplasma gondii isolate Iraq-Hasbraa16/Iraq (PV931645.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
17. Toxoplasma gondii isolate Iraq-Hasbraa17/Iraq (PV931646.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
18. Toxoplasma gondii isolate Iraq-Hasbraa18/Iraq (PV931647.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
19. Toxoplasma gondii isolate Iraq-Hasbraa19/Iraq (PV931648.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
20. Toxoplasma gondii isolate Iraq-Hasbraa20/Iraq (PV931649.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
21. Toxoplasma gondii isolate Iraq-Hasbraa21/Iraq (PV931650.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
22. Toxoplasma gondii isolate CT-1/USA (U17347.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
23. Toxoplasma gondii isolate Tg10/China (KX008033.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
24. Toxoplasma gondii strain RK1/Iraq (LC749846.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
25. Toxoplasma gondii isolate Group3/Pakistan (OM510428.1)	TTACCCAATCCTGATTCCGGGAGGTAGTGACAAGC
26. Toxoplasma gondii isolate Iraq-Camel 4/Iraq (PQ891929.1)	TTCTACCCTCCTGATTCAGGGAGGTAGTGACGGAA
27. Toxoplasma gondii isolate W-N4/Australia (KM875436.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGTTACAA
28. Toxoplasma gondii isolate IQ-TB1/Iraq (MH636821.1)	TTACCCAATCCTGATTCAGGGAGGTAGTGACAAGA
29. Toxoplasma gondii isolate /USA (AY663792.1)	TTACCCAATCCTGATTCAGGGAGGTAGAATGACAA
30. Toxoplasma gondii isolate Iraq-Ghirban 20/Iraq (PV596110.1)	TTACCCAATCCTGATTCAGGGACCGGTAGTGACAA

Figure 6. MSA for study and global NCBI-GenBank *T.gondii* isolates.

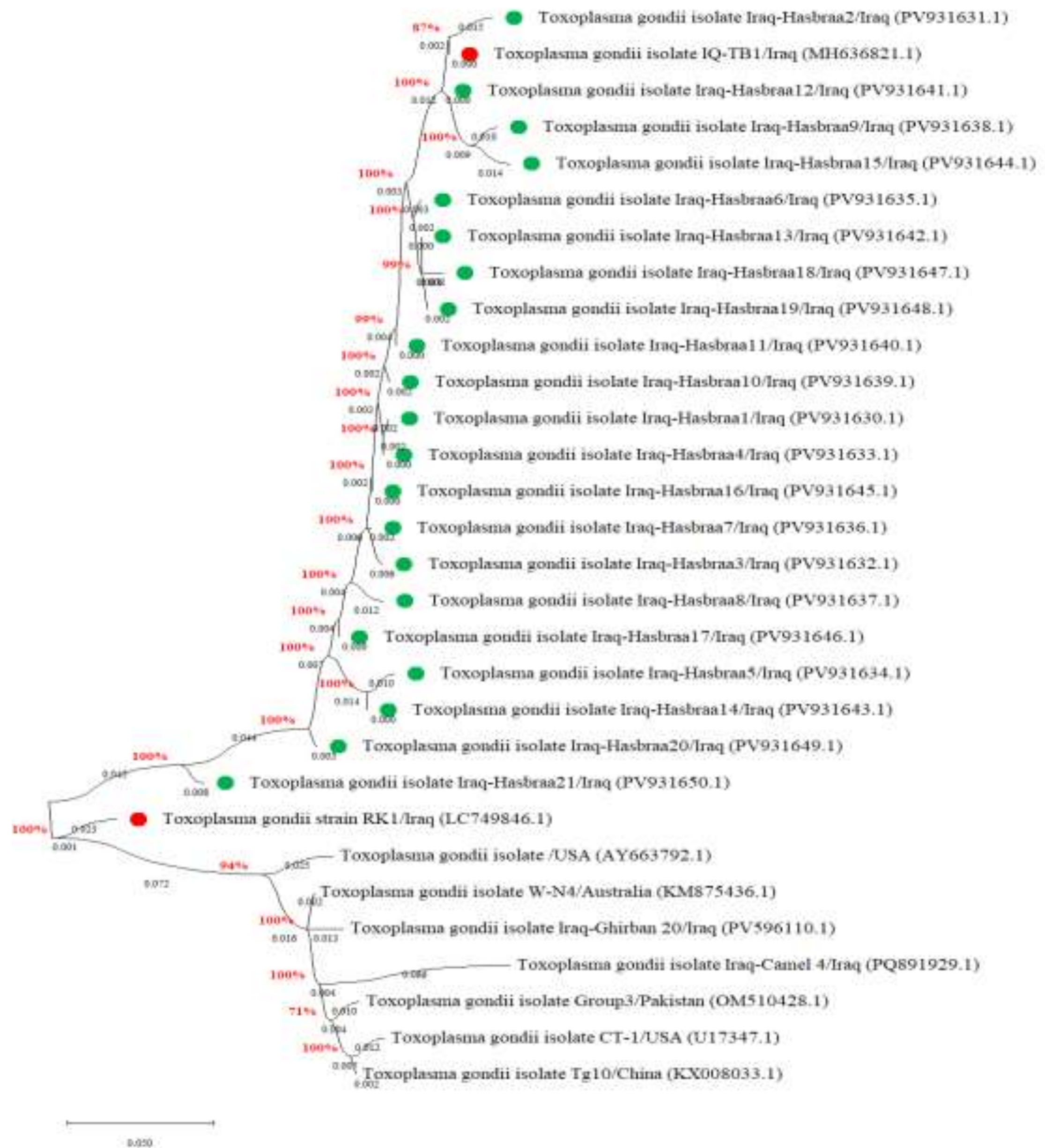

Figure 7. Phylogenetic tree analysis of the current study and global NCBI-GenBank *T.gondii* isolates.

For *MTHFR-C677T* gene polymorphism, Hardy Weinberg equilibrium demonstrate significant variation among the observed and expected genotypes (Table 4). In infected individuals, frequency of dominant wild type homozygous CC and mutant homozygous TT was increased significantly ($p<0.0242$; 95%CI: 40.88 to 117.1; and $p<0.0314$; 95%CI: 37.97 to 50.97, respectively) more than expected; whereas, the frequency of mutant heterozygous CT was reduced significantly ($p<0.0268$; 95%CI: 68.09 to 97.09) in study population.

Table 4. MTHFR-C677T C>T rs1801133 genotyping by Hardy Weinberg equilibrium.

Genotype	Observed	Expected	p-value	95%CI
CC	82 (82%) *	76 (76%)	0.0242	40.88 to 117.1
CT	8 (8%)	21 (21%) *	0.0268	68.09 to 97.09
TT	10 (10%) *	3 (3%)	0.0314	37.97 to 50.97

However, the results of genotypic frequency revealed a significant higher frequency of mutant homozygous TT (p<0.0414; 95%CI: 54.18 to 90.42) and heterozygous CT (p<0.0341; 95%CI: 34.47 to 64.76) in infected women (23.81% and 19.05%, respectively) compared to non-infected women (12.43% and 11.24%, respectively) in contrast to that recorded in the dominant homozygous CC (p<0.011; 95%CI: 55.18 to 188.7) shown a significant decreasing in infected women (57.14%) compared to non-infected one (76.33%), (Table 5).

Table 5. Frequency of *MTHFR-C677T* genotypes and alleles among study population.

MTHFR-C677T	Infected	Non-infected	p-value	95%CI
TT	5 (23.81%) *	21 (12.43%)	0.0414	54.18 to 90.42
CT	4 (19.05%) *	19 (11.24%)	0.0341	34.47 to 64.76
CC	12 (57.14%)	129 (76.33%) *	0.011	55.18 to 188.7

For allele frequency, the mutant T allele was higher significantly (p<0.0152; 95%CI: 192.1 to 247.9) in infected (45.24%) than non-infected (10.61%) women; while, the wild type C allele was reduced significantly (p<0.0351; 95%CI: 147.9 to 292.1) in infected (54.76%) more than non-infected (89.39%) women (Table 6).

Table 6. Frequency of *MTHFR-C677T* alleles among study population.

MTHFR-C677T	Infected	Non-infected	p-value	95%CI
T	19 (45.24%)	38 (10.61%)	0.0152	192.1 to 247.9
C	23 (54.76%)	320 (89.39%)	0.0351	147.9 to 292.1

4. Discussion

This study showed that the seroprevalence of human toxoplasmosis was 37.89% with a significant prevalence of mild infection when compared with the moderate and severe infections. In comparison with the findings of other Iraqi studies, there was 39% in Baghdad province by ELISA (Naizi *et al.*, 1990), 29.41% in Duhok province by ELISA (Razzak *et al.*, 2005), 5% in Wasit province by ELISA (Al-Saidi, 2009), 94% in Baghdad province by ELISA (AL Mossa, 2009), 43.33% in Baghdad province by ELISA (Mahmood *et al.*, 2010), 35.1% by latex agglutination test and 77.78% by ELISA (Al-Hindawi and Al-Shanawi, 2015), 48% in Qadisiyah province by ELISA (Hadi *et al.*, 2016), 47.73% in Erbil city by ELISA (Abdullah and Mahmood, 2017), 81% in Baghdad by ELISA (Rahi and Al-Rodaynee, 2017), 80% in Samarra city by ELISA (Al-Ammash *et al.*, 2018), 44% in Diyala by ELISA (Darweesh *et al.*, 2018), 17.8% in Wasit province by ELISA (Al-Sray *et al.*, 2019), 28.46% in Duhok (Mikaeel and Al-Saeed, 2019), 42.6% by LAT and 26.6% by ELISA in Babylon province (Mohammed and Al-Janabi, 2019), 23.81% in Najaf by ELISA (Al-Tufaili and Amer, 2020), 52.3% in Maysan province by ELISA (Mijbel and Alsaady, 2023), and 41.14% in Duhok by ELISA (Mustafa *et al.*, 2024). Globally, there was 1.4% using ELISA in KSA (Mohajab *et al.*, 2020), 24.7% in Jordan (Abo-Shehada *et al.*, 2021), 47.4% by ELISA in Turkey (Halici-Ozturk *et al.*, 2021), 41.2% by ELISA in India (Deka *et al.*, 2022), 3.6% by ELISA in China (Gao *et al.*, 2024), and 25.3-62.2% in Iran by ELISA (Maisarah *et al.*, 2024; Asfaram *et al.*, 2025; Bahador *et al.*, 2025). However, this variation in seroprevalence of human toxoplasmosis might be attributed to several factors such as specific region or population being studied, time frame, as well as sensitivity and specificity of the diagnostic test kit.

Our molecular results identified that 11.05% of study women were having positive reactivity to human toxoplasmosis. These findings were similar with that detected by Darweesh *et al.* (2018) who revealed that the total molecular positive result of *T. gondii* was 10.7%. In Wasit province, Al-Sray *et al.* (2019) demonstrated that 7.06% were positive by PCR targeting *B1* gene. Mikaeel and Al-Saeed (2019) reported that 8.33% of study samples were shown positive amplification by PCR assay targeting *B1* gene. In Maysan province, molecular testing of 40 women that 10% were positives (Allamy and Alsaady, 2023). In Libya, Gashout *et al.* (2016) collected blood samples from the healthy 364 pregnant women to be tested by the RT-PCR that demonstrated 11.81% positive samples. In contrast to findings of this study, molecular testing a total of 350 clinical suspected women in Al-Najaf province by PCR targeting three genes (*B1*, *18S rRNA*, and *B30*) detected respectively that toxoplasmosis was found in 20.28%, 18.85% and 17.71% at a rate of sensitivity 98.61%, 91.66%, and 87.32% (Al-Hadraawy and Hadi, 2017). In Thi-Qar province, molecular testing of aborted women showed that 40.83% of samples were positive by PCR (Ali *et al.*, 2019). Of the 137 samples tested in KSA, the *B1* gene could be amplified in 41% by PCR (Bin Dajem and Almushait, 2012). The study of Elamin *et al.* (2012) demonstrates the use of two groups of women in Sudan using some molecular methods of detection of the DNA of the organism in the form of the *B1* gene indicating the value of 19.1% of the study group and 22.3% of the control group. Results of PCR on the sample set of 34 women who aborted their pregnancy in Egypt and 48 asymptomatic occupational staff indicated that 26.5%, 35.4%, and 19.7% of them were infected with *Toxoplasma* (Ibrahim *et al.*, 2017). According to Duarte *et al.* (2020), a positive result of the *T. gondii* was observed in 16 of the samples with a primer to the *B1* gene (5.5%) in blood and 2.5% at the site of the placental tissue. The findings of a molecular diagnosis of toxoplasmosis in a totally 500 females of Mahzesaran province (Iran) revealed that the prevalence of PCR approach turned out to be 4.2% (Rahimi Esboei *et al.*, 2021). However, differences in obtained between our study and other above studies might be related to the phase of infection (acute / chronic, symptomatic / asymptomatic), type of applied molecular assay, and gene targeted for primer detection.

Phylogenetically, we recorded that 17 of 21 study *T. gondii* isolates have a marked identity to Iraqi *T. gondii* isolate (GenBank ID: LC749846.1) obtained from cats in Mosul city in Nineveh province; while, 4 of 21 study *T. gondii* isolates have a significant identity to another Iraqi *T. gondii* isolate (GenBank ID: MH636821.1) obtained from birds of Dulmaj marsh in Wasit province. Cats represent the primary definitive host for *T. gondii*, which become infected due to consuming infected prey like rodents and birds (Abdul Hafeez *et al.*, 2022). Infected cats shed oocysts in their feces which contaminate the environment and transmit to human through various routes including accidental ingestion (Khan *et al.*, 2023). On the other hand, although birds act as an intermediate host harboring the parasite and potentially infecting cats, they cannot consider a direct source of human infection in the same way as contaminated cat feces or undercooked meat from infected animals (Zanet *et al.*, 2023).

The nuanced interplay between host genetics and parasitic metabolic requirements underscores the complexity of host-pathogen interactions and highlight the need for personalized approaches in managing parasitic diseases. In the presents study, *MTHFR-C766T* gene polymorphism was carried out to evaluate the frequency between (CC) wild type allele and mutant (CT) allele either in homozygous or heterozygous condition, using ARMS PCR that is highly sensitive and more reliable technique for SNP analysis. Our findings demonstrated by the Hardy Weinberg equilibrium that the frequency of dominant wild type homozygous CC and mutant homozygous TT was increased significantly in infected women more than expected. However, genotypic frequency of mutant homozygous TT and heterozygous CT was higher in infected women than non-infected women; whereas for allele frequency, the mutant T allele was higher in infected than non-infected women. These findings indicate that infected asymptomatically

pregnant women are at the risk unexpected reproductive illness and termination of pregnancy as recorded by various researchers (Rafiei et al., 2017; Xie et al., 2017; Loizidou et al., 2021).

5. Conclusion

This study indicates serologically a marked high seroprevalence of anti-*T. gondii* IgG antibodies, particularly mild phase; and molecularly, an existence of mutant acute human toxoplasmosis. Phylogenetically, the study isolates have a significant identity to another Iraqi isolates identified in cat and bird suggesting the role of these hosts in transmission of infection and the isolate might circulate between different hosts including human. Genetic and allele frequencies of *MTHFR-C766T* gene demonstrate a significant trend to *T. gondii* infection; however, furthermore studies are greatly needed due to the lack of such studies in Iraq.

REFERENCES

Abdul Hafeez, M., Mehdi, M., Aslam, F., Ashraf, K., Aleem, M. T., Khalid, A. R., and Almutairi, M. M. (2022). Molecular characterization of Toxoplasma gondii in cats and its zoonotic potential for public health significance. *Pathogens*, *11*(4), 437.

Abdullah, H. M., and Mahmood, M. A. (2017). seroprevalence of *Toxoplasma gondii* among pregnant women in Erbil city/Kurdistan Region/Iraq. Polytech J, 7(3), 54-63.

Abo-Shehada, M. N., Khalil, R., Abu-Halaweh, M., Sweis, K., Amr, Z., and Billeh, L. (2021). Seroprevalences of *Toxoplasma gondii* and Neospora caninum infections in Jordanian women who had a recent spontaneous abortion. Revista Brasileira de Parasitologia Veterinária, 30(3), e008821.

Adem, D., and Ame, M. (2023). Toxoplasmosis and its significance in public health: a review. *J. Biomed. Biol. Sci*, *2*(1), 1-20.

AL Mossa, H. (2009). Toxoplasmosis in Iraqi women: a retrospective study. Kerbala Journal of Medicine, 2(5), 697-701.

Al-Ammash, M. S. J., Al-Shaibani, K. T. M., and Al-Abodi, H. R. J. (2018). Investigating the prevalence of infection with *Toxoplasma gondii* in men and women in Samarra city, Iraq. Plant Archives, 18(2), 2501-2508.

Al-Graibawi, M. A., Yousif, A. A., Gharban, H. A., and Zinsstag, J. (2021). First serodetection and molecular phylogenetic documentation of Coxiella burnetii isolates from female camels in Wasit governorate, Iraq. *Iraqi Journal of Veterinary Sciences,* 35 (Supplement III), 47-52.

Al-Hadraawy, S. K., and Hadi, F. A. (2017). Immunological and molecular study of Toxoplasma gondii in Al-Najaf Governorate-Iraq. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(4), 482-492.

Al-Hindawi, N. G., and Al-Shanawi, F. A. (2015). Seroprevalence of *Toxoplasma gondii* and cytomegalovirus in aborted women in Baghdad-Iraq. Iraqi journal of science, 649-655.

Ali, A. T., Mahdi, D. S., and Awad, A. H. (2019). Molecular Study of Toxoplasmosis in Spontaneous Aborted Women in Thi-Qar Province, Iraq. Indian Journal of Public Health Research and Development, 10(8).

Allamy, S. S., and Alsaady, H. A. M. (2023). Using Antibody rapid test, RT-qPCR, and RFLP/PCR for detecting *Toxoplasma gondii* in the placenta of abortion women and determining the genotype in Maysan province, Iraq. Journal of Wasit for Science and Medicine, 16(3), 28-43.

Al-Saidi, M. A. (2009). Serological detection of Toxoplasmosis among women in wasit province. Journal of Wasit for Science and Medicine, 2(1), 150-156.

Al-Sray, A. H., Sarhan, S. R., and Mohammed, H. A. (2019). Molecular and serological characterization of toxoplasma gondii in women in wasit province. *Advances in Animal and Veterinary Sciences*, *7*(8), 657-663.

Al-Tufaili, N., and Amer, R. (2020). Evaluation of commercial Linked immune-sorbent assay (ELISA) for detecting sero-prevalence of *Toxoplasma gondii* antibodies in Iraqi women. Systematic Reviews in Pharmacy, 11(12).

Araszkiewicz, A. F., Jańczak, K., Wójcik, P., Białecki, B., Kubiak, S., Szczechowski, M., and Januszkiewicz-Lewandowska, D. (2025). MTHFR Gene Polymorphisms: A Single Gene with Wide-Ranging Clinical Implications—A Review. *Genes*, *16*(4), 441.

Asfaram, S., Zarei, Z., Teimoorpour, R., Heidari, Z., Iranpour, S., Azghani, P., and Molaei, S. (2025). Serological Evaluation and Molecular Genotyping of *Toxoplasma gondii* in Pregnant Women in Meshkin-Shahr District, Northwestern Iran. Iranian Journal of Parasitology, 20(1), 44.

Atif, I., Touloun, O., and Boussaa, S. (2024). Toxoplasma Gondii in humans, animals and in the environment in Morocco: a literature. *generations*, *20*, 21.

Bahador, A., Babazadeh, T., Maleki Chollou, K., Moqadam, H., Zendeh, M. B., Valipour, B., and Behniafar, H. (2025). Seroprevalence and risk factors associated with Toxoplasmosis in nomadic, rural, and urban communities of Northwestern Iran. Frontiers in Public Health, 13, 1516693.

Berrett, A. N. (2018). *Latent Toxoplasma gondii Infection Moderates the Association Between the C677T MTHFR Polymorphism and Cognitive Function in US Adults*. Brigham Young University.

Bin Dajem, S. M., and Almushait, M. A. (2012). Detection of *Toxoplasma gondii* DNA by PCR in blood samples collected from pregnant Saudi women from the Aseer region, KSA. Annals of Saudi medicine, 32(5), 507-512.

Bullen, H. E., Bisio, H., and Soldati-Favre, D. (2019). The triumvirate of signaling molecules controlling Toxoplasma microneme exocytosis: Cyclic GMP, calcium, and phosphatidic acid. *PLoS pathogens*, *15*(5), e1007670.

Darweesh, N. H., Hussein, R. A., Salman, S. T., and Shaker, M. J. (2018). Immunological and Molecular study of Toxoplasma gondii from aborted women in Diyala/Iraq. *Scientific Journal of Medical Research*, *2*(6), 75-82.

Deka, S., Kalita, D., Paul, M., Badoni, G., and Mathuria, Y. P. (2022). Seroprevalence and determinants of ToRCH pathogens in pregnant women in the sub-Himalayan region. Cureus, 14(2).

Dixit, B., Meshram, S., Jha, A. K., and Khare, R. (2024). Parasitic fauna associated with reproductive disorders. *Principles and Practices of Canine and Feline Clinical Parasitic Diseases*, 161-172.

Duarte, P. O., Oshiro, L. M., Zimmermann, N. P., Csordas, B. G., Dourado, D. M., Barros, J. C., and Andreotti, R. (2020). Serological and molecular detection of *Neospora caninum* and *Toxoplasma gondii* in human umbilical cord blood and placental tissue samples. Scientific reports, 10(1), 9043.

Elamin, M. H., Al-Olayan, E. M., Omer, S. A., Alagaili, A. N., and Mohammed, O. B. (2012). Molecular detection and prevalence of *Toxoplasma gondii* in pregnant women in Sudan. Afr. J. Microbiol. Res, 6(2), 308-311.

Farhan, S. M., Adi, Y. A., Alani, Z. K., AlYasari, J. T. O., and AlTimimi, H. M. (2024). Prevalence of Toxoplasma gondii Infections in Pregnant Women and Small Ruminants: A Molecular Diagnostic Approach. *Integrative Biomedical Research*, *8*(8), 1-6.

Gao, J., Huo, L., Zhu, S., Xu, W., Lu, Q., Wang, X., and Jiang, J. (2024). Seroprevalence of *Toxoplasma gondii* infection among pregnant women in the first trimester in China. Acta Tropica, 260, 107482.

Gashout, A., Amro, A., Erhuma, M., Al-Dwibe, H., Elmaihub, E., Babba, H., and Abudher, A. (2016). Molecular diagnosis of *Toxoplasma gondii* infection in Libya. BMC infectious diseases, 16, 1-8.

Hadi, H. S., Kadhim, R. A., and Al-Mammori, R. T. (2016). Seroepidemiological aspects for *Toxoplasma gondii* infection in women of Qadisiyah province, Iraq. International journal of pharmtech research, 9(11), 252-259.

Hakimi, M. A. (2022). Epigenetic reprogramming in host-parasite coevolution: the toxoplasma paradigm. *Annual review of microbiology*, *76*, 135-155.

Hiraoka, M., and Kagawa, Y. (2017). Genetic polymorphisms and folate status. *Congenital anomalies*, *57*(5), 142-149.

Ibrahim, H. M., Mohamed, A. H., El-Sharaawy, A. A., and El-Shqanqery, H. E. (2017). Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt. Asian Pacific Journal of Tropical Medicine, 10(10), 996-1001.

Khan, B., Channo, A., Rajput, R., Qazi, A., Akbar, H., Zaynub, N., and Hassan, M. (2023). Animals to human transmission of intestinal diseases: a review of the mechanism and factors involved. *Zoonosis, Unique Scientific Publishers, Faisalabad*, *Pakistan*, *4*, 29-45.

Kim, J., Kim, H., Roh, H., and Kwon, Y. (2018). Causes of hyperhomocysteinemia and its pathological significance. *Archives of pharmacal research*, *41*(4), 372-383.

Li, J., Duan, H., Ramaswamy, H., and Wang, C. (2025). A Comprehensive Review of Fortification, Bioavailability, and Health Benefits of Folate. *International Journal of Molecular Sciences*, *26*(16), 7703.

Loizidou, E. M., Kucherenko, A., Tatarskyy, P., Chernushyn, S., Livshyts, G., Gulkovskyi, R., and Livshits, L. (2021). Risk of recurrent pregnancy loss in the Ukrainian population using a combined effect of genetic variants: a case-control study. *Genes*, *12*(1), 64.

Lozano, T. D. S. P., Benitez, A., Santos, J. C. D., Navarro, I. T., Nagata, W. B., Pinto, M. D. S., and Bresciani, K. D. S. (2024). Seroprevalence of Toxoplasma gondii and Associated Risk Factors in Pregnant Women in Araçatuba, São Paulo, Brazil: A Multi-Level Analysis. *Microorganisms*, *12*(11), 2183.

Mahmood, S. H., Hassani, H. H., and Zghair, K. H. (2010). Detection of B1 gene of *Toxoplasma gondii* in blood of pregnant and abortive women infected with this parasite. Iraqi J Med Sci, 8(3), 42-8.

Maisarah, A., Mohamad, S., Husain, M., Abdullah, S., and Noordin, R. (2024). Seroprevalence and sociodemographic characteristics of *Toxoplasma gondii* infection in patients with psychiatric disorders. Acta Tropica, 255, 107241.

Maltsev, D. V., and Hurzhii, O. O. (2022). Recurrent ocular toxoplasmosis infection in a patient with a selective deficiency of NK T-cells and cytotoxic CD8+ T-cells associated with a genetic folate cycle deficiency. *Journal of Ophthalmology (Ukraine)/Oftal'mologičeskij Žurnal*, (4).

Mijbel, Z. K., and Alsaady, H. A. M. (2023). Seroprevalence of *Toxoplasma gondii* and Its Effect on the Levels of Dopamine and Adrenaline in Humans. Journal of Chemical Health Risks, 13(3).

Mikaeel, F. B., and Al-Saeed, A. T. M. (2019). Seroprevalence and molecular detection of *Toxoplasma gondii* among women in Duhok province/Iraq. Journal of Duhok University, 22(2), 85-92.

Mir, R., Elfaki, I., Javid, J., Barnawi, J., Altayar, M. A., Albalawi, S. O., and AbuDuhier, F. M. (2022). Genetic determinants of cardiovascular disease: the endothelial nitric oxide synthase 3 (eNOS3), Krüppel-like factor-14 (KLF-14), methylenetetrahydrofolate reductase (MTHFR), MiRNAs27a and their association with the predisposition and susceptibility to coronary artery disease. *Life*, *12*(11), 1905.

Mohajab, A. H., Alshehri, H. Z., Shati, R. O., Alshehri, A. A., Alafghani, M. A., Alasmari, A., and Oraif, A. (2020). Anti-toxoplasma antibody prevalence and cost-effectiveness in pregnant women at the King Abdulaziz University Hospital, Jeddah, KSA. Cureus, 12(1).

Mohammed, L. J., and Al-Janabi, M. S. (2019). Seroprevalence of toxoplasmosis in aborted women in Babylon Province, Iraq. Medical Journal of Babylon, 16(3), 188-191.

Mustafa, K. M., Mohammed, A. B., Mero, W. M., Mustafa, K., and Mohammed, A. B. (2024). Seroprevalence of *Toxoplasma gondii* antibodies and associated risk factors among women in Zakho City, Iraq. Cureus, 16(3).

Naizi, A. D., Omer, A. R., Al-Hadithi, T. S., and Aswad, A. (1990). Prevalence of *Toxoplasma* antibodies in Iraqi pregnant women in Baghdad. In Zoonotic and parasitic diseases: proceedings of the Third International and Pan-Arab Seminar held in Amman, Jordan, 17-20 Oct. 1989. IDRC, Ottawa, ON, CA.

Rafiei, S. T., Mohammadian, T., and Rasi, H. (2017). Common Polymorphisms in MTHFR and Prothrombin Gene in Iranian Women with Abortions at Different Ages. *Obstet. gynecol. int. j*, *8*(2), 00286.

Rahi, A.A., and Al-Rodaynee, S.H. (2017). Diagnosis of *Toxoplasma gondii* in pregnant women at Baghdad province. World Journal of Pharmaceutical and Life Sciences, 3(7), 52-54.

RahimiEsboei, B., Rahimi, M. T., Ghorbani, A., and Mahdavi, S. (2021). Genetic characterization of *Toxoplasma gondii* in women of reproductive age in the North of Iran. Journal of Nursing and Midwifery Sciences, 8(2), 74-78.

Ramírez-Flores, C. J., and Mondragón-Flores, R. (2025). Comprehensive analysis of Toxoplasma gondii migration routes and tissue dissemination in the host. *PLOS Neglected Tropical Diseases*, *19*(7), e0013369.

Razooqi, M. A., Gharban, H. A. J., and Al-Kaabi, M. A. F. (2022). Molecular and seroprevalence of toxoplasmosis in goats' blood and milk in Iraq. *Archives of Razi Institute*, *77*(5), 1749.

Razzak, A. H., Wais, S. A., and Saeid, A. Y. (2005). Toxoplasmosis: the innocent suspect of pregnancy wastage in Duhok, Iraq. East Mediterr Health J, 11(4), 625-32.

Sanchez, S. G., and Besteiro, S. (2021). The pathogenicity and virulence of Toxoplasma gondii. *Virulence*, *12*(1), 3095-3114.

Saxena, A. K., Agarwal, M., Kumar, A., and Singh, C. K. (2020). Genetic heterogenicity of MTHFR C677T allele modulate hormonal dysfunction associated risk factors in the cases of male infertility. *International Journal of Development Research*, *10*(12), 42700-42705.

Xie, X., Zhang, Y., Xin, L., Leng, J., Lu, Y., and Xue, Y. (2017). Relationship of folate metabolism related enzymes MTHFR and MTRR gene polymorphisms with unexplained recurrent spontaneous abortion. *Int J Clin Exp Pathol*, *10*(3), 3746-3752.

Ye, S., Lunghi, M., and Soldati-Favre, D. (2022). A signaling factor linked to Toxoplasma gondii guanylate cyclase complex controls invasion and egress during acute and chronic infection. *MBio*, *13*(5), e01965-22.

Zanet, S., Veronesi, F., Giglia, G., Baptista, C. R. P., Morganti, G., Mandara, M. T., and Ferroglio, E. (2023). The dangerous side of being a predator: Toxoplasma gondii and Neospora caninum in birds of prey. *Pathogens*, *12*(2), 271.