

Detection Study: Prevalence of toxoplasmosis in aborted women infected with *Toxoplasma gondii* in Baghdad province, with implications for oncology

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ABSTRACT

Toxoplasmosis is a common parasitic disease caused by *Toxoplasma gondii*, which can cause fetal abnormalities and spontaneous abortions. The prevalence of toxoplasmosis in aborted women in Iraq may vary depending on factors such as geographic location, sample size, and diagnostic methods used. This study aimed to determine the prevalence of toxoplasmosis in aborted women infected with *T. gondii* in Baghdad Province, and to provide insight into the impact of the disease on reproductive health.

Blood samples were collected from 100 women who had experienced previous abortions in Baghdad province. The samples were tested for the presence of *T. gondii* using the Mini Vidas assay. The overall prevalence of *T. gondii* infection among aborted women in Baghdad Province. The study also revealed that the highest incidence of abortion was reported among women aged 20 years-29 years (46%), compared to the younger age group (14 years-19 years) where it was recorded at 22%. This study found that Toxoplasmosis was more prevalent in urban women (71%) than rural women (29%). The number of births did not have a significant effect on the prevalence of Toxoplasmosis among the study participants. This study found that the majority of abortions occurred in the first trimester, with 68% of women experiencing abortions during this stage compared to other trimesters. Furthermore, the total number of cases studied, only 6 cases (6%) showed evidence of recent infection with *T. gondii* (as indicated by seropositivity for IgM antibodies). This suggests that recent infection with the parasite may not be a major contributor to the high rate of abortions observed in the study population. On the other hand, 38 cases (38%) were found to have evidence of past exposure to the parasite (as indicated by seropositivity for IgG antibodies). This suggests that prior exposure to *T. gondii* may be more common among women who have experienced abortions.

Keywords: toxoplasmosis, *Toxoplasma gondii*, aborted women, trimester stages, immunoglobulin IgM, immunoglobulin IgG

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INTRODUCTION

T. gondii is an intracellular obligate parasite that causes toxoplasmosis, a zoonosis affecting warm-blooded animals and humans worldwide, with domestic cats being the ultimate hosts [1]. Humans can contract the disease by consuming tissue cysts in meat, oocysts from contaminate d soil, water, or food, or directly from the feces of cats and other feline animals [2]. Prenatal *T. gondii* infection can have serious negative effects on unborn children, mothers, and developing fetuses [3]. From a public health perspective, the focus was on determining the precise anatomical distribution of *T. gondii* in naturally and experimentally infected lambs. This was achieved by analyzing fresh and frozen samples of various parts of the meat [4]. Cats are the exclusive hosts capable of excreting oocysts, which are resilient to environmental factors, while humans, birds, and other mammals serve as intermediate hosts [5].

Toxoplasmosis, caused by the *T. gondii* parasite, affects approximately one-third of the global population [6]. Congenital toxoplasmosis can result from *T. gondii* infection during pregnancy, leading to severe effects on the fetus [7]. Despite advancements in knowledge of congenital toxoplasmosis prevention and therapy, acute *T. gondii* infection during pregnancy and its potentially fatal consequences for the fetus and newborn continue to occur globally [8]. Accurate differentiation between recent and past infection is essential when both *T. gondii*-specific IgM and IgG are present in a pregnant woman's serum. To determine the age of infection, testing for *T. gondii*-specific IgG avidity is a vital method. However, interpreting the results of this test can be challenging [9]. Therefore, this study was aimed to identify the serological prevalence of toxoplasmosis in abortion women in Baghdad province based on immunoglobulin IgG and immunoglobulin IgM antibodies, age groups, residence, the period and habitual abortion period, and trimester stages.

MATERIALS AND METHODS

Blood samples were collected from female participants between the ages of 16 and 40 who attended a public clinic in Baghdad province between September 2018 and June 2019. An information sheet was created, and a questionnaire was administered to collect the necessary data.

The blood samples were collected using disposable syringes from One TXM strip and one TXM SPR were used for each sample, the radial vein, and the resulting serum was subjected to serological control, or standard that needed to be examined. Once the tests that included Mini Vidas to measure IgG and IgM titers. The necessary SPRs were taken out, the storage pouch was centrifugation process was carried out at 3000 RPM for 5 minutes meticulously resealed. The TXM code on the instrument to ensure proper serum extraction. The Mini Vidas is an automated identified the test. The standard was recognized by S1 and tested immunoassay system used for diagnostic testing. To measure IgG twice. The positive control was recognized by C1 if it was to be and IgM titers for the detection of Toxoplasma, the BioMerieux tested, and C2 identified the negative control if it had to be VIDAS® TOXO IgG II (TXG) assay was used.

The current study included 100 pregnant women who received written on the TXM reagent strips in the space given, and the private care at multiple hospitals located in the governorate of calibrator was combined [11]. Descriptive statistics, such as Baghdad, and who were in different stages of pregnancy. The assay frequency distribution, and percentage, have been used to (ELFA) combines a two-step enzyme immunoassay sandwich summarize and describe the demographic and clinical technique with fluorescence detection. The Solid Phase Receptacle characteristics of the study participants. Percentage is calculated (SPR*) serves as both the solid phase and pipetting device. Sealed by taking the frequency in the category divided by the total reagent strips contain predisposed reagents, and the equipment number of participants and multiplying by 100%. Chi square automatically completes all assay steps. During the assay, the sample test was used to examine the association between Toxoplasmosis is cycled in and out of the SPR for a set time period following a and abortion in women infected with *T. gondii*. For all analysis, dilution step. Anti-*T. gondii* IgG antibodies present in the specimen statistical significance was considered at highly significant level will bind to the *T. gondii* antigen that is coated on the interior of the p-value of <0.01, significant level p-value of <0.05 and specimen using VIDAS® TOXO IgG II (TXG) and VIDAS® TOXO insignificant level p-value>0.05. All statistical analysis was done IgM (TXM) assays [10].

Specimen collection and preparation

Serum or plasma (EDTA) was collected for both VIDAS TOXO IgG II (TXG) and VIDAS TOXO IgM (TXM) assays. Compatibility of the collection tube was checked before sample collection. For VIDAS TOXO IgG II (TXG) assay, sera inductive at 56 °C for 30 min were used.

VIDAS TOXO IgG II (TXG) Procedure

Necessary reagents were taken out of the fridge and left for 30 minutes to reach room temperature. One TXG strip and one TXG SPR were used to test each sample, control, or calibrator. After removing the necessary SPR, the storage pouch was resealed. The test code was entered by typing it or choosing TXG. The calibrator was verified by S1 and tested twice. A vortex-style mixer was used to combine the calibrator, control, and samples. 100 m of calibrator, a control, or a sample was pipetted into the sample well. The SPR and strips were placed into the device and the assay was started as instructed in the operation's instructions. The instrument executed each assay step automatically. The SPR and strips were removed from the instrument after the assay was finished and placed in the proper recipient for disposal.

VIDAS TOXO IgM (TXM) Procedure

Standard procedures were followed to obtain whole blood and separate the serum for VIDAS TOXO IgM (TXM) assay. Heating the serum was not advised. Samples containing particle matter were cleared by centrifugation or filtration prior to testing. The samples were kept at 2°C-8°C for up to five days if testing could not be done on the same day they were collected. Specimens were frozen at 256°C if prolonged storage was necessary, and the recommended number of freeze-thaw cycles was one. The usage of plasma was not demonstrated for this test.

To conduct the VIDAS TOXO IgM (TXM) assay, necessary parts were taken out of the kit and left to warm up to room temperature.

tested. The necessary sample identification numbers were written on the TXM reagent strips in the space given, and the private care at multiple hospitals located in the governorate of calibrator was combined [11]. Descriptive statistics, such as Baghdad, and who were in different stages of pregnancy. The assay frequency distribution, and percentage, have been used to (ELFA) combines a two-step enzyme immunoassay sandwich summarize and describe the demographic and clinical technique with fluorescence detection. The Solid Phase Receptacle characteristics of the study participants. Percentage is calculated (SPR*) serves as both the solid phase and pipetting device. Sealed by taking the frequency in the category divided by the total reagent strips contain predisposed reagents, and the equipment number of participants and multiplying by 100%. Chi square automatically completes all assay steps. During the assay, the sample test was used to examine the association between Toxoplasmosis is cycled in and out of the SPR for a set time period following a and abortion in women infected with *T. gondii*. For all analysis, dilution step. Anti-*T. gondii* IgG antibodies present in the specimen statistical significance was considered at highly significant level will bind to the *T. gondii* antigen that is coated on the interior of the p-value of <0.01, significant level p-value of <0.05 and specimen using VIDAS® TOXO IgG II (TXG) and VIDAS® TOXO insignificant level p-value>0.05. All statistical analysis was done by using SPSS computer program version 16 and Excel application.

RESULTS

The overall seroprevalence of *T. gondii* infection has been recorded as 39 (39.0%), with seroprevalence of acute Toxoplasma-specific IgM antibodies as categorized by age groups.

Frequency of aborted women with toxoplasmosis according to age groups is shown in Table 1. The study found that out of the total number of aborted women with Toxoplasmosis, the highest percentage was observed in the age group of 20 years-29 years. Specifically, 19 (19.0%) of the women in this age group were diagnosed with Toxoplasmosis. However, when considering all age groups (14 years-19 years, 20 years-29 years, and 30 years-40 years old), the total number and percentage of abortion women with Toxoplasmosis were recorded as 39 (39.0%).

Table 2 presents a detailed breakdown of the frequency of women who had abortions due to toxoplasmosis, categorized by the specific trimester of their pregnancy. According to the recorded data in this table, the highest number and percentage of women who had abortions due to toxoplasmosis occurred during the first trimester, with a total of 30 cases (30.0%).

Table 3 displays the correlation between the number of women who had abortions due to toxoplasmosis and their levels of Immunoglobulin IgG antibodies. The recorded data in this table indicates that during the first trimester of pregnancy, 15 women (15.0%) tested positive for VIDAS Toxo IgG antibodies and had abortions due to toxoplasmosis.

Table 4 displays the correlation between the number of women who had abortions due to toxoplasmosis and their levels of Immunoglobulin IgM antibodies. The recorded data in this table indicates that out of all the women who had abortions due to toxoplasmosis and were tested with VIDAS Toxo IgM antibodies, only 3 cases (3.0%) tested positive.

Table 5 displays the correlation between the number of women who had abortions due to toxoplasmosis and their place of residence. The recorded data in this table indicates that a higher number and percentage of women who had abortions due to toxoplasmosis during the first trimester of their pregnancy resided in urban areas, with a total of 30 cases (30.0%). In comparison, the number of women who had abortions due to toxoplasmosis in rural areas was 9 (9.0%).

Tab. 1. Distribution of study participants by number of women who had abortion due to toxoplasmosis, categorized by age groups

Number of Abortions	Age groups			Total
	(14 years-19 years)	(20 years-29 years)	(30 years-40 years)	
Non	5 (5.0%)	10 (10.0%)	9 (9.0%)	24 (24.0%)
1	8 (8.0%)	19 (19.0%)	12 (12.0%)	39 (39.0%)
2	5 (5.0%)	12 (12.0%)	5 (5.0%)	22 (22.0%)
3	2 (2.0%)	3 (3.0%)	3 (3.0%)	8 (8.0%)
4	1 (1.0%)	0 (0.0%)	3 (3.0%)	4 (4.0%)
5	1 (1.0%)	2 (2.0%)	0 (0.0%)	3 (3.0%)
Total	22 (22.0%)	46 (46.0%)	32 (32.0%)	100 (100.0%)

Tab. 2. Distribution of study participants by number of women who had abortion due to Toxoplasmosis, categorized by trimester stages

Number of Abortions	Trimester Stages			Total
	First	Second	Third	
Non	9 (9.0%)	13 (13.0%)	2 (2.0%)	24 (24.0%)
1	30 (30.0%)	7 (7.0%)	2 (2.0%)	39 (39.0%)
2	19 (19.0%)	2 (2.0%)	1 (1.0%)	22 (22.0%)
3	5 (5.0%)	2 (2.0%)	1 (1.0%)	8 (8.0%)
4	3 (3.0%)	1 (1.0%)	0 (0.0%)	4 (4.0%)
5	2 (2.0%)	1 (1.0%)	0 (0.0%)	3 (3.0%)
Total	(%68.0) 68	26 (26.0%)	6 (6.0%)	100 (100.0%)

Tab. 3. Distribution of study groups by number of women who had abortion and presence of immunoglobulin IgG antibodies

Number of Abortions	Immunoglobulin IgG		The Total
	Positive (8+)	Negative (4 ≤ 8)	
Non	5 (5.0%)	19 (19.0%)	24 (24.0%)
1	15 (15.0%)	24 (24.0%)	39 (39.0%)
2	9 (9.0%)	13 (13.0%)	22 (22.0%)
3	4 (4.0%)	4 (4.0%)	8 (8.0%)
4	3 (3.0%)	1 (1.0%)	4 (4.0%)
5	2 (2.0%)	1 (1.0%)	3 (3.0%)
Total	38 (38.0%)	62 (62.0%)	100 (100.0%)

Tab. 4. Distribution of study groups by number of women who had abortion and presence of immunoglobulin IgM antibodies

Number of Abortions	Immunoglobulin IgM		Total
	Positive (0.65+)	Negative (0.55 ≤ 0.65)	
Non	3 (3.0%)	21 (21.0%)	24 (24.0%)
1	2 (2.0%)	37 (37.0%)	39 (39.0%)
2	1 (1.0%)	21 (21.0%)	22 (22.0%)
3	0 (0.0%)	8 (8.0%)	8 (8.0%)
4	0 (0.0%)	4 (4.0%)	4 (4.0%)

5	0 (0.0%)	3 (3.0%)	3 (3.0%)
Total	6 (6.0%)	94 (94.0%)	100 (100.0%)

Tab. 5. Distribution of study groups by number of women who had abortion and place of residency

Number of Abortions	Residency		Total
	Urban	Rural	
Non	16 (16.0%)	8 (8.0%)	24 (24.0%)
1	30 (30.0%)	9 (9.0%)	39 (39.0%)
2	15 (15.0%)	7 (7.0%)	22 (22.0%)
3	5 (5.0%)	3 (3.0%)	8 (8.0%)
4	3 (30%)	1 (1.0%)	4 (4.0%)
5	2 (2.0%)	1 (1.0%)	3 (3.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

DISCUSSION

The present study was conducted from (September 2018 and June 2019) in a public clinic located in the Baghdad province, Iraq. A total of 100 serum samples were analyzed to determine the prevalence of toxoplasmosis among women who had abortions. The results in Table 1 indicate that out of all the women who had abortions, 19 (19.0%) were diagnosed with toxoplasmosis. Further analysis revealed that the prevalence of the disease was highest among women between the ages of 20 and 29, with a total of 39 (39.0%) cases across all age groups (14 years-19 years, 20 years-29 years, and 30 years-40 years old). It was found that 22 (22.0%) of the most infected women were younger than 20 years old, while 46 (46.0%) were in the age bracket of 20 years to 29 years old. These findings highlight the vulnerability of younger married women to the disease. This study's results are consistent with regarding the highest seroprevalence of IgG in the age range of 20 years to 29 years (22.66%), while the age range of 40 years had the lowest seroprevalence (16.66% for IgG and 0% for IgM). Additionally, the study's findings align with where the majority of age groups were in the (16 years-21 years) range, accounting for 33.3% of all age groups. However, these results differ from which reported a higher prevalence of IgG (30%) and IgM (20.68%), with the highest levels among participants aged (36-45) years [13, 14]. It can be concluded that there is a significant association between age group and *T. gondii* infection status in the study population, (p-value<0.001). Specifically, the results suggest that the prevalence of *T. gondii* infection is highest in the 20 years-29 years age group, and that there is a significant difference in the prevalence of infection between this group and the other two age groups. Additionally, the data suggests that there is a significant association between *T. gondii* infection status and the presence of toxoplasma IgG antibodies, but not IgM antibodies, as the proportion of women with positive IgG antibodies was significantly higher among those who were infected with *T. gondii* compared to those who were not infected. The prevalence of toxoplasmosis infection increases with age, but the disease's prevalence varies considerably between regions and countries, based on economic and social status, dietary habits, and

health standards. In some countries, better farming practices and hygiene have resulted in a decrease in the disease's prevalence [15]. Table 2 shows a positive association between trimester stages and the incidence of toxoplasmosis in abortion women. Specifically, the first trimester had a significantly higher number and percentage (68.0%) of cases compared to the second (26.0%) and third (6.0%) trimesters. These findings support observations that housewives and young women (ages 25 years-31 years) in their first trimester are more likely to contract toxoplasmosis [16]. This study found that all investigated BOH-afflicted women had experienced one or more abortions, with the highest percentage (48.8%) having had a single abortion. This is consistent with other studies, including suggesting potential explanations such as immunity development or pathogenic causes [17]. Similarly found that among 70 investigated women, the majority had experienced one or two abortions, with a small minority having three or more [18]. This study analyzed age ranges: 18 years-24 years (35.1%), 25 years-31 years (57.1%), 32 years-38 years (4.3%), and 39 years-45 years (2.9%). The majority of toxoplasmosis cases occurred in the first trimester (81.4%), followed by the second (14.3%) and third (4.3%) trimesters. Primary *T. gondii* infection during pregnancy is uncommon, but diagnosis is challenging, and vertical transmission to the fetus may cause congenital toxoplasmosis. Vertical transmission and its effects on the fetus are affected by gestational age [19]. Housewives who handle potentially contaminated meat and vegetables during meal preparation may increase the seroprevalence of toxoplasmosis [14]. Table 3 shows the correlation between the number of women with toxoplasmosis and their IgG immunoglobulin levels. One trimester had a high percentage (15.0%) of women with toxoplasmosis, as detected by VIDAS Toxo IgG antibodies. Table 4, shows a correlation between the number of abortion-related toxoplasmosis cases and Immunoglobulin IgM levels, with a high proportion of 3% cases. This finding is consistent with the study by but the study of which did not find any cases of abortion-related toxoplasmosis using a VIDAS test IgM Toxo antibody [20, 21]. This study was conducted between September 2020 and April 2021 in both urban and rural areas of the Iraqi province of Al-Najaf, where 190

blood samples were collected and tested for *T. gondii* IgG and IgM antibodies using the enzyme-linked immunosorbent assay and the Rapid Diagnostic immunochromatographic test. Each of these two assays used 5 milliliters of blood. The ICT test was used to detect IgG antibodies, and it found 80 (42.1%) positive samples, while no IgM positive samples were found. The ELISA test was used to detect both IgG and IgM antibodies, and 27 (33.7%) samples were found to be positive for IgG, and 4 (5%) samples were found to be positive for IgM. The estimated prevalence of toxoplasmosis was investigated, and the findings align with the study by in their study, 72 of the 82 sera samples had chronic toxoplasmosis (92.7%), with high IgG antibody titers detected in all sera [22]. Four samples also showed notable IgM antibody titers. Acute toxoplasmosis was identified in the group with antibodies against *T. gondii*-specific IgG and IgM. The AI percent was 50 or less in 16 samples, while 2 samples had an AI percent of more than 50 (89% and 11%, respectively). In the chronic group, the mean AI percent at serum titers of 1:200, 1:400, 1:800, 1:1600, 1:3200, and 1:6400 with detectable IgG antibody titers was 74.3, 82.5, 76.5, and 79.2, respectively, with a decrease in AI as serum dilution increased. These results are consistent with the findings of which reported significant seroprevalences of IgG (26.66%) and IgM (10%) antibodies among pregnant women in rural areas who were diagnosed with toxoplasmosis [12]. A comparison of these findings with those of revealed that 114 out of 273 (41.8%) patients showed evidence of prior infection, while 71 out of 273 (26%) had no

infection [23]. Proper testing for other infectious disorders that could jeopardize fetal development was performed in 172 out of 273 (62%) of the women. Living in rural areas and consuming raw meat were independently associated with an increased risk of *T. gondii* infection during pregnancy, according to a valid regression model analysis (OR=2.89, 95% CI: 1.42-5.9, p=0.004; and OR=2.07, 95% CI: 1.03-4.18, p=0.04, respectively). In conclusion, living in rural areas is an independent risk factor for *T. gondii* infection during pregnancy. Table 5 shows that more women who had toxoplasmosis-positive abortions were from the urban area (30.0%) compared to the rural area (9.0%). This finding is consistent with [24], discovery of high prevalence of toxoplasmosis in Iraq's Najaf province. Regular checkups are necessary for early detection of infections. Further research should be conducted in the AL-Najaf province, including surveys of *T. gondii* in animals, food, and water. Infection rates vary by age, region, and socioeconomic status. While study found high infection rates among women who had abortions in the Kurdistan region report disagrees, showing clusters of infection in rural areas of urbanized municipalities that necessitate proper healthcare measures [18, 25]. Toxoplasma infection occurs through consuming contaminated meat or water, contact with cat feces, or accidentally ingesting polluted dirt. While some regions have seen a decline in infection rates, disparities persist due to various factors. Healthcare actions are necessary for women living in rural and outlying areas where clusters of infection occur.

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