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Original Article

SEROLOGICAL AND IMMUNOHISTOCHEMICAL DIAGNOSIS OF TOXOPLASMOSIS AMONG ABORTED WOMEN IN SORAN DISTRICT, KURDISTAN- IRAQ

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ABSTRACT

The CLIA assay explored that 19.6% of women who underwent abortions showed positive anti-*Toxoplasma* IgG antibodies, indicating a past or chronic infection, while 1.2% displayed positive anti-*Toxoplasma* IgM, suggesting an acute or recent infection. All placental tissue from CLIA-seropositive women showed a positive reaction by IHC, indicating the presence of the parasite antigen. This study found that the highest rate of abortion occurred among women aged 26-35 years, at 22.9% (30/131). Most pregnancies ended in abortion during the first trimester. The consumption of undercooked meat was most significantly affecting the seroprevalence of anti-*Toxoplasma* IgG antibodies (P value = 0.008). In comparison, the factor influencing anti-*Toxoplasma* IgM antibodies was the level of education, with a P value of 0.031. This research revealed a high rate of toxoplasmosis among women who experienced pregnancy loss. It is advisable to raise awareness about risk factors and to test pregnant women during their gynecological care.

KEYWORDS: *Toxoplasma gondii*, Seroprevalence, Chemiluminescence Immunoassay. Immunohistochemistry, Risk factors, Aborted women,

1. INTRODUCTION

The protozoan parasite *Toxoplasma gondii* causes a zoonotic condition, which causes toxoplasmosis. This species is part of the phylum Apicomplexa, which consists of intracellular coccidian parasites found worldwide (Brito *et al*., 2023). It belongs to the suborder Eimeriorina, which includes several genera that infect vertebrates, such as *Sarcocystis* and *Toxoplasma*, along with various types of coccidia. Many of these coccidia are known to produce cysts (Swar and Shnawa, 2020). The parasite life cycle involves sexual reproduction in its definitive host (cats) and asexual reproduction in intermediate hosts (humans and animals). Cats shed millions of oocysts in their feces daily for three weeks. The fresh feces are not contagious, as oocysts require 1 to 5 days to be sporulated and become infective (Durdu and Bohîlțea,2023).

One of the main causes of abortion in pregnant women is *T. gondii*. Congenital toxoplasmosis occurs when a mother transmits the parasite to her unborn child during pregnancy. The risk of transmission increases as pregnancy progresses, about 2-15% in the first trimester, 18% in the second, and up to 70% in the third. Early infections can lead to fetal damage or miscarriage, while later infections may show no immediate symptoms, though they can result in neurological, eye, or hearing issues later on. If a mother contracts the infection before the 24th week, she is at higher risk for complications, especially if the strain is virulent (Rico-Torres *et al*.,2016; Kheirandish *et al*., 2019). Clinical problems such as spontaneous abortion, premature labor, stillbirth, and fetal abnormalities can arise when toxoplasmosis develops during pregnancy (Mohammed and Al-Janabi, 2019).

According to Robert-Gangneux and Belaz (2016), humans can get infected by consuming undercooked meat having *Toxoplasma* tissue cysts or sporulated oocysts. Additionally, congenital transmission can occur when tachyzoites are transferred from the mother to the fetus via the placenta (Vimercati *et al*., 2020). Although toxoplasmosis is usually without symptoms, it can become severe or even fatal during pregnancy if it affects the fetus, and or in immunocompromised persons. Symptoms will typically appear after the baby is born. Chronic inflammatory lesions of the placenta are a major challenge caused by toxoplasmosis (Kim *et al*., 2015).

This parasite can infect any nucleated cell found in warm-blooded mammals. Several microneme proteins have been proposed to be necessary for host cell recognition and invasion (Singer *et al*., 2023). *T. gondii* enters the intestinal epithelium and spreads throughout the body after being consumed as bradyzoites or oocysts. It is regarded as one of Europe's top three foodborne parasites with significant public health implications (Calero-Bernal *et al*., 2025).

Tachyzoites multiply quickly in the early stages of infection, infiltrate nucleated host cells, and spread to many organs, such as the heart, muscles, placenta, eyes, and central

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nervous system (Cabo *et al*., 2024). This phase results in inflammation and lysis of host cells. Eventually, host immunity causes tachyzoites to change into bradyzoites and create tissue cysts that last a long time (Wu *et al*., 2025). The consequence of toxoplasmosis relies on the equilibrium between the parasite's virulence and the status of the immune response of the host (Sanchez and Besteiro, 2021).

Human toxoplasmosis is diagnosed by the serological tests that determine the immunological status of the patient by detecting antibodies specific for *T. gondii* in their sera, especially in pregnant women, to know if the infection is acquired during gestation or prior to conception, as the fetus is at risk of developing congenital *Toxoplasma* infection (Durdu and Bohîlțea,2023).

This research aimed to investigate the presence of anti-*Toxoplasma* IgG and IgM antibodies and the potential risk factors related to toxoplasmosis among women of reproductive age who have aborted in Soran city, also to detect the parasite's antigens in their placental tissue using immunohistochemical analysis. Notably, this aspect has not been addressed in previous research within our area, highlighting the importance and novelty of this investigation.

2. MATERIALS AND METHODS

Study design and study subjects:

This investigation is a cross-sectional study. Two hundred and fifty women who experienced spontaneous abortion and were admitted to Soran Maternity Teaching Hospital in Soran district, Kurdistan, Iraq, from December 2024 to June 2025, it was enrolled in the study. The study population included women of various ages, ethnicities, and gestational ages undergoing abortion. The participants were interviewed after giving verbal consent. A questionnaire was organized for gathering epidemiological information (age, educational level, and residency), clinical data (gestational age and abortion frequency), and risk factors for toxoplasmosis, for example, having cats, consumption of undercooked meat, recent blood transfusions, and eating unwashed vegetables.

Sample collection and serum preparation:

A total of 250 blood specimens from spontaneously aborted females were checked for anti-*Toxoplasma* IgG and IgM antibodies by Chemiluminescent Immunoassay (CLIA). In aseptic conditions, 5 mL of blood was drawn from the antecubital vein by sterile, disposable syringes. The blood specimens were kept in gel tubes (IMPROVACUTER, Improve Medical, USA) for serum separation. The blood specimens were centrifuged for 15 minutes at 3000 rpm, and the serum was collected and stored at −20°C for the next examination (Shani *et al*., 2012). Additionally, 80 placental tissue samples were used from the seropositive aborted women for the confirmation of the parasite antigen in their placenta by the immunohistochemistry (IHC) technique. Twenty grams of each placental tissue were removed (Yi *et al*., 2020). After that, the tissue was fixed and subjected to further histological processing for 48 hours in sterile screw-capped containers containing buffered 10% formalin (Hamad *et al*.,2022).

Chemiluminescence immunoassay (CLIA)

The CLIA assay was performed using a 1000 series Maccura Chemiluminescence Analyzer utilizing an automated chemiluminescent immunoassay kit (Maccura Biotechnology Co., Ltd., China). The manufacturer's instructions were followed for processing and interpreting the sample. Signal-to-cut-off ratio (S/CO) was used to identify the anti-*Toxoplasma* IgM CLIA test; values <0.9 were regarded as negative, values ≥1.1 as positive, and values between 0.9 and 1.1 as inconclusive. In accordance with the WHO International Standard (NIBSC code: 01/600), the anti-*Toxoplasma* IgG yielded results in international units per milliliter (IU/mL). A value of less than 1.70 IU/mL was regarded as negative, more than 2.30 IU/mL as positive, and between 1.70 and 2.29 IU/mL as borderline, indicating the need for retesting.

Immunohistochemistry (IHC)

To detect *T. gondii* in the placental tissues, small parts were fixed in 10% neutral buffered formalin for 24 hr. Then, 4 µm thin sections were prepared from the paraffin-embedded blocks (Hamad *et al*.,2022). For the IHC study, the Dako EnVision detection immunohistochemistry kit (Envision FLEX, Dako, K8000, Denmark) was utilized in accordance with the manufacturer's guidelines. Sections of paraffin blocks were incubated with a polyclonal rabbit anti-*T. gondii* primary antibody (MBS373041, MyBiosource, USA; 1:100 dilution in EnVision™ FLEX Antibody Diluent, K8006), followed by HRP-labeled secondary antibody. Visualization was completed using the chromogen 3,3′-Diaminobenzidine (DAB). All sections were counterstained with Mayer's haematoxylin (Bio-Optica, Italy), dehydrated in ethanol, cleared in xylene, and mounted using DPX. Slides were viewed under a light microscope, and positive immunoreactivity appeared as brown deposits (Bolon, 2014).

Statistical analysis:

Data analysis was performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, IL, USA), which included both descriptive and analytical statistics. The chi-square test was utilized to investigate the relationship among various factors. Also, with the GraphPad Prism software (San Diego, CA, USA version 10.4), Fisher's exact test was applied. The supplied data contained information about proportion and frequency. A P-value < 0.05 is considered the significance level.

3. RESULTS

Out of 250 blood samples of the aborted women, 52 (20.8%) were positive for anti-*Toxoplasma* IgG or IgM antibodies, and 49 (19.6%) were seropositive for anti-*T. gondii* IgG using the CLIA assay, whereas just three samples (1.2%) had a seropositive rate for IgM antibodies, as presented in Table 1.

Table 1: Anti-*Toxoplasma* IgG and IgM antibodies seropositivity rate among 250 aborted pregnant females utilizing the automated CLIA system.

Test	Number of positive samples	%
CLIA IgG	49	19.6
CLIA IgM	3	1.2
Total	52	20.8

Regarding age, the maximum seroprevalence of IgG and IgM antibodies was observed among those aged 26-35 years, at 20.6 % (27/131) and 2.3% (3/131), respectively, while the age group of 36–45 years recorded the lowest seropositive rates for IgG and IgM, at 15.6% and 0.00%, respectively. Statistically, those age groups showed no significant difference ($p>0.05$)

(Table 2). In terms of residency, IgG antibodies were most prevalent in urban areas (19.8%), followed by rural areas (19.4%), with non-significant differences, whereas IgM was most prevalent in urban areas (3%), demonstrating no meaningful difference (P-value =0.064), as shown in Table 3.

Our findings revealed that most women who had first-trimester abortions displayed the highest levels of IgG and IgM antibodies, at 21% and 2%, respectively. This was followed by women who aborted in the third trimester for anti-*Toxoplasma* IgG at 20%, and those in the second trimester at 17.2%, based on gestational stages. In terms of abortions, the largest range for IgG and IgM was observed in 1-2 abortions, which were 35% and 3%, respectively, as depicted in Table 2.

Concerning educational status, the maximum prevalence of seropositive anti-*Toxoplasma* IgG was observed among women with university degrees (24.2%), followed by women with secondary education (22%) and illiterate participants (18.5%). Women with primary education showed a lower prevalence of 17.1%, although these differences were not statistically significant. Whereas we observed significant differences among the different educational levels when IgM antibodies were examined (P-value=0.031). Acute cases were identified among women with secondary education (2%) and those with university education (6%), as shown in Table 2.

Table 2: Comparison of Anti-Toxoplasma IgG and IgM with some patients' and environmental factors

	Total samples		**CLIA IgG +ve**		**CLIA IgM +ve**	
Variables	**No.**	**Total positive NO (%)**	**Sero Positive No (%).**	**P Value**	**Sero positive No (%).**	**P Value**
			Age group			
16-25	68	14(20.5)	14(20.5)		0(0)	
26-35	131	30(22.9)	27(20.6)	**0.732**	3(2.3)	**0.252**
36-45	51	8(15.6)	8(15.6)		0(0)	
Total	250	52(20.8)	49(19.6)	χ^2=0.62	3(1.2)	χ^2=2.75
			Abortions No.			
2-Jan	165	38(23)	35(21.2)		3(1.8)	
4-Mar	60	11(18.3)	11(18.3)	**0.535**	0(0)	**0.457**
≥5	25	3(12)	3(12)		0(0)	
Total	250	52(20.8)	49(19.6)	χ^2= 1.25	3(1.2)	χ^2=1.56
			Sources of water			
Tap water	93	20(21.5)	18(19.4)		2(2.1)	
springs	80	17(21.3)	16(20)	**0.999**	1(1.2)	**0.777**
well	77	15(19.5)	15(19.5)		0(0)	
Total	250	52(20.8)	49(19.6)	χ^2=0.01	3(1.2)	χ^2= 1.64
			Education			
Illiterate	97	18(18.5)	18(18.5)		0(0)	
Primary	70	12(17.1)	12(17.1)	**0.809**	0(0)	**0.031***
Secondary	50	12(24)	11(22)		1(2)	
University	33	10(30.3)	8(24.2)		2(6)	
Total	250	52 (20.8)	49(19.6)	χ^2=0.97	3(1.2)	χ^2=8.87

Statistical significance (P-value < 0.05), Chi-square (χ2) test:

Moreover, the prevalence of anti-*Toxoplasma* IgG antibodies was greater in females who had cats than in those who did not (22% versus 15.1%); this difference indicated no significant effect(P-value=0.241). Conversely, females without any contact with cats exhibited a greater prevalence of anti-*Toxoplasma* IgM antibodies than those who had contact (2.3% versus 0.6%), and this difference, too, was not statistically significant (P=0.237), as shown in Table 3.

According to the source of water, users of wells had a percentage of anti-Toxoplasma IgG antibodies, 15/77 (19.5%), followed by users of tap water for both anti-*Toxoplasma* IgG and IgM as 18/93 (19.4%) and 2/93 (2.1%) antibodies, respectively. Also, springs users recorded 16/80 (20%) anti-*Toxoplasma* IgG, as shown in Table 3. Among women who had experienced abortions, 11 out of 27 (40.7%) were noted to be seropositive for anti-*Toxoplasma* IgG after consuming undercooked meat. In contrast, only 38 out of 223 (17%) women who refrained from eating undercooked meat tested positive, too was found to differ significantly (P-value=0.008). Additionally, among those who did not consume undercooked meat, 3 out of 223 (1.3%) showed the presence of anti-*Toxoplasma* IgM, as presented in Table 3.

Aborted women who consumed unwashed veggies showed seropositivity for both IgG and IgM,18/110 (16.4%) and 2/110 (1.8%), respectively. The findings presented a correlation with the consumption of unwashed vegetables regarding the acute cases among the aborted women, as proved by the anti-*Toxoplasma* IgM antibodies, as depicted in Table 3. Also, 7/60 (11.7%) were seropositive for anti-*Toxoplasma* IgG among those with a blood transfusion history, as presented in Table 3.

Table 3: Comparison of Anti-Toxoplasma IgG and IgM with some patients and environmental factors.

Variables	Total samples No.	Total positive No (%)	CLIA IgG +ve				CLIA IgM +ve			
			Sero positive No%	OR	95%CI	P Value	Sero positive No%	OR	95% CI	P Value
Gestation age										
1st trimester	152	35(23)	32(21)				3(2)			
2nd trimester	93	16(17.2)	16(17.2)	0.77	0.40-1.49	**0.762**	0(0)	0	0.00-1.87	**0.376**
3rd trimester	5	1(20)	1(20)				0(0)			
Total	250	52 (20.8)	49(19.6)				3(1.2)			
Contact with cats										
Yes	164	37(22.6)	36(22)	1.58	0.79-3.21	**0.241**	1(0.6)	0.25	0.02-2.25	**0.272**
No	86	15(17.4)	13(15.1)				2(2.3)			
Total	250	52(20.8)	49(19.6)				3(1.2)			
Eating undercooked meat										
Yes	27	11(40.7)	11(40.7)	3.34	1.49-7.55	**0.008***	0(0)	0.11	0.00-9.66	**0.999**
No	223	41(18.4)	38(17)				3(1.3)			
Total	250	52(20.8)	49(19.6)				3(1.3)			
Consumption of unwashed vegetables										
Yes	110	20(18.2)	18(16.4)	0.68	0.36-1.28	**0.266**	2(1.8)	2.57	0.29-37.54	**0.58**
No	140	32(23)	31(22.1)				1(0.7)			
Total	250	52(20.8)	49(19.6)				3(1.2)			
Blood transfusion history										
Yes	60	7(11.7)	7(11.7)	0.46	0.18-1.09	**0.093**	0(0)	0	0.00-3.66	**0.999**
No	190	45(23.5)	42(22.1)				3(1.8)			
Total	250	52(20.8)	49(19.6)				3(1.2)			
Residency										
Urban	101	23(22.7)	20(19.8)	0.97	0.52-1.79	**0.999**	3(3)	0.6	0.00-0.77	0.064
Rural	149	29(19.4)	29(19.4)				0(0)			
Total	250	52(20.8)	49(19.6)				3(1.2)			

Statistical significance (P-value < 0.05), Fisher exact test used, OR (Odd ratio) and CI (Confidence intervals).

Immunohistochemical diagnosis of the parasite:

The antigen of *T. gondii* was detected in the placental tissues analyzed by immunohistochemistry assay. Images in Figures 1A, B, and C illustrate the chorionic plate region, showing a positive expression of anti-*T. gondii* antibody (black arrows). A significant and pervasive *T. gondii* tachyzoite infection within the afflicted placenta was indicated by the extensive and intense staining that covered a considerable amount of the tissue sections under examination. Also, *T.gondii* tachyzoite protozoa were observed in large blood vessels (yellow arrow). Also, the chorionic villi's blood vessel lumina, *T. gondii* tachyzoites (black arrow) were strongly expressed. With a mostly intravascular localization, the protozoan forms were restricted to the vascular compartments. They were not discernible in the surrounding villous stroma or trophoblastic layers (yellow arrow), as presented in Figure 2: A, B, C&D. Generally, the number of tachyzoites was varied in different samples of placenta depending on the case of the aborted women. Intensive numbers were observed in some of them.

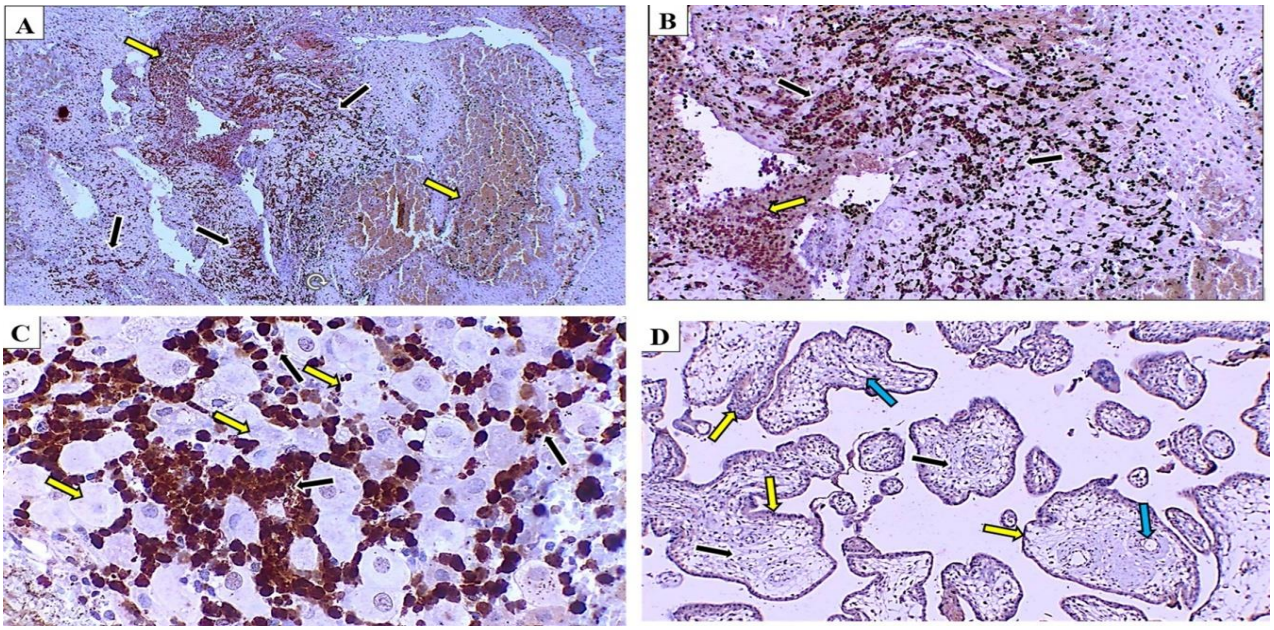

Figure 1: Photomicrographs of the placenta infected with *T.gondii*. **A and B** show a strong expression of *T. gondii* tachyzoites (black arrow) observed in the chorionic plate region, indicating severe infection. Also, tachyzoites were detected in large blood vessels (yellow arrow). **C.** Strong expression of tachyzoites (black arrow) in the chorionic plate region. The tachyzoites were localized around trophoblast cells, with clustering suggestive of active parasitic invasion. Additionally, numerous trophoblasts exhibited loss of nuclear detail (yellow arrow), indicating trophoblastic necrosis. **D.** The negative control section of the placental tissue showed no reaction. The placental villi (black arrow) appear as characteristic finger-like projections, with trophoblast cells (yellow arrow). Within the core of the villi, fetal blood vessels (blue arrow). All sections were stained with 3,3-diaminobenzidine (DAB) and Hematoxylin. Magnification: A: 40x, B &D: 100x, C: 400x.

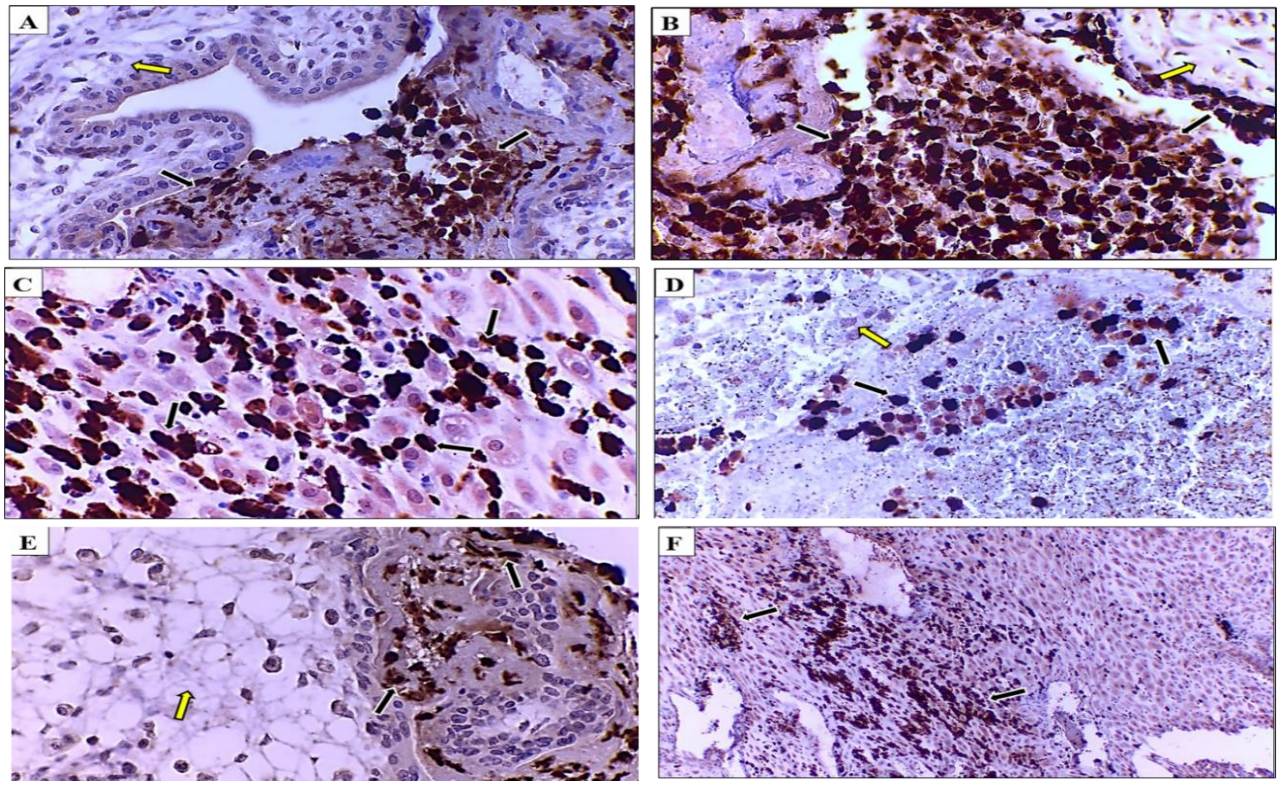

Figure 2: Image of a placenta with *T. gondii* infection. **A and B.** A strong expression of *T. gondii* tachyzoites (black arrow) within the lumina of blood vessels in the chorionic villi. The tachyzoites appeared only in the vascular compartments, with no detectable presence in the surrounding villous stroma or trophoblastic layers (yellow arrow). **C.** Strong expression of tachyzoites (black arrow) in the villous trophoblast layer, between trophoblast cells. **D and E.** A strong expression of tachyzoites (black arrow) within the blood vessel lumina of the chorionic villi. The protozoan forms were confined to the vascular compartments, with no detectable presence in the surrounding villous stroma or trophoblastic layers (yellow arrow). **F.** Strong expression of tachyzoites (black arrow) between trophoblast cells in the villous trophoblast layer. All sections were stained with DAB and Hematoxylin. Magnification power for A, B, C, D, and E is 400X, and for F is 100X.

4. DISCUSSION

Toxoplasmosis is usually asymptomatic or manifests as a mild clinical illness that is difficult to detect. However, if toxoplasmosis occurs during pregnancy, the tachyzoites may be transmitted to the fetus, potentially causing severe damage (Remington *et al*., 2006). Untreated congenital toxoplasmosis can severely harm the fetus, leading to serious consequences. Therefore, to avoid intrauterine infection and other consequences associated with the infection, it is essential to check pregnant women for the presence of anti-*Toxoplasma* IgG and IgM periodically (Vueba *et al*., 2020).

This investigation used CLIA to detect anti-*Toxoplasma* IgG antibodies among the aborted women, revealing an overall seropositivity of 19.6%. Only 1.2% were positive for IgM, indicating a low rate of recent (acute) infection and suggesting chronic exposure due to persistent IgG levels. These findings are approximately comparable to previous studies of Abdullah *et al*. (2024), who found a prevalence of toxoplasmosis IgG of 20.6% among women in Duhok by ELISA. Another study also reported a percentage of Anti-*T. gondii* IgG antibodies in 20% of pregnant women in Yemen using electrochemiluminescence immunoassay (Al-Adhroey *et al*., 2019). Moreover, the prevalence of anti-*Toxoplasma* antibodies documented in this study is greater than the rates found in previous research. For instance, Mizuri and Mero (2020) reported a seroprevalence rate of 11.58% for anti-*Toxoplasma* IgG and 0.63% for IgM in Zakho city. Also, in Dhouk, a serological screening conducted by CLIA indicated that 10% (7 out of 70) of aborted women were positive for anti-*Toxoplasma* IgG, while only 2.8% (2 out of 70) were positive for IgM antibodies (Al-Saeed *et al*., 2016).

Conversely, previous studies from various cities in Kurdistan, Iraq, have indicated a higher prevalence in women. In Erbil, 26% and 5% of aborted females were found to have Anti-*Toxoplasma* IgG and IgM, respectively, according to ELISA results (Ahmed and Khudhair, 2023). Additionally, a significant seroprevalence of anti-*Toxoplasma* IgG (32.46%) and IgM (8.68%) was observed among females in Zakho city (Mustafa *et al*., 2024). The variances observed in the outcomes of numerous studies might be due to several factors, such as differing sample sizes, locations, distribution of stray cats, levels of hygiene and education, immune health, and socioeconomic conditions.

Based on age group, the highest IgG seropositivity in the current investigation was seen in the 26–35 age group (20.6%). And this is comparable to Al-Sray *et al*. (2019), who found the highest seropositivity in the age interval of 20–29 years (19.9%) among women in Wasit province of Iraq. This result is also like that of Vueba *et al*. (2020), who reported the highest range in the 26-35 interval as (63.8%), The high percentage of females who suffering abortion and were infected with toxoplasmosis, particularly in the age interval of 35-36 years, can be attributed to the large sample size of 131 women in this age range included in the present study, as it is a common age of pregnancy. It is important to note that a random sample of women who had undergone an abortion was selected for the research.

The current study identified a significant correlation between anti-*Toxoplasma* IgM seropositivity and residency in urban versus rural areas. Specifically, there were three cases recorded in the urban area, while no cases were found in rural areas (P-value = 0.034). This disparity may be linked to dietary habits, as more women in urban areas tend to eat out at restaurants and consume fast food. It is important to note the key risk factors identified previously, which include the consumption of raw and unwashed fruits and vegetables, dining at restaurants, and the presence of stray cats in the area. Additionally, there is a troubling lack of awareness about toxoplasmosis (Alzaheb, 2018; Alghafari, 2025). Another study highlighted that the primary cause of infection is eating contaminated food, particularly dairy products that may contain tachyzoites and meat items that harbor tissue cysts of *T. gondii* (Marín-García *et al*., 2022).

Also, the current investigation showed that the highest level was in urban areas, anti-*Toxoplasma* IgG in 19.8%, followed by rural areas, 19.4%, with non-significant differences. This finding was in line with a study conducted by Hamad and Kadir (2013) in Erbil, which found non-significant differences between females living in different residential areas (urban and rural). Moreover, it is similar to that of an Iranian investigation that documented pregnant women living in urban regions had a greater seroprevalence than those residing in rural zones (89.7% IgG, 100% IgM vs. 10.3% IgG, 0.0% IgM (Sharifi *et al*., 2019). Nevertheless, these findings are in contrast to those of Mikaeel and Al-Saeed (2019), who mentioned the highest range was in rural areas (69.8%), and according to a study done in Babylon City, the highest range was also in rural areas, 59% (Al-Jbouri *et al*., 2020). Discrepancies in toxoplasmosis seroprevalence across studies may stem from factors such as age, education, hygiene practices, diagnostic methods, dietary habits, environmental conditions, and cultural influences affecting *T. gondii* exposure and interactions with cats. These variations often reflect the demographic differences of participants and geographical locations studied (Johnson,2020; Wodage *et al*.,2023).

Female illiterates had the highest toxoplasmosis seropositivity, 18(18.6%) for IgG, compared to 6% for IgM among women who completed their university education by CLIA. A statistical observation was also noted by the CLIA assay that recorded (P-value=0.031). and is comparable with Mizuri and Mero (2020), in Zakho city, who found 17.75% for illiterates. Following Mikaeel and Al-Saeed (2019), housewives (61.4%) have a higher range than employees. A study in Erbil by Ahmed and Khudhair (2023) reported illiterate females as having the greatest rates of 26.2% for IgG, among other educational levels. Lack of health literacy, restricted access to healthcare facilities, and a lack of knowledge about preventive measures may all contribute to the higher seropositivity rates among illiterate women (Alghafari, 2025; Ait Hamou and Laboudi, 2021).

Furthermore, our results revealed that ladies who had abortions during the first trimester had the greatest seropositivity for toxoplasmosis, with anti-*Toxoplasma* IgG in 21%. Sadiqui *et al*. (2018) and AlIbady (2024) all reported similar findings. Also based on the gestational period, another study involving 57 cases in the first trimester found that 5 cases (8.8%) tested positive for IgG antibodies against *T. gondii*, with 1 case (1.8%) showing anti-*Toxoplasma* IgM antibodies, as determined by CLIA. In the second trimester, among 10 cases, 2 (20%) were positive for anti-*Toxoplasma* IgG, while 1 case (10%) had IgM antibodies, with no seropositive cases identified in the third trimester (Al-Saeed *et al*., 2016). In the present work, females who had 1-2 abortions had the highest anti-*Toxoplasma* IgG seropositivity (21.2%). This result was in contrast with the research of Al-Jbouri *et al*. (2020), who stated that women of Babylon city have the highest abortion rate at 82% with 1-2 repeated abortions due to Cytomegalovirus (CMV) and *T. gondii*, this may be due to (CMV) is associated with a significant percentage of repeated miscarriages, often without noticeable symptoms during the acute phase. CMV can persist in the body and cross the placenta, potentially impacting the fetus through breastfeeding. This is like *T. gondii*, which causes a lifelong latent infection by modulating the immune system after the initial infection (Mocanu *et al*., 2022). Additionally, *T. gondii* triggers the secretion of pro-inflammatory cytokines and chemokines, including IL-6, TNF-α, and IFN-γ. These substances play a role in the degradation and inflammation of tissues. The inflammatory reaction within the placenta may hinder its function, decrease blood circulation to the fetus, and ultimately lead to a miscarriage. Also, an imbalanced Th1 response, especially in the 1st trimester, can lead to abortion (Moghaddami *et al*., 2024).

In this study, 22% of the seropositive ladies had IgG, and 0.6% had IgM, based on having a cat with no significant differences, and this is comparable to a study in Zhako, Iraq, where 26.97 % of women who had cats were seropositive

(Mustafa *et al*., 2024). Also, Research in Akre City, Iraq, found that pregnant women who had cats had increased anti-*Toxoplasma* IgG (61.90%) and anti-*Toxoplasma* IgM antibodies (9.52%) (Shukri *et al*., 2020). And according to the WHO Eastern Mediterranean Region, contact with cats was examined as a risk factor, with 34.8% for IgG (Rabaan *et al*., 2023). The cats are the final hosts for *T. gondii.* They serve as the main source of environmental contamination with the oocysts, which are considered the infective form of the *T.gondii* after sporulation (Shani *et al*., 2012).

According to water sources, users of springs have the highest percentage among the three types of water, for IgG 16 (20%), and this is incomparable to a Sudanese study that discovered that tap water has the highest range, 29.4% (Mustafa *et al*., 2019). Also, disagree with Nadia *et al*. (2023), regarding the tap water users, the prevalence of toxoplasmosis among pregnant females in Cameroon was 30.2%, whereas in Yemen, the percentage was 20.9% for improved municipal pump (tap water) (Al-Adhroey *et al*., 2019).

The current investigation detected that among the seropositive women who reported eating undercooked meat, 40.7% had IgG, and there was a significant connection in IgG (P-value=0.003). Although this finding is comparable to the results of Mohammed *et al*. (2018) in Omdurman, which was 26%. Also, in Akre, according to research, which is higher than the present study by Shukri *et al*. (2020), it was discovered that IgG and IgM were found to be positive in 54.83%, 9.67% respectively. and with Abdelbaset *et al*. (2020) as 66.7% in El-Minya, Egypt. Additionally, undercooked meat in Algeria was found by Sebaa *et al*. (2024) as a percentage of 13.6% among women infected with toxoplasmosis.

According to women who consumed unwashed vegetables, they had IgG levels of 16.4% and IgM levels of 1.8%, and this is comparable to Golek (2023), who reported unwashed vegetables at 16.1% in Amedi. Also, Barzinij (2021) concluded that the total seroprevalence of *T. gondii* in Kirkuk among women consuming unwashed vegetables was 26.3% and 14.3% for both IgG and IgM, respectively. And Mahmoud *et al*. (2019) mentioned that a high number of ladies who consumed unwashed vegetables showed positive anti-*Toxoplasma* of 83.9 % in Libya.

Additionally, based on blood transfusion history, the anti-*Toxoplasma* IgG percentage was recorded as 11.7% in our study. This is comparable to the findings of Al-Adhroey *et al*. (2019), who revealed that 10% of pregnant females infected with toxoplasmosis in Dhamar, Yemen, had a history of blood transfusions. Also, another study of Nadia *et al*. (2023) that 8.7% of Cameroonian pregnant women who expressed anti-*Toxoplasma* positive antibodies had received a blood transfusion. Similarly, Teweldemedhin *et al*. (2019) mentioned that 15.8% of pregnant females who were positive for *T. gondii* in Ethiopia had a history of blood transfusions.

Immunohistochemical analysis in this study revealed *T. gondii* tachyzoites in every placental tissue examined, indicating a 100% positive rate. Parasites were primarily located in the lumina of chorionic villous blood vessels. However, they were also occasionally found in the trophoblastic layers and the villous stroma surrounding them. These findings support the theory that hematogenous spread results in tissue invasion and demonstrate both intravascular and extravascular dissemination of *T. gondii* within the placenta (Kim *et al*., 2015). The high sensitivity of IHC in detecting *T. gondii* aligns with previous human studies. For example, reported positive IHC results in placentas from women who experienced miscarriage, showing visible tachyzoites and pseudocysts along with necrosis, leukocytic infiltration, and hemorrhage. Similarly, Al-Kaeebi *et al*. (2023) and other studies demonstrated 100% IHC positivity in PCR-confirmed aborted placentas, emphasizing IHC as a dependable method even with varying parasite loads. Furthermore, Khalil *et al*. (2016) found no significant difference (P>0.05) in the effectiveness of ELISA, ELFA, and IHC tests for detecting *Toxoplasma* infection in women who had abortions when the tests were compared to one another. However, IHC showed a higher sensitivity (100%) in confirming *T. gondii* infection in women who had undergone abortions. In 2024, a recent study conducted in Kirkuk governorate, Iraq, determined that immunohistochemistry is a more sensitive immunological method compared to serological assays for directly diagnosing *T. gondii* in the placental tissue of women who experienced abortions (Ahmed *et al*., 2024). Even with other parasitic infections, the immunohistochemistry technique shows accuracy in the identification of the immune cell subtypes' responses in hydatid cyst disease and in the detection of histopathological changes in the liver of infected mice (Hamad *et al*., 2022).

CONCLUSION

The prevalence of anti-*Toxoplasma* IgG and IgM antibodies among the aborted women enrolled in the current study was relatively high among women of reproductive age, with no significant seroprevalence among residency participants, indicating the wide distribution of toxoplasmosis. Additionally, the relationship between the seroprevalence of anti-*Toxoplasma* antibodies in females and certain risk factors, including contact with cats, was statistically nonsignificant. In contrast, it was significant for those who frequently consume undercooked meat, and was also significantly associated with education level. Moreover, the current data indicate that IHC assists in precise diagnosis and improves our understanding of the pathological mechanisms involved in vertical transmission of congenital toxoplasmosis. Educating the local population is vital for preventing toxoplasmosis, and pregnant women should regularly check for T*oxoplasma* infection. The high prevalence of anti-*Toxoplasma* antibodies among pregnant residents in rural and urban areas highlights the necessity of enhancing maternal health programs to prevent toxoplasmosis regardless of their location.

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Ethical Approval:

This investigation has been accepted by the Scientific Research Ethics Committee of the Faculty of Science at Soran University (Approval no: (1226) 28 / October 2024).

Author Contributions:

H.D.Z: performed the experimental methods, analyzed the data, and prepared the initial draft of the thesis. S.B. S: supervised the project, data analysis, revised, and prepared the final version of the thesis.

Conflict of Interest:

We declare that there is no conflict of interest.

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