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# Endemicity Of *Toxoplasma Gondii* Infections Among Aborted Women in Erbil, The Kurdistan Region of Iraq

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#### ABSTRACT

The current seroprevalence survey was carried out to evaluate the endemic status of Toxoplasma (T.) gondii, which is deemed a ubiquitous zoonotic protozoan pathogen among aborted women in Erbil, the Kurdistan region of Iraq. To meet the requirements of the study, the sera of 270 miscarried women, who attended the emergency department of Maternity Teaching hospital-Erbil from October 20, 2021, to May 1, 2022, were examined serologically using enzyme-linked immunosorbent assay (ELISA). The study revealed that 27.03% of aborted individuals had an inactive infection (IgG), whilst 0.74% of them were harbours of active infections (IgM). The highest percentage of inactive infection (36.19%) was recorded in the age group 31-40 years, whilst the lowest percentage (17.64%) was recorded in the age group 14-20 years. Regarding active infections, a very low percentage (less than 1%) was recorded. Concerning recurrent abortions, there was a significant difference (P<0.05) between various age groups, but there was no significant difference (P>0.05) pertaining to the concentrations of IgG in their sera. The pregnant women in the first trimester were exposed to abortion (86.66%) more than those in the second trimester (13.33%). The highest percentage (57.7%) of abortions was recorded in women with an elementary level of education,



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while 19.25% and 22.96% of abortions were recorded for illiterate and university levels, respectively. Ultimately, it could be extracted that Toxoplasmosis is still reckoned a threat to the community of pregnant women in the Kurdistan region of Iraq.

### 1. Introduction

Toxoplasmosis in animals and humans is caused by an obligate intracellular protozoan parasite termed *Toxoplasma (T.) gondii*, which was discovered for the first time in the North African rodent, *Ctenodactylus gundi*. Usually, the natural cycle occurs between cats as definitive hosts and mice or rats as intermediate hosts, whilst mammals such as humans, cattle, pigs, sheep, and goats serve as intermediate hosts during the accidental cycle (Elmore *et al.*, 2010; Kamal, Ahmed, Abdellatif, Tawfik, & Hassan, 2015; Calero-Bernal & Gennari, 2019). The aforementioned mammals, including humans, acquire the infection via consuming food contaminated with sporulated oocysts containing sporozoites shed by infected cat excreta (Saadatnia & Golkar, 2012). On the other hand, humans can also get the infection when they consume raw or partially cooked beef, pork, and mutton containing infective *T. gondii* cysts (bradyzoites) (Torrey & Yolken, 2013).

Pathogenicity of this moderately benign protozoan depends on strain virulence, host susceptibility and the host site of the parasite (Calero-Bernal & Gennari, 2019). Regarding acquired postnatal Toxoplasmosis, the blood-borne parasite invades different tissues to form cysts after parasitemia. Consequently, various clinical symptoms such as fever, lymphadenopathy, muscle ache, hypotension, leukopenia, pneumonia, meningoencephalitis, myocarditis, and hepatitis may occur (Randall & Hunter, 2011; Belluco, Simonato, Mancin, Pietrobelli, & Ricci, 2018).

In contrast to the acquired postnatal Toxoplasmosis in adults, congenital Toxoplasmosis contracted by transplacental transmission is frequently severe and could be incurable (McLeod *et al.*, 2014). It occurs approximately in 0.1 to 0.5% of pregnancy cases. The severity of the disease is associated with the fetus's age at infection and the protected antibodies produced by the mother. The typical clinical manifestations that appear in intrauterine-infected children include intracerebral



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calcification, microcephaly, convulsions, hydrocephaly, mental retardation, chorioretinitis, and even stillbirth (McAuley, 2014; Wang *et al.*, 2017; Tyebji, Seizova, Hannan, & Tonkin, 2019).

Epidemiologically, Toxoplasmosis is rampant worldwide, including in the Kurdistan region of Iraq, and studies have pointed out that 20-75% of different global populations harbour *T. gondii* asymptomatically (Torrey & Yolken, 2013; Abdullah & Mahmood, 2017; Al-Sray, Sarhan, & Mohammed, 2019). Usually, infection is common where felids are numerous, hygiene is poor, and the climate is suitable, enhancing the viability of faecal oocysts for a long period in the environment (Montoya & Liesenfeld, 2004).

Due to the above-said reasons and to check-up the annual incidence of Toxoplasmosis among aborted women in Erbil, the Kurdistan region of Iraq, this applied study, exploiting enzyme-linked immunosorbent assay (ELISA), has come into existence.

# 2. Materials and Methods

## 2.1 Study site

This serological study was done in the emergency department of the Maternity Teaching hospital of Erbil from October 20, 2021, to May 1, 2022. For this purpose, blood specimens were collected from 270 women during the study period, followed by performing ELISA in the Research Center of Salahaddin University.

### 2.2 Application of questionnaire sheet

The profile of respondents was recorded in a questionnaire sheet to incorporate some epidemiological aspects associated with the occurrence of abortion in the community. In this regard, the sheet embraced residency of aborted women, history of miscarriage, gestation age, pet handling, and educational level.

### 2.3 Recruitment of patients

After taking written and signed consent from each patient (n=270), they were categorized into four age groups; 14-20, 21-30, 31-40, and 41-48. Moreover, the survey protocol was approved by the Ethical Committee of the Biology Department-College of Science-Salahaddin University-Erbil.



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### 2.4 Collection of blood specimens

5 ml of blood was taken from each participant. The collected blood was centrifuged at 3000 rpm for 5 minutes to separate the sera, which were then divided into 2 parts and placed into Eppendorf tubes labelled with names and numbers. The sera were then transported inside an ice box to Salahaddin University Research Center and stored at -80 c° in the freezer to measure anti-*Toxoplasma gondii* antibodies (IgM and IgG) later by ELISA (Manufacturer's protocol of ACON Laboratories, Inc. San Diego, USA).

### 2.5 Serodetection of Anti-Toxoplasma gondii antibodies using ELISA

In order to perform the aforementioned serological technique, two types of kits were used for qualitative and quantitative detection of Anti-*Toxoplasma gondii* IgM and IgG. The kits, imported from ACON Laboratories, Inc. San Diego, USA, were *Toxoplasma* IgM ELISA Kit and *Toxoplasma* IgG ELISA Kit. According to the manufacturer instructions, the definition of positive IgM and IgG *Toxoplasma gondii* antibodies are defined as serum IgM and IgG concentrations > 1.1 IU/ml and > 10 IU/ml, respectively. While negative IgM and IgG *Toxoplasma gondii* antibodies are defined as the serum IgM and IgG concentrations < 0.9 IU/ml and < 10 IU/ml, respectively. Nonetheless, the suspected results that should be re-evaluated are the IgM and IgG serum concentrations which are between 0.9-1.1 IU/ml and 9-11 IU/ml, respectively.

## 3. Statistical analysis

The obtained data were statistically analyzed using computer programs, SPSS version 8 and GraphPad Prism 8. Median and range (25-75 % quartile range) were applied for descriptive statistics of age and IgG concentrations. The Chi-square test was used to compare IgG seropositivity and seronegativity among pet handlers and non-pet handlers and also applied to differentiate between IgG and IgM seropositivity, recurrent abortion based on the four age groups. For comparison of IgG concentrations One-Way NOVA was used. The significant difference was defined to be less than 0.05.



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### 4. Results

The obtained data were calculated and statistically analyzed, and the following results were extracted:

### 4.1 Serodetection of infection

The employed serological assay confirmed the inactive (27.03%) and active (0.74%) infections in aborted women (n=270) incorporated in the study. Details are displayed in table (1).

Table (1): Demonstration of Anti-*Toxoplasma* IgG and IgM in the sera of aborted women who attended the Maternity Teaching Hospital-Erbil during the study

Immunoglobulin type	Total cases	Positive cases	Percentage (%)	
lgG	270	73	27.03	
lgM	270	2	0.74	
Total	270	75	27.77	

period.

### 4.2. Infectivity based on age groups

As demonstrated in table (2), the highest percentage of inactive infection (36.19%) was recorded in the age group; 31-40 years, whilst the lowest percentage (17.64%) was recorded in the age group; 14-20 years. Regarding active infections, a very low percentage was recorded.

Table (2): Rampancy of Anti-Toxoplasma IgG and IgM among aborted women inaccordance with their ages.

Age group	Cases examined	IgG positive		IgM	<u>positive</u>	
(Years)		Cases	Percentage (%)	Cases	Percentage (%)	
14-20	34	6	17.64	0	0	
21-30	117	25	21.36	1	0.85	
31-40	105	38	36.19	1	0.95	
41-48	14	4	28.57	0	0	
Total	270	73	-	2	-	



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### 4.3 Susceptibility of different age groups to recurrent abortion

According to the analyzed data exhibited in table (3), there was a significant difference (P<0.05) between various age groups in their vulnerability to repeated miscarriage. On the other hand, there was no significant difference (P>0.05) between the same age groups in the availability of IgG concentrations in their sera.

Table (3): Association of women ages with the recurrence of abortion plus concentration of IgG in their sera.

Age group (Years)	<u>Recu</u> <u>1-3 times</u> Cases	rrent abort <u>4-6 times</u> Cases	<u>ion</u> <u>7-9 times</u> Cases	<u>P-value</u>	<u>lgG concentration</u> <u>(IU/ml)</u> Median/range	<u>P-value</u>
14-20	34	0	0		274.3 (41.27-291.5)	
21-30	110	7	0	0.0033**	261.6 (104.4-295.1)	0.9513
31-40	85	18	2		267.2 (83.12-286.7)	
41-48	12	1	1	P<0.05	272.5 (261-325)	P>0.05
Total	241	26	3			
Percentage (%)	89.25	9.62	1.11			

\*\* Denote significant difference

### 4.4 Infection vulnerability and pet handling

The statistical analysis concluded that there was no significant difference (P>0.05) between pet handlers and non-pet handlers pertaining to their susceptibility to getting infections with Toxoplasmosis table (4).

Table (4): Comparison between pet handlers and non-pet handlers regarding thepresence of Anti-Toxoplasma antibodies (IgG) in their sera.

Pet handling	Positive cases/ Percentage (%)	Negative cases/ Percentage (%)	Total	P-value	
Pet handlers	9 (31.03)	20 (68.96)	29		
Non-pet handlers	64 (26.55)	177 (73.44)	241	0.6079	
				P>0.05	
Total	73	197	270		



#### 4.5. Pregnancy period and incidence of abortion

The survey revealed that aborted women in the first trimester are more prone to abortion as compared to those in the second trimester figure (1).



Figure (1): Comparison between first trimester and second trimester of pregnancy concerning the percentage of abortion incidence.

### 4.6 Association of abortion incidence with educational levels

The highest percentage (57.7%) of abortions was recorded in women with elementary education levels, while 19.25% and 22.96% of abortions were recorded for illiterate and university levels, respectively figure (2).





Figure (2): Association of abortion occurrence with different educational levels of women.

#### 4.7. Residency and miscarriage incidence

As shown in figure (3), women living in the urban areas were more vulnerable to abortion (64.07%) compared to the rural areas (35.92%).



#### Aborted women in urban and rural areas

Figure (3): Comparison between women residing in urban and rural areas concerning their susceptibility to abortion.



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### 5. Discussion

According to the previous epidemiological surveys, Toxoplasmosis is rampant and endemic in the Kurdistan region (Razzak, Wais, & Saeid, 2005; Khoshnaw, 2010; Abdullah & Mahmood, 2017) and the rest parts of Iraq (Alewy, Ali, & Hammady, 2010; Al-Dory, 2011; Kadir, Ghalib, Othman, & Ahmed, 2011; Al-Sray *et al.*, 2019) and neighbouring countries (Jumaian, 2005; Al-Harthi, Jamjoom, & Ghazi, 2006; Tamer, Dundar, & Caliskan, 2009; Hajsoleimani, Ataeian, Nourian, & Mazloomzadeh, 2012). The current serodetection of Toxoplasmosis in the aborted women (n=270) incorporated in the study revealed that 27.03% of respondents had chronic infections (harbours of IgG), whilst 0.74% of them had newly acquired infections (carriers of IgM), so the total infection rate was 27.77% table (1).

It is noteworthy to mention that our findings differ from other studies conducted in this field in Iraq. For example, the survey of Abdullah and Mahmood (2017) recorded 34.98% as inactive infection rate, 12.93% as active infection rate, and 47.91% as total infection rate, which are deemed higher than our data. Khoshnaw's (2010) data were very close to Abdullah and Mahmood's (2017), which means higher than our findings. In contrast, the results of Al-Dory (2011) were closer to our data. He recorded 26.1% as chronic infections, 3.1% as recent infections, and 29.2% as total infections. The variability in data obtained in different studies could be back to the abundance of semi-stray cats, following public health rules, ecological factors affecting maturation and survival of oocysts, habits of beef and mutton consumption, the status of innate immunity, distribution of women ages within age groups, and contact with members of felids (Ocak, Zeteroglu, Ozer, Dolapcioglu, & Gungoren, 2007; Weiss & Dubey, 2009; Tehrani-Sharif, Jahan, Alavi, & Khodami, 2015; Al-Sray *et al.*, 2019).

Concerning susceptibility of age groups for chronic infection, it was found that 31-40 years old was the highest (36.19%), whilst the recent infections of 21-30 and 31-40 years old were 0.85% and 0.95%, respectively table (2). The present epidemiological survey has underlined the frequency of abortion in each group as well. So, there was a significant difference P<0.05 among the age groups. At the same time, there was no significant difference between all age groups (P>0.05) pertaining to serum IgG concentrations table (3). In this regard, global studies have recorded various



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outcomes. Perhaps the disparity is attributed to the frequency and dose variations of exposure of each age group to the pathogen (Tamer *et al.*, 2009; Alewy *et al.*, 2010). On the other hand, no significant difference (P>0.05) was recorded between pet handlers and non-pet handlers for their susceptibility to Toxoplasmosis table (4). However, the empirical surveys about this zoonotic ailment on feline, bovine, ovine, and caprine are very rare in the Kurdistan region, but we can imagine that women as intermediate hosts get most infections during the accidental cycle from consumption of beef and mutton containing bradyzoites in addition to contaminated vegetables with sporulated oocysts as compared to direct transmission to them from cats (Saadatnia & Golkar, 2012; Torrey & Yolken, 2013).

The survey demonstrated that pregnant women in the first trimester are more prone to miscarriage (86.66%) as compared to pregnant women in the second trimester (13.33%) figure (1). In this regard, previous local and international investigations have recorded various results, probably due to the fluctuation of hormones during gestation trimesters (Tamer *et al.*, 2009; Muqbil & Alqubatii, 2014).

Nonetheless, the highest percentage (57.7%) of abortion was recorded in women who had elementary levels of education figure (2), but earlier epidemiological surveys in this domain have recorded diverse outcomes. While some studies reported a non-significant difference (P>0.05) between different educational levels, others concluded that illiterate women are more susceptible to Toxoplasmosis as compared to other educational levels (Hajsoleimani *et al.*, 2012; Malarvizhi, Viswanathan, Lavanya, Arul, & Moorthy, 2012). So, the issue is still contentious, and extra studies are inevitable on huge populations around the world.

Ultimately, in light of the current study, women who live in the urban areas were more vulnerable to abortion (64.07%) as compared to the rural areas (35.92%), as displayed in figure (3). In this regard, global researchers differ in recording their results. Some investigators have not observed any significant difference (P>0.05) statistically between rural and urban women, whilst other researchers in some countries have concluded that pregnant women who dwell in rural areas are more susceptible to abortion as compared to pregnant women who reside in cities. These distinctions may be back to socio-economical differences between rural and urban



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communities to a minimal level in some countries nowadays (Nash, Chissel, Jones, Warburton, & Verlander, 2005; Pappas, Roussos, & Falagas, 2009; Kamal *et al.*, 2015).

## 6. Conclusions

In light of the analyzed data of the present research work, it could be extracted that Toxoplasmosis is still reckoned a threat to the community of pregnant women in the Kurdistan region of Iraq. The majority of aborted women in Erbil and its environs are in the first trimester (31-40 years old), and they have IgG in their sera. Moreover, women who are at the level of elementary education are more exposed to miscarriage, and, finally, urban women are more susceptible to abortion as compared to rural women. Having said that, pet handling does not play any role in susceptibility to Toxoplasmosis.

### Acknowledgements

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## **Conflict of interest**

The authors acknowledge that there is no conflict of interest regarding this research work.

## **Statement of novelty**

Owing to the endemicity and zoonotic nature of Toxoplasmosis in our region and the occurrence of miscarriage and severe pathological disorders in newborns because of infection with this ailment, particularly in pregnant women, some local investigations and various international researches have been conducted in this domain. In this regard, annual follow-up is unavoidable to determine the incidence of the disease among the population. Hence, this survey came into existence covering extra epidemiological parameters that had not been elaborated on in the previous studies in Erbil.



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رەگ داكوتانى پێكانەكانى Toxoplasma gondii لەناو ئافرەتانى منداڵ لەبارچوو لە ھەولێر/ھەرێمى كوردستان عێڕاق

#### پوخته:



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سەردانى بەشى فرياكەوتنى نەخۆشخانەى ئافرەتان و منداڵبوونى فێركارى ھەولێريان كردووە لە بەروارى 20 ى تشرينى يەكەمى 2021 تاكوو 1 ى مايسى 2022 لە رێگەى پشكنينى ئامێرى (ELISA). توێژينەوەكە دەريخست كەوا (%27.03) ى ئافرەتانى منداڵ لەبارچوو ھەڵگرى يارێزبەندى گڵۆبيولينى جۆرى IgG بوون، لە كاتێكدا تەنھا (%0.74) ى ئەو ئافرەتانە ھەڵگرى پارێزبەندى گڵۆبيولينى جۆرى IgM بوون. بەرزترین رێژەی توشبوونی نا چالاک IgG کە %36.19 بوو لە کۆمەڵەی تەمەنی 40-31 ساڵ تۆماركرا، بەڵام كەمترين رِێژەيان كە (%17.64) بوو لە كۆمەڵەى تەمەنى 20-14 ساڵ تۆماركرا. سەبارەت بە تووشبوونى چالاک IgM رێژەيەكى زۆر كەم تۆماركرا كە كەمتربوو لە %1. ھەروەھا دەربارەي ئەو ئافرەتانەي كە زياتر لە جارێک منداڵيان لەبارچووبوو جياوازيەكى كاريگەر (P<0.05) بەدى دەكرا لە نێوان كۆمەڵەكانى تەمەن، لەكاتێكدا ئەو جياوازيە بەدى نەدەكرا بۆ چرى گڵۆبيولينى پارێزبەندى جۆرى lgG لە سيرەمى ئافرەتانى منداڵ لەبارچوو بۆ كۆمەڵە جياوازەكانى تەمەن. رێژەى (%86.66) ی ئەو ئافرەتانەی تووشی لەبارچوونی منداڵ بووبوون لە سێەکی يەکەمی سکپريدا بوون له کاتێکا تەنھا رێژەی (%13.33) يان لە سێەکی دووەمی سکپريدا بوون. بەرزترين رێژەی لەبارچوون که (/57.7%) بوو لهو ئافرەتانەدا تۆمارکرا که بروانامەی سەرەتاييان ھەبوو، بەلام رێژەکانی (%19.25) و (22.96) بەدواى يەكدا تۆماركرا بۆ نەخوێندەوارەكان و ھەڵگرانى برووانامەى زانكۆو پەيمانگاكان. له کۆتاييدا، دەتوانين بڵێين تووشبوون به Toxoplasma gondii هێشتا به مەترسى دادەنرێت بۆ سەر ئافرەتانى دووگيان لە ھەرێمى كوردستانى عێراق.

# توطن إصابات المقوسة القوندية Toxoplasma gondii بين النساء المجهضات في أربيل/ إقليم كردستان العراق

### الملخص:

هذا المسح الحالي للأنتشار المصلي نفذ لتقييم حالة التوطن للمقوسة القوندية Toxoplasma gondii والتي تعد ممرضا أوليا مشتركا، و منتشرا بين النساء المجهضات في أربيل/ إقليم كردستان العراق. لأكمال متطلبات الدراسة، امصال 270 من النساء المجهضات اللواتي حضرن قسم الطوارئ التابع المستشفى الأمومة التعليمي في أربيل من 20 من التشرين الأول 2021 الى الأول من مايس 2022 قد فحصت مصليا من خلال أختبار في أربيل من 20 من التشرين الأول 2021 الى الأول من مايس 2022 قد فحصت مصليا من خلال أختبار في أربيل من 20 من التشرين الأول 2021 الى الأول من مايس 2022 قد فحصت مصليا من خلال أختبار (ELISA). الدراسة أظهرت أن (%20.00) من المجهضات لديهن إصابات غير فعالة (الكلوبيولين المناعي من نوع 10g)، بينما (%30.00) حاملات للأصابة الفعالة (الكلوبيولين المناعي من نوع 20g)، بينما الأولي المحابة الفعالة (الكلوبيولين المناعي من نوع 20g)، بينما الأريف 2021)، سجلت لدى المجموعة العمرية 13-00 سنة، بينما النسبة الأدنى



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(17.64%) سجلت لدى المجموعة العمرية 14-20 سنة. بخصوص الإصابات الفعالة، سجلت نسبة منخفضة جداً أقل من 17. فيما يتعلق بالإجهاضات المتكررة، كان هناك فرق هام بنسبة 5% بين مختلف المجموعات العمرية، ولكن ليس هناك فرق هام بنسبة 190 بين مختلف المجموعات ولكن ليس هناك فرق هام بنسبة 190 بين مختلف المجموعات ولكن ليس هناك فرق هام بنسبة 190 بين مختلف المجموعات والعمرية، ولكن ليس هناك فرق هام بنسبة 190 بين مختلف المجموعات والعمرية، ولكن ليس هناك فرق هام بنسبة 190 بين مختلف المجموعات والعمرية، ولكن ليس هناك فرق هام بنسبة أكثر من 5% فيما يخص تراكيز الكلوبيولين المناعي من نوع 190 في المصالهن. إن النساء الحوامل في الثلث الأول من الحمل قد تعرضن للأسقاط (60.6%) اكثر من 190 في الثلث الثاني (13.3%). النسبة العالية من الأسقاط سجلت لدى النساء اللواتي يحملن الشهادة الأبتدائية، في الثلث الثاني (13.3%). والمحموم العالية من الأسقاط سجلت لدى النساء اللواتي يحملن الشهادة الأبتدائية، بينما (19.5%) و (13.3%). من الأحماض سجلت لدى غير المتعلمات و حاملات الشهادة الأبتدائية، بينما (19.5%) و (19.5%) من الأحماض سجلت لدى غير المتعلمات و حاملات الجامعية على الندا (19.5%). والتر الأحماض سجلت لدى غير المتعلمات و حاملات الشهادة الأبتدائية، بينما (19.5%) من الأحماض سجلت لدى غير المتعلمات و حاملات الشهادات الجامعية على التوالي. أخيرا، يمكن الأستخلاص بأن الأصابة بالمقوسة القوندية ما زالت تعد تهديدا لجالية النساء الحوامل في إقليم كردستان العراق.