MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1, 2026, Pages: 2355-2359
ELSEVIER

Gene Expression of virulence factors ROP1 and ROP2 in women infected with *Toxoplasma gondii* mixed with Herpes Simplex Virus

Alquraishy D. A. Kh.[1] and Altamemy A.K. Aakool[2]
[1]Wasit University, College of Science, Department of Pathological analysis, Iraq
[2]Wasit University, College of Science, Department of Biology, Iraq
Email: duaa.akh49@gmail.com, Email: altamemy1959@gmail.com

Abstract

Toxoplasma gondii (*T. gondii*) is a pervasive protozoan parasite associated with serious pregnancy complications, such as spontaneous abortion. Rhoptry Protein1 (ROP1) and Rhoptry Protein2 (ROP2) are a major virulence factor which is used in the host cell invasion and formation of parasitophorous vacuoles. The purpose of this research was to examine the relation between *T. gondii* infection and the intensity of the ROP1 and ROP2 gene expression and the frequency of adverse pregnancy outcomes. The present research was carried out on 250 women, 100 of whom were pregnant women (Group 1), 100 of whom had a history of spontaneous abortion (Group 2), and 50 of whom were controls (Group 3). Initial screening for toxoplasmosis was performed using a rapid IgG antibody test. Confirmation of active infection and detection of Herpes Simplex Virus (HSV) co-infection was carried out using Real-Time PCR targeting the *T. gondii* B1 gene and HSV DNA, respectively. ROP1 and ROP 2gene expression was quantified via RT-qPCR in *T. gondii*-positive samples and compared across groups. The rapid IgG test showed seropositivity in 23.0% (23/100) of pregnant women and 21.0% (21/100) of aborted women, while all controls were seronegative (0/50), a statistically significant difference (p=0.001). Real-Time PCR confirmed active *T. gondii* infection in 19.0% (19/100) of pregnant women and 16.0% (16/100) of aborted women, with all controls testing negative (p=0.005). HSV was detected in 7.0% (7/100) of pregnant women and 17.0% (17/100) of aborted women (p=0.002). ROP1 gene expression was significantly higher (p=0.053) in pregnant women with *T. gondii* infection (Mean ± SD: 8.15 ± 1.33) and those with mixed *T. gondii*/HSV infection (7.76 ± 1.02) compared to aborted women with *T. gondii* infection only (6.12 ± 1.41) and aborted women with mixed infection (5.65 ± 1.4), while Mean of ROP2 Gene expression were (Mean ± SD: 7.30 ± 1.16) in pregnant women with *T. gondii* infection only and those with mixed *T. gondii*/HSV infection (6.38 ± 1.2) compared to aborted women with *T. gondii* infection only (6.75 ± 0.68) and aborted women with mixed (*T. gondii* and Herpes) infection (5.90 ± 1.3), the mean levels was non-significant higher (p-value=0.24) in both group (pregnant women with *T. gondii* infection only, pregnant women with mixed (*T. gondii* and Herpes) infection) in compared to both (aborted women with *T. gondii* infection only and aborted women with mixed (*T. gondii* and Herpes) infection, as determined by Duncan's post-hoc test. Finally, the high increase in the ROP1 expression level may also be used as a possible molecular marker in predicting the risk of abortion in pregnant women infected with *T. gondii*.

Keywords: Herpes Simplex Virus, *Toxoplasma gondii*, ROP, Abortion, Real-Time PCR, Gene Expression, Pregnancy.

1. Introduction

T. gondii is an apicomplexan obligatory intracellular parasite that is worldwide distributed and can infect virtually all warm-blooded animals, including humans **(Ramírez-Flores and Mondragón-Flores, 2025).** All over the world, the prevalence rates of seroprevalence are uneven, as they depend on the geographical location, dietary habits, and the socioeconomic status. In most areas, high percentage of the population carries a chronic and latent infection which is usually asymptomatic in immunocompetent individuals **(Pappas *et al.*, 2009).**

Nevertheless, primary infection acquired throughout pregnancy is a major risk to the development of the fetus. The parasite is capable of passing through the placental barrier resulting into congenital toxoplasmosis which could be in the form of spontaneous abortion, stillbirth, or extreme neurological and ocular sequelae in the newborn **(Montoya and Liesenfeld, 2004).** The *T. gondii* infection is a complicated interaction between the virulence of the parasites, the size of inoculum, and the immune response of the host to achieve the clinical outcome. Most of the studies and diagnostic practices have traditionally been done on serological markers (IgG, IgM, IgA), and the avidity tests to differentiate between the most recent and the previous infection **(Robert-Gangneux and Dardé, 2012).** Although they are invaluable tools, they give little information on the parasitic load and the molecular pathogenicity mechanisms that govern an active infection.

The introduction of the molecular methodologies, especially Polymerase Chain Reaction (PCR) and Real-Time PCR, has transformed the direct direction regarding parasitic DNA in clinical samples, providing high specificity as well as sensitivity to diagnose active toxoplasmosis **(Costa and Bretagne, 2012; Kumar *et al.*, 2021).** The pathogenicity related to *T. gondii* is majorly due to its advanced invasion machinery, housed in distinctive secretory organelles called micronemes and rhoptries.

During the invasion of the host cell, rhoptry proteins (ROPs) are discharged, which are essential in the creation of the parasitophorous vacuole (PV) that shields the parasite from host defences **(Ben Chaabene et al., 2021).** One of these has been pointed out to be Rhoptry Protein (ROP1) and (ROP2). ROP1, even though not necessary for invasion in all strains, is part of the irreversible alteration of the host cell membrane and the formation of PV, which contributes to the successful formation of an intracellular niche of a parasite **(Butterworth *et al.*, 2022).** Although the process of invasion is accompanied by the insertion of ROP2 into the parasitophorous vacuole membrane (PVM) exposing its N-terminal end into the host cell cytoplasm **(Reese and Boothroyd, 2009).**

The later research found a close connection between this N-terminus of the ROP2 protein that interacts with the host cell mitochondrial import machinery **(Sinai, 2014; El Hajj *et al.*, 2007)**, where the levels of this virulence factor can directly affect the contact of the parasite with the host, which can change the severity of the infection and consequently, the immune and pathological responses.

The maternal immune response to *T. gondii* is of a delicate balance. Robust T-helper 1 (Th1) response, characterized through the production of (interferon-gamma (IFN-γ)) is essential in the control of parasitic proliferation **(Sana *et al.*, 2022).** How certain parasitic virulence factors such as ROP1and ROP2 might modulate this local immune environment is of increasing interest, it is suggested that changes in the expression of genes might be the difference between an infection successfully being controlled by the host or resulting in placental damage and fetal demise. The structural role and interaction with active kinases that modulate host immune responses and improve parasite survival is the importance of ROP2 in the complex virulence strategy in the parasite which is demonstrated by the close relationship between ROP2 and the active kinase ROP18 that phosphorylates other proteins thereby triggering a series of virulence-related events **(Qiu *et al.*, 2009).** More so, one cannot ignore the effects of co-infections in pregnancy.

Another typical pathogen that is linked with poor pregnancy outcomes is that of Herpes Simplex Virus (HSV) **(Brown *et al.*, 2005).** *T. gondii* and HSV co-infection have a potential effect on the alteration of the clinical outcome due to the synergistic or antagonistic effects of immune modulation that remains poorly characterized in the case of abortion.

Although many studies have developed epidemiological relation involving *T. gondii* seropositivity and abortion, some studies have utilized PCR to identify the presence of the parasite in the aborted tissues, a notable gap in the literature is the quantitative expression of major parasitic virulence genes in the clinical samples in clearly defined patient cohorts.

Practically, there is a gap in research done on the relationship between the degree of ROP1 gene expression and the clinical pregnancy outcome. Thus, the case-control study was aimed at filling this gap. We intended to not only find out the prevalence of active *T. gondii* and

HSV infection among pregnant and aborted women using sophisticated molecular methods but also to determine the level of expression of the gene ROP1 in *T. gondii*-positive patients quantitatively.

Our hypothesis assumed that the risk of spontaneous abortion is linked to the differential expression of the ROP1 gene, and it gives a new molecular insight into the pathogenesis of pregnancy-associated toxoplasmosis.

2. Materials and Methods

2.1. Study Design and Participants

The study was conducted from [February 2024 to December 2024]. A total of 250 women were recruited and categorized into three groups:

- **Pregnant Women:** 100 women with ongoing pregnancies.
- **Aborted Women:** 100 women who had recently experienced a spontaneous abortion.
- **Healthy Control:** 50 healthy, non-pregnant women with no history of recent abortion or known *T. gondii* infection.

Informed consent was obtained from all participants. The study protocol was approved by the Institutional Ethical Committee of [Health, Higher Education and Scientific Research in Iraq]

Source of funding: Self-funding

2.2. Sample Collection and Initial Serological Screening

Peripheral blood samples (5 ml) were collected from each participant under aseptic conditions. Serum was separated for initial screening of *T. gondii* IgG antibodies using a commercial rapid diagnostic test cassette (OnSite Toxo IgG Rapid Test, CTK Biotech, USA), performed according to the manufacturer's instructions.

2.3. DNA Extraction and Real-Time PCR for Pathogen Detection

Genomic DNA was extracted from 200 µl of whole blood using the gSYAN DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's protocol. The concentration and purity of DNA were verified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). Real-Time PCR was performed to detect *T. gondii* and HSV DNA using a Bio-Rad MiniOpticon Real-Time PCR System. The reaction mixture for *T. gondii* detection (20 µl) contained 5µL DNA template, 1µL each of forward and reverse primers targeting the B1 gene **(12)**, 1µL of a FAM-labeled TaqMan probe, and 10µL of RealMOD™ Probe 2X qPCR Mix (INtRON, Korea). The thermocycling conditions were: 95°C for 5 min, followed by 40 cycles of 95°C for 20 sec and 60°C for 30 sec. Detection of HSV was performed similarly using specific primers and probe **(Kessler, *et al.*, 2000).** The primer sequences used were**:**

T. gondii Forward TCCCCTCTGCTGGCGAAAAGT 113 bp
Reverse AGCGTTCGTGGTCAACTATCGATTG
Probe FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA

HSV Forward CATCACCGACCCGGAGAGGGAC 92 bp
Reverse GGGCCAGGCGCTTGTTGGTGTA
Probe FAM-CCGCCGAACTGAGCAGACACCCGCGC-TAMRA

2.4. RNA Extraction, cDNA Synthesis, and Gene Expression Analysis (RT-qPCR)

Total RNA was extracted from *T. gondii*-positive blood samples using the AccuZol Total RNA Extraction Kit (Bioneer, Korea). Extracted RNA was treated with DNase I (Promega, USA) to remove genomic DNA contamination. cDNA was synthesized from 100 ng of DNase-treated RNA using the M-MLV Reverse Transcriptase kit (Bioneer, Korea) with random hexamer primers. The expression of the *ROP1* gene was quantified by RT-qPCR using the GoTaq® qPCR Master Mix (Promega, USA) on the Bio-Rad MiniOpticon system. The GAPDH gene was used as an endogenous control for normalization. The primer sequences used were:

- *ROP1-F:* ACGCAAACGCTGATGACTTC
- *ROP1-R:* TCTGTGACAAGAGCAGCTTCTC
- *GAPDH-F:* AAAACATCGCCCTGACAACG
- *GAPDH-R:* TTTTGAGGCGTTCCAGAAGC

The relative gene expression was calculated using the 2^–ΔΔCT method **(14)**. The pregnant women with *T. gondii* mono-infection group were used as the calibrator.

2.5. Statistical Analysis

Data were analyzed using SPSS software (Version 26, IBM, USA). Continuous variables (e.g., age, gene expression) were presented as Mean ± Standard Deviation (SD) and compared using one-way ANOVA followed by Duncan's post-hoc test for multiple comparisons. Categorical variables (e.g., residency, infection status) were presented as numbers and percentages (%) and compared using the Chi-square (χ^2) test. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Prevalence of mixed infection with *T. gondii* and HSV.

The rapid IgG test identified 23% (23/100) of pregnant women and 21% (21/100) of aborted women as seropositive, while all controls were negative (p=0.001). Real-Time PCR, confirming active infection, detected *T. gondii* DNA in 19% (19/100) of pregnant women and 16% (16/100) of aborted women (p=0.005). HSV DNA was found in 7% (7/100) of pregnant women and 17% (17/100) of aborted women (p=0.002). All control samples were negative for both pathogens **(Table 3-1 & 3-4).**

Table (3-1): Rapid diagnosis test (cassette) IgG of Toxoplasmosis in pregnant, aborted women and healthy women.

Result	Pregnant women *n* = 100	Aborted women *n* = 100	Healthy control *n* = 50
Positive, *n* (%)	23 (23.0%(	21 (21.0%)	0
P value	0.001 S		
X²	13.487		

¥: Chi-square test; S: significant at P ≤ 0.001.

Table (3-2): Real Time PCR diagnosis *of Toxoplasma gondii* and Herpes simplex virus in pregnant, aborted women and healthy women

Result	Pregnant women *n* = 100	Aborted women *n* = 100	Healthy control *n* = 50
Real Time PCR *Toxoplasma gondii*			
Positive, *n* (%)	19 (19.0%(	16 (16.0%)	0
P value	0.005 S		
X^2	10.548		
Real Time PCR Herpes simplex virus			
Positive, *n* (%)	7 (7.0%(	17 (17.0%)	0
P value	0.002 S		
X^2	12.399		

¥: Chi-square test; S: significant at $P \leq 0.001$.

3.3. ROP1 and ROP2 Gene Expression Analysis

The analysis of virulence gene expression revealed a significant finding for *ROP1*. The mean *ROP1* expression level was significantly higher (p=0.053) in pregnant women with *T. gondii* mono-infection (8.15 ± 1.33) and those with mixed infection (7.76 ± 1.02) compared to aborted women with *T. gondii* mono-infection (6.12 ± 1.41) and aborted women with mixed infection (5.65 ± 1.4). Duncan's post-hoc test assigned the same superscript 'A' to the pregnant groups and 'B' to the aborted groups, confirming a significant difference between these two clusters **(Table 3-3, Figure 1).**

Table (3-3): ROP1 Gene expression in different groups

Groups		***ROP1 Gene expression***
Pregnant with *T. gondii* only	**Mean ± SD**	**8.15 ± 1.33^{A}**
	Range	5.75-11.45
Pregnant with mixed (*T. gondii* and *Herpes*) infection	**Mean ± SD**	**7.76** $\pm 1.02^{A}$
	Range	5.65-8.75
Aborted with *T. gondii* only	**Mean ± SD**	**6.12 ± 1.41^{B}**
	Range	2.53-8.40
Aborted with mixed (*T. gondii* and *Herpes*) infection	**Mean ± SD**	**5.65** $\pm 1.4^{B}$
	Range	0.80-8.75
p-value		**0.053** †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SD: standard deviation; †: one way ANOVA

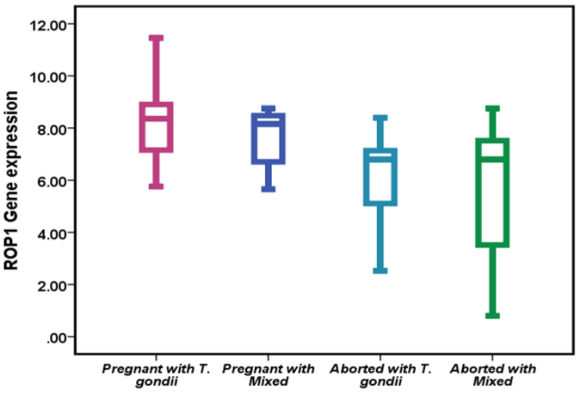

Figure 1: ROP1 Gene Expression in Study Groups (A bar chart showing significantly higher ROP1 expression in infected pregnant women (Groups: Pregnant with *T. gondii* only, Pregnant with mixed infection) compared to infected aborted women (Groups: Aborted with *T. gondii* only, Aborted with mixed infection)).

In contrast, the expression levels of the ROP2 gene did not show a statistically significant difference across the same groups (p=0.241) (Table 3-4, Figure 2).

Table (3-4): ROP2 Gene expression in different groups

Groups		***ROP2 Gene expression***
Pregnant with *T. gondii* only	**Mean ± SD**	**7.30 ± 1.16**^{A}
	Range	5.92-8.92
Pregnant with mixed (*T. gondii* and Herpes) infection	**Mean ± SD**	**6.38** ± 1.2^{A}
	Range	4.92-8.30
Aborted with *T. gondii* only	**Mean ± SD**	**6.75 ± 0.68**^{A}
	Range	5.50-7.52
Aborted with mixed (*T. gondii* and Herpes) infection	**Mean ± SD**	**5.90** ± 1.3^{A}
	Range	2.03-9.58
p-value		**0.241** †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SD: standard deviation; †: one way ANOVA

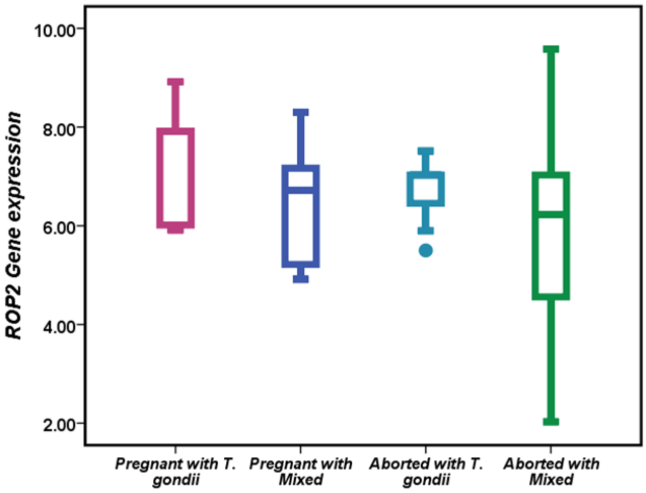

Figure 2: ROP 2 Gene Expression in Study Groups (A bar chart showing non- significant higher ROP2 expression in infected pregnant women (Groups: Pregnant with *T.gondii* only, Pregnant with mixed infection) compared to infected aborted women (Groups: Aborted with *T. gondii* only, Aborted with mixed infection)).

4. Discussion

This study provides compelling evidence linking the molecular pathology of *T. gondii* to adverse pregnancy outcomes, specifically through the differential expression of the parasitic virulence gene *ROP1*. Our findings confirm a significant prevalence of active *T. gondii* infection in both pregnant and aborted women, with Real-Time PCR offering a more precise picture of active parasitemia than serology alone, also demonstrate for the first time in a clinical cohort that women who maintained their pregnancies despite *T. gondii* infection exhibited significantly higher levels of *ROP1* gene expression compared to women who suffered spontaneous abortions.

The seroprevalence of 21-23% is comparable to intermediate levels of endemicity observed in various regions **(Foroutan *et al.*, 2024).** The greater detection of active T. gondii infection in the aborted group found through the PCR method (16%) than the control group (0%) confirms the previously tested linkage between acute toxoplasmosis and pregnancy loss **(El Deeb *et al.*, 2012).**

Independent risk posed by this virus is emphasized by the fact that the HSV infection rate among the aborted population (17%) is much higher than the rate among the pregnant women (7%) **(Brown *et al.*, 2005).** The fact that our cohort has mixed infections is also a source of complexity because interactions between two or more pathogens may exacerbate inflammatory responses at the placenta **(Megli and Coyne, 2022).**

The central and most novel finding of this study is the differential expression of the *ROP1* gene. The significantly lower *ROP1* expression in aborted women, regardless of HSV mixed infection status, challenges a straightforward assumption that higher virulence factor expression always equates to worse pathology. There is a proposal for two non-mutually exclusive hypotheses to explain this inverse correlation.

First, higher ROP1 levels in the pregnant group may reflect a more robust and effective host immune response. ROP1 is a known immunodominant antigen **(Lekutis *et al.*, 2000).** An aggressive Th1-driven immune response, necessary to control the parasite, might also upregulate certain parasitic genes as part of a stress response or could be more effective at lysing parasites with high ROP1 expression, leaving a population with lower ROP1 expression to persist. The controlled, high-level expression in pregnant women may represent a balanced host-parasite interaction where the parasite is kept in check without triggering catastrophic placental inflammation.

Second, lower *ROP1* expression in the abortion group could be a strategic adaptation of the parasite to evade host immunity **(Butterworth *et al.*, 2022).** Strains or phenotypic variants with downregulated "classical" virulence factors might adopt a more stealthy approach, favoring chronic persistence. However, this stealth could come at the cost of altered tropism or a failure to properly establish a controlled intracellular niche, leading to erratic growth and the release of parasitic products that incite a localized, destructive inflammatory cascade at the maternal-fetal interface.

This is an uncontrolled inflammation which has been widely documented to cause pregnancy losses **(Mor and Cardenas, 2010).** The parasite, upon manipulating its gene expression, in this case, inadvertently triggers pathological host response, which leads to abortion. This is in line with the research findings that the result of the infection is greatly dictated by the strain of the parasite and the interaction between the effects of the parasite and the host signaling pathways **(Hunter and Sibley, 2012).**

The fact that there is no significant difference in the expression of ROP2 indicates that the action of ROP1 in that case is different. ROP2 is a component of a protein complex that is engaged in the recruitment of host mitochondria to the PV and possesses varied immunological characteristics **(Pernas *et al.,* 2014).** It suggests that not every rhoptry protein is equally related to the pregnancy clinical outcome, which indicates the importance of the ROP1 in particular.

Finally, this paper does not limit itself to identification but creates a quantitative correlation between a central parasitism virulence gene and a fatal clinical event. The present research has demonstrated that low-level expression of the *T. gondii* ROP1 gene is closely related to spontaneous abortion.

This makes the expression of ROP1 a possible new prognostic biomarker. The measurement of ROP1 levels with a combination of standard PCR diagnostics would allow stratifying the abortion risk among pregnant women with *T. gondii* infection and, therefore, monitor and manage them more closely. The next direction of the research should be to understand the exact molecular processes through which the ROP1 expression affects the host-pathogen standoff in the placenta and provide new possibilities to make therapeutic intervention.

References

Ben Chaabene, R., Lentini, G., & Soldati-Favre, D. (2021). Biogenesis and discharge of the rhoptries: Key organelles for entry and hijack of host cells by the Apicomplexa. Molecular microbiology, 115(3), 453-465.

Brown, Z. A., Gardella, C., Wald, A., Morrow, R. A., & Corey, L. (2005). Genital herpes complicating pregnancy. Obstetrics and gynecology, 106(4), 845–856 .

Butterworth, S., Moreira-Souza, A. C. A., Broncel, M., Song, O.-R., Young, J. C., Russell, M. R. G., Torelli, F., Wagener, J., Lockyer, E. J., & Treeck, M. (2022). *Toxoplasma gondii* virulence factor ROP1 reduces parasite susceptibility to murine and human innate immune restriction. PLoS Pathogens, 18,(12)

Costa, J. M., & Bretagne, S. (2012). Variation of B1 gene and AF146527 repeat element copy numbers according to Toxoplasma gondii strains assessed using real-time quantitative PCR. Journal of clinical microbiology, 50(4), 1452-1454.

El Deeb, H. K., Salah-Eldin, H., Khodeer, S., & Allah, A. A. (2012). Prevalence of *Toxoplasma gondii* infection in antenatal population in Menoufia governorate, Egypt. Acta Tropica, 124(3), 185-191.

El Hajj, H., Lebrun, M., Fourmaux, M. N., Vial, H., & Dubremetz, J. F. (2007). Inverted topology of the *Toxoplasma gondii* ROP5 rhoptry protein provides new insights into the association of the ROP2 protein family with the parasitophorous vacuole membrane. Cellular microbiology, 9(1), 54-64.

Foroutan, M., Majidiani, H., Hassanipour, S., & Badri, M. (2024). *Toxoplasma gondii* seroprevalence in the Iranian blood donors: A systematic review and meta-analysis. Heliyon, 10(6)

Hunter, C. A., & Sibley, L. D. (2012). Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. Nature Reviews Microbiology, 10(11), 766-778.

Kessler, H. H., Mühlbauer, G., Rinner, B., Stelzl, E., Berger, A., Dörr, H. W., ... and Rabenau, H. (2000). Detection of herpes simplex virus DNA by real-time PCR. Journal of clinical microbiology, 38(7), 2638-2642.

Kumar, S., Gupta, S., Mohmad, A., Fular, A., Parthasarathi, B. C., & Chaubey, A. K. (2021). Molecular tools-advances, opportunities and prospects for the control of parasites of veterinary importance. International Journal of Tropical Insect Science, 41(1), 33-42.

Lekutis, C., Ferguson, D. J., & Boothroyd, J. C. (2000). *Toxoplasma gondii*: identification of a developmentally regulated family of genes related to SAG2. Experimental parasitology, 96(2), 89-96.

Lin, M. H., Chen, T. C., Kuo, T. T., Tseng, C. C., & Tseng, C. P. (2000). Real-time PCR for quantitative detection of Toxoplasma gondii. Journal of clinical microbiology, 38(11), 4121-4125.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408.

Megli, C. J., & Coyne, C. B. (2022). Infections at the maternal–fetal interface: an overview of pathogenesis and defence. Nature Reviews Microbiology, 20(2), 67-82 .

Montoya, J. G., & Liesenfeld, O. (2004). Toxoplasmosis. Lancet (London, England), 363(9425), 1965–1976 .

Mor, G., & Cardenas, I. (2010). The immune system in pregnancy: a unique complexity. American journal of reproductive immunology, 63(6), 425-433.

Pappas, G., Roussos, N., & Falagas, M. E. (2009). Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. International journal for parasitology, 39(12), 1385-1394.

Pernas, L., Adomako-Ankomah, Y., Shastri, A. J., Ewald, S. E., Treeck, M., Boyle, J. P., & Boothroyd, J. C. (2014). *Toxoplasma* effector MAF1 mediates recruitment of host mitochondria and impacts the host response. PLoS biology, 12(4), e1001845.

Qiu, W., Wernimont, A., Tang, K., Taylor, S., Lunin, V., Schapira, M., ... & Sibley, L. D. (2009). Novel structural and regulatory features of rhoptry secretory kinases in *Toxoplasma gondii*. The EMBO journal, 28(7), 969-979.

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

Reese, M. L., & Boothroyd, J. C. (2009). A helical membrane-binding domain targets the *Toxoplasma* ROP2 family to the parasitophorous vacuole. Traffic, 10(10), 1458-1470.

Robert-Gangneux, F., & Dardé, M. L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical microbiology reviews, 25(2), 264–296 .

Sana, M., Rashid, M., Rashid, I., Akbar, H., Gomez-Marin, J. E., & Dimier-Poisson, I. (2022). Immune response against toxoplasmosis—some recent updates RH: Toxoplasma gondii immune response. International journal of immunopathology and pharmacology, 36, 03946320221078436.

Shwab, E. K., Saraf, P., Zhu, X. Q., Zhou, D. H., McFerrin, B. M., Ajzenberg, D., ... & Su, C. (2018). Human impact on the diversity and virulence of the ubiquitous zoonotic parasite *Toxoplasma gondii.* Proceedings of the National Academy of Sciences, 115(29), E6956-E6963.

Sinai, A. P. (2014). The *Toxoplasma gondii* parasitophorous vacuole membrane: A multifunctional organelle in the infected cell. Toxoplasma gondii, 375-387.