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Evaluation of the Sensitivity Three of Immunological and Molecular Diagnostic Techniques for the Diagnosis of Toxoplasmosis in Women in Wasit/ IRAQ

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Abstract: Background and objective: Toxoplasmosis is widely recognized as a highly widespread parasitic infection in humans, and is usually asymptomatic, capable of vertical transmission from mother to fetus . Clinical problems associated with toxoplasmosis during pregnancy include spontaneous miscarriage, stillbirth, and congenital form is one of the most important clinical aspects of this disease . This study aimed to determine the prevalence of toxoplasmosis in women infected with *T. gondii* in Wasit Province, and to provide insight into the impact of the disease on reproductive health and determine anti-*toxoplasma* antibodies, and *Toxoplasma* DNA.

Methods: One hundred and twelve women who were examined for laboratory testing were enrolled in this cross-sectional study. The prevalence of toxoplasmosis in women in Iraq may vary depending on factors such as geographic location, sample size, and diagnostic methods used Blood samples were tested for specific anti-*toxoplasma* IgM and IgG antibodies using an enzyme-linked immuno-sorbent assay (ELISA) and VIDAS

TOXO IgG II Procedure and Confirm at detection of *T. gondii* by PCR.

Results: Of 64 sera examined of female 14 (21.8 %) were seropositive for anti-*toxoplasma* IgG by Minivoids TOXO IgG device. Using ELISA test, 25 (22.3%) of the sera examined were seropositive for anti-*toxoplasma* IgM. The ELISA test finding for anti-*toxoplasma* IgM the sera 4 sample only examined were subsequently subjected to PCR. Thus, PCR analysis for detecting *T. gondii* DNA in the blood of female was positive in 4 (16 %) of the DNA samples.

Conclusion: The finding of this study indicates that ELISA and PCR is a more sensitive molecular techniques than a serological assay for the direct diagnosis of *T. Gondii* in the blood women.

Keywords: Toxoplasmosis, Enzyme-Linked immunosorbent Assay, PCR, Minivoids TOXO IgG device.

1. 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 (Shaukat et al., 2022). 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 (Wilking et al., 2016). Prenatal *T. gondii* infection can have serious negative effects on unborn children, mothers, and developing fetuses (Parvin et al., 2022). 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 (Thomas et al., 2022). 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 (Madireddy et al., 2022).

Toxoplasmosis, caused by the *T. gondii* parasite, affects approximately one-third of the global population (Ansari-Lair et al., 2017) Congenital toxoplasmosis can result from *T. gondii* infection during pregnancy, leading to severe effects on the fetus (Kieffer et al., 2008) . 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 (McAuley. 2014). 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. T o 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 (Goldstein et al., 2008) . Therefore, this study was aimed to identify the serological prevalence of toxoplasmosis in women in Wasit province based on immunoglobulin IgG and immunoglobulin IgM antibodies, age groups, residence, and abortion period .

2. Materials and Methods

Blood samples were collected from female participants between the ages of 18 and 41 who attended a public clinic in Wasit province between from 2020 to 2022 . An information sheet was created, and a questionnaire was administered to collect the necessary data . The blood samples were collected using disposable syringes from the radial vein, and the resulting serum was subjected to serological tests that included Mini Vidas to measure IgG titers. the centrifugation process was carried out at 3000 RPM for 5 minutes to ensure proper serum extraction. the Mini Vidas is an automated immunoassay system used for diagnostic testing . To measure IgG and IgM titers for the detection of *Toxoplasma*. the current study included 112 women who received private care at multiple hospitals located in the governorate of waist. Anti-*T. gondii* IgG antibodies present in the specimen will bind to the *T. gondii* antigen that is coated on the interior of the specimen using VIDAS® TOXO IgG II (VIDAS® TOXO IgG.

2.1. Specimen collection and preparation

Serum or plasma (EDTA) was collected for both, VIDAS TOXO IgG II. Compatibility of the collection tube was checked before sample collection.

2.2. VIDAS TOXO IgG II 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 Sprite 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.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The collected serum samples from female were tested for the presence of the specific IgM antibodies by using Clinotech Too ELISA IgM (Biotech Diagnostics Company, Germany). Clinotech Too IgM ELISA kits are microwell ELISA test designed for the qualitative detection of IgM antibodies to *Gondi* in human serum. The guidelines provided by the manufacturer carried out ELIZA.

2.4. Confirmatory testing for *T. gondii* by Polymerase chain reaction (PCR)

2.4 . 1.DNA extraction

Extraction DNA was extracted from whole blood samples, using DNA extraction kit, (High Pure PCR Template Preparation Kit) according to, the manufacture protocol Polymerase chain reaction was carried out according (Burg et al., 1989). The purity of DNA extracted from whole blood samples was measured by nanodrop and kept at 70 C until PCR was performed.

2.4.2. Primers

Two PCR primers were used to *Gondi* in human serum TR1

(5-ACGAACACTCGCAGAGATGA-3) and TR2 (5-GATCCTTTTGCACGGTTGTT-3)

was designed for B1 Gene.

2.4.3. PCR master mix

The PCR mixture was made in PCR tubes that had the kit with the PCR components . The remaining components were added to the reaction mixture in accordance with the company's instructions, which are displayed in Table (2-1).

Table (2-1): PCR master mix components

Master mix components	Volume 1 Sample (all)
Master Mix	12.5 all
Forward primer	1.25 all
Reverse primer	1.25 3M
sterile deionized water	7 all
DNA	3 all
Total volume	25

2.4.4. Programs of PCR Thermocycler

Each of 35 cycles of PCR thermal was consisted of an initial denaturation cycle, for 3min at 94 C^{o},denaturation of DNA for 30 seconds at 94 C^{o}, annealing for 30 seconds at 45 C^{o}, extension for min at 72 C^{o} . 15 all of the amplified PCR product was analyzed on 1.5% agarose gel electrophoresis and visualized under transluminal or after Ethidium bromide staining .

3. Results and Discussions

3.1. ELISA and Rapid Test

Seropositivity of anti-*Toxoplasma* IgG antibodies showed in 64 pregnant women . The positive infection was 14 patients with a rate of 21.8% and the healthy women were 50 samples with a percentage of 78.2% in rapid test manual (Minivoids TOXO IgG device). While in ELISA anti-*Toxoplasma* IgM antibodies showed in 112 pregnant women. Positive infection was 25 patients with a rate of 22.3 % and the healthy women were 87 samples with a percentage of 77.7 %. As demonstrated in Table (3-1)

Table (3-1): A comparison of anti-*Toxoplasma* IgG antibodies seropositivity using Rapid test and (ELISA) tests.

Infection Status	ELIZA Test		Minivoids TOXO IgG device	
	Number	Percentage %	Number	Percentage %
Negative	87	77.7	50	78.2
Positive	25	22.3	14	21.8
Total	112	100	64	100

Freuqency of women with toxoplasmosis according to age groups is shown in Table (3-2). the study found that out of the total number of women with Toxoplasmosis, the highest percentage was observed in the age group of 36 years-41 years. Specifically, 19 (52.6 %) of the women in this age group were diagnosed with Toxoplasmosis . However, when considering all age groups (18 years-23 years, 24 years-29 years, and 30 years-35 years old), the total number of women with Toxoplasmosis were recorded as 25 (22.3 %) .

Table (3-2): Distribution of study participants by number of women infection due to toxoplasmosis, categorized by age group

Age group	Total	IgM+%
18-23	30	4 (13.3%)
24-29	41	9 (21.9%)
30-35	22	2 (9%)
36-41	19	10 (52.6%)
Total	112	25 (22.32%)

Table (3-3) presents a detailed and their relationship to age and housing for patients in an urban or rural environment Where the percentage of single projections was 16% and ranged between ages (28-40) years and they lived in a good urban environment, while the percentage of repeated projections was 13% and ranged between ages (36-43) years and they lived in the environment of districts and sub-districts .

Table (3-3): Distribution of study participants by number of women who had abortion due to toxoplasmosis, categorized by age group

Drop Type	Number	Age Group
Single Drop live in urban	4 (16 %)	28-40
Frequent Dropping live in rural	3 (13 %)	36-41

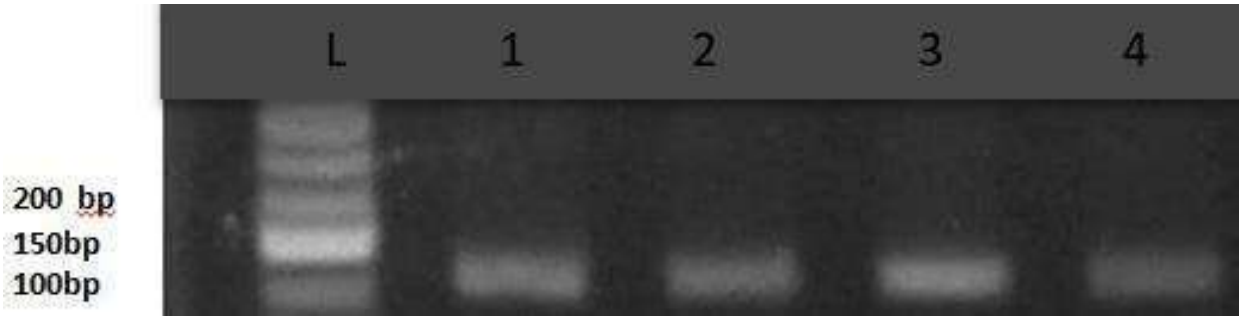
The increased percentage of infections significant that suggests a lack of culture, knowledge, and understanding of the causes of this disease, how it spreads among patients, and how cats can spread among family members, or those who work in gardens where they leave their litter The high rate of infection among field animals , which is a source of infection, could potentially be the cause. Additionally, the percentage was high in high-standard areas because of cat breeding, and women are more vulnerable to illness because, they come into direct touch with meat and vegetables when cooking, which might expose them to infection-causing germs .

3.2. PCR test

PCR findings to the 4 samples screened for DNA female are shown in Table (3-4). PCR was positive in 3.57 % of the blood screened for *T. gondii* DNA organism. PCR technique was used to confirm the infection with *T. gondii* by detection of *T. gondii* DNA in the blood of female. *Toxoplasma gondii* DNA was successfully extracted and analyzed by PCR. Thus, positive samples were detected by presenting a 120 bp band of amplification test on 1.5% agarose gel (Figure 3-1) .

Table (3-4): Interpretation of the four PCR results obtained for each patient sample

Tests	Positive samples	Total
PCR	4 (16 %)	112



(Figure 3-1). PCR amplification of the B1 gene of *Toxoplasma gondii* genome. Lane 1.Marker, Mol. wt. marker (100 bp ladder), lane 1, 2 ,3 and 4 are positive samples.

Between 2020 and 2022, was 176 blood samples were drawn from both urban and rural parts ofthe Iraqi province of Wasit. The enzyme-linked immunosorbent assay and the Rapid Diagnostic Test were used to check for *T. gondii* IgG and IgM antibodies Five milliliters of blood were utilized in each of these two assays The Minivoids TOXO IgG device test was used to identify IgG antibodies, and it found 14 (21.8%) positive samples. IgM antibodies were detected using the ELISA technique, and samples were positive for IgM The study examined the expected incidence of toxoplasmosis and found that 72 out of 82 sera samples had chronic toxoplasmosis (92.7%), with high IgG antibody titers found in all sera . (Ali. 2010; Albay & Ali. 2024) The most common methods used in current studies, and research to confirm the existence of toxoplasmosis include the direct detection of parasites in body fluids or tissues, the culture and

isolation of protozoa in mice or tissue culture, and the detection of antibodies against IgM, IgG, and IgA in serum or other body fluids (Flori et al., 2009). The detection of IgM antibodies is usually necessary for the diagnosis of acute toxoplasmosis, which is why ELISA was chosen for the identification of IgM and IgG anti-*Toxoplasma* substances . It is generally noted that in the case of acute illnesses, IgM antibody levels rise approximately one to two weeks after the sickness begins (Wilson et al., 1997) . The current investigation Toxoplasmosis Prevalence in women was assessed by analysis of 176 serum samples. A total of instances across all age categories (18–23 years, 24–29 years, and 30–35 years old), additional research showed that the condition was most prevalent in women aged 36–41 was 10 (52.6%) . These results demonstrate how susceptible younger married women are to the illness. According to the results of this study, the age group of 30 to 35 years old had the lowest seroprevalence2 (9% for IgM), while the age group of 36 to 42 years old had the highest seroprevalence of IgG (41.3%) . These findings, however, are different from those that showed a greater prevalence of IgG (30%) and IgM (20.68%), with the greatest levels found in people who were between the ages of 36 and 42 (Nowakowska et al., 2006; Left et al., 222020; Mohammed. 2018). (Nowakowska et al., 2006; Left et al., 2020; Mohammed. 2018) .

It can be concluded that there is a significant association between age group and *T. gondii* infection status in the study population (Beaumont et al.,2024) . We may conclude that the study population's age group and *T. gondii* infection status is significantly correlated. Infection with toxoplasmosis is more common as people age, although the frequency of the disease varies greatly between nations and regions depending on factors like dietary practices, health standards, and social and economic standing . In several nations, the occurrence of the disease has decreased as a result of improved farming methods and hygiene. In several nations, the disease's frequency has decreased as a result of improved farming methods and sanitation (Robert-Bagneux and Dared. 2012)

The incidence of toxoplasmosis in women who have had abortions is positively correlated with thestages of the trimesters . These results corroborate findings indicating young women in their first trimester, including housewives, had a higher risk of contracting toxoplasmosis (ages 25–31) (Amedi. 2013). This supports the findings of earlier investigations and raises the possibility of disease reasons or immune development as explanations (Morris and Croxson. 2004). The seroprevalence of toxoplasmosis may rise if housewives prepare meals while handling possibly contaminated meat and vegetables (Mohammed. 2018). The relationship between women's IgG immunoglobulin levels and the number of toxoplasmosis cases. As indicated by VIDAS Too IgG antibodies, a significant proportion of women experienced toxoplasmosis during one trimester This finding is in line with a study that used an IgM Too antibody VIDAS test to detect no cases of toxoplasmosis linked to abortion. (Ali. 2010; Jaber and Noori. 2021) .

Findings of high toxoplasmosis prevalence in Iraq's Wasit province infection rates vary by age, region, and socioeconomic status; however, a study in the Kurdistan region found high infection rates among women who had abortions; however, Najaf province disagrees, demonstrating clusters of infection in rural areas of urbanized municipalities that require appropriate healthcare measures. Regular checkups are necessary for early detection of infections (Al-Saeed. 2016; Antinarelli et al., 2021; Albay & Ali. 2024). Infection with *Toxoplasma* can be contracted by eating or drinking tainted food or water, coming into touch with cat excrement, or inadvertently swallowing contaminated soil. Disparities still exist because of a number of causes, even if infection rates have decreased in some areas. For women residing in rural and distant locations where infection clusters develop, healthcare measures are required. The PCR in the blood of women by age group was 52.6% higher in those over 20. The current investigation concurred with the research by (Remington et al., 2001; Al-Gargoyle. 2014).

The variations in outcomes the variations in parasite strains may be a significant factor in triggering the host immune response to the parasite, considering the variations in the specificity and sensitivity of the diagnostic technique and each host's reaction to the parasite strain (Suzuki

and John. 1994). The varied numbers of sick individuals in each age group may have an impact on the current result. Additionally, people may have been exposed to *Toxoplasma* as children through cats. Additionally, soil exposure has caused anti-*Toxoplasma* antibodies to accumulate in humans at varying percentages, which can result in a persistent infection with toxoplasmosis (Spalding et al., 22005) .

The current study's findings validate that a significant proportion of females actually have a *T. gondii* infection. ELISA (IgM) tests obtained from females revealed four positive samples using PCR technique based TaqMan probe for detection of *T. gondii*, and all positive blood samples by Minivoids TOXO IgG device. This finding is consistent with other previous investigations that have demonstrated that a positive PCR result does not necessarily translate into a positive serology that shows local antibody production (Talabani et al., 2009; Al-Sanjay and HHussein.2012). The gold diagnostic standard for *T. gondii*, PCR, was used to test the sensitivity and specificity of ELISA experiments. In identifying anti-*toxoplasma* IgM, the investigations demonstrated the sensitivity and specificity of ELISA. There was a strong and noteworthy correlation between PCR and anti-*toxoplasma* antibodies IgM. A basilophorous vacuole (PV) is created when the intracellular parasite *T. gondii* enters mammalian cells via an action-dependent mechanism The PV is mostly modified by the parasite. Many cell types found in warm-blooded vertebrates can be affected by the parasite (Dobrowolski and Sibley, 1996) .

Polymerase chain reaction has been advised to overcome false negative serologic testing, particularly in compromised immune individuals (Savva et al.,1990). For *T. gondii* infection, a number of PCR-based methods have been created as substitute diagnostic assessments. These methods utilize the gene sequences that are most constant between strains of *T. gondii*, such as ribosomal DNA, the P30 (SAG1) gene, and the repetitive B1 gene (Ellis. 1998). Additionally, in some immunocompromised people, the test may not detect *T. gondii* infection because the titers of particular anti-*Toxoplasma* antibodies may not increase in these patients. In fact , PCR identification of *T. gondii* DNA reduces the issues encountered with serodiagnosis techniques. Consequently, a female infection is ruled out by the negative PCR and ELISA results (Abbas et al ., 2014). In this case, molecular testing that could identify circulating parasites would be extremely useful. While direct detection of *T. gondii* in blood or other clinical samples definitively establishes the parasite presence and leads to the diagnosis of primary, reactivated, or chronic toxoplasmosis, a positive serological result simply indicates exposure (Bastien. 2002) The PCR- based method described in this study provides a rapid, sensitive, and quantitative way of detecting *T. gondii* in clinical specimens .

References

1. Abbas, HH, Yassir DAL, Manal BAL. Detection Toxoplasma gondii by real-time PCR in abortive and pregnant women in AL-MUTHANNA province. J Inter Accad Res Mult 2014;2: 310-7.
2. Al-Gargoyle NAS. Sero epidemiological and Molecular Study for Toxoplasma gondii Among Diabetic Mellitus Patients in Babylon Province M.Sc. Thesis. College of Science. University of Babylon. 2014; p125
3. Ali SH. Prevalence of toxoplasmosis among pregnant women in Najaf city. Kufa J Vet Med Sci. 2010; 1:101-108.
4. Albay, K., & Ali, Q. A. N. (2024). Detection Study: Prevalence of toxoplasmosis in aborted women infected with toxoplasma gondii in Baghdad province, with implications for oncology. Onko logia I Radiotherapies, 18(2).
5. Al-Saeed ATM. Detection of toxoplasmosis among women with abortion using molecular and serological tests in Duhok City. Duhok Med J. 2016; 10:56-68

6. Al-Sanjay RA, Hussein TH. Using species-specific PCR technique to detect Toxoplasma gondii in broiler chickens. Iraqi J Vet Sci 2012; 26:53-6.
7. Amedi H. Seroprevalence screening for TORCH infections among pregnant women in Duhok. M.Sc. thesis. University of Duhok; 2013.
8. Ansari-Lair M, Farahani H, Mohammadi F. Association of Toxoplasma gondii infection with schizophrenia and its relationship with suicide attempts in these patients. Trop Med Int Health. 2017; 22:1322–1327.
9. Antinarelli LMR, Silva MR, Guimarães RJPSE, Terror MS, Lima PE, et al. Rural residence remains a risk factor for Toxoplasma infection among pregnant women in a highly urbanized Brazilian area: a robust cross-sectional study. Trans R Soc Trop Med Heeg. 2021; 115:896-903
10. Bastien P. Molecular diagnosis of toxoplasmosis. Trans R Soc Trop Med Heeg. 2002; 96:205-15.
11. Beaumont, E., Brodeur, J., Thomas, F., Dujon, A. M., Consortium Signature, & Lupien, S. J. (2024). Toxoplasma gondii infection in people with schizophrenia is related to higher hair glucocorticoid levels. Frontiers in Psychiatry, 15, 1286135.
12. Burg JL, Grover CM, Poletti P, Boothroyd J. Direct and sensitive detection of a pathogenic protozoan, Toxoplasma gondii, by polymerase chain reaction. J Clin Microbic 1989; 27(8): 1787-92.
13. Ellis JT. Polymerase chain reaction approaches for the detection of Neosporin caninum and Toxoplasma gondii. Int J Parasite 1998; 28: 1053-60.
14. Goldstein EJC, Montoya JG, Remington JS. Management of Toxoplasma gondii Infection during Pregnancy. Clin Infect Dis. 2008; 47:554–66.
15. J. M. Dobrowolski and L. D. Sibley, “Toxoplasma Invasion of Mammalian Cells Is Powered by the Actin Cytoskeleton of the Parasite,” Cell, vol. 84, no. 6, pp. 933–939, Mar. 1996, Doi: 10.1016/S0092-8674(00)81071-5
16. Jaber KA, Noori RA. Comparisons of Toxoplasma gondii Prevalence in Rural and Urban Areas of Al-Najaf Province of Iraq Using Serological Methods. Arch Razi Inst. 2021; 76:1695-1701.
17. Kieffer F, Wallon M, Garcia P, Tullie P, Peyron F, et al. Risk factors for retinochoroiditis during the first 2 years of life in infants with treated congenital toxoplasmosis. Pediatric Infect Dis J. 2008; 27:27–32.
18. Left RM, AL-Khalida EK, AL-Jacory MKA. Seroprevalence of Toxoplasmosis Gondii Infection among Pregnant Women in Karbala Governorate 2014-2017. Medico-legal Update. 2020; 20:1086-1090.
19. M. Wilson, J. S. Remington, C. Clavet, G. Varney, C. Press, and D. Ware, “Evaluation of Six Commercial Kits for Detection of Human Immunoglobulin M Antibodies to Toxoplasma gondii,” 1997.
20. Madireddy S, Rivas Chacon ED, Mangat R. Toxoplasmosis. In: Stat Pearls (Internet). Treasure Island (FL): Stat Pearls Publishing; 2022
21. McAuley JB. Congenital Toxoplasmosis. J Pediatric Infect Dis Soc. 2014; 3:30-35.
22. Mohammed BA. Seroprevalence of Toxoplasma gondii among pregnant women in Tikrit city, Salahaldeen Province. Med J Tikrit Univ. 2018; 24:170-176.
23. Morris A, Croxson M. Serological evidence of Toxoplasma gondii infection among pregnant women in Auckland. N Z Med J. 2004; 117:1-7.

24. Nowakowska, D., Stray-Pedersen, B., Spiewak, E., Sabala, W., MacAfee, E., & Wilczynski, J. (2006). Prevalence and estimated incidence of Toxoplasma infection among pregnant women in Poland: a decreasing trend in the younger population. Clinical Microbiology and infection, 12(9), 913-917.

25. P. Flori, G. Chene, M.-N. Varlet, and R. T. M. Sung, "Serologies de la toxoplasmosis chez la femme enceinte: toxoplasmosis et pieces," Ann Biol Clin (Paris), vol. 67, no. 2, pp. 125–133, Mar. 2009, Doi: 10.1684/abc.2009.0308.

26. Parvin I, Das SK, Ahmed S, Rahman A, Shahid ASMSB, et al. Toxoplasma gondii Infection Is Associated with Low Birth Weight: Findings from an Observational Study among Rural Bangladeshi Women. Pathogens. 2022; 11:336.

27. Remington JS, McLeod R, Tullie P. Toxoplasmosis, Infectious diseases of the fetus and newborn infant; in Remington JS, Klein J (5thed.). W. B. Saunders, Philadelphia, Pa 2001; pp 205

28. Robert-Bagneux F, Dared ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbial Rev. 2012; 25:264- 296.

29. Savva D, Morris JC, Johnson JD, Holliman RE. Polymerase chain reaction for detection of Toxoplasma gondii. J Med Microbial. 1990; 32: 25-1.

30. Shaukat T, Awan UA, Mahmood T, Afzal MS, Wasif S, et al. Epidemiology of Toxoplasmosis among the Pakistani Population: A Systematic Review and Meta-Analysis. Pathogens. 2022; 11:675.

31. Spalding SM, Amender MRR, Klein CH, Ribeiro LC. Serological screening and toxoplasmosis exposure factors among pregnant women in south of Brazil. Rev Soc Bras Med Trop, 2005;38:173-7.

32. Suzuki Y, John K. Effect of the strain of Toxoplasma gondii on the development of toxoplasmosis encephalitis in mice treated with antibody by interferon-gamma. Parasite Res 1994; 80:125-30.

33. Talabani H, Ashraf M, Yera H, Delair E, Ancelle T, Tullie P, Brazin, AP, et al. Contributions of immunoblotting, real-time PCR, and the Goldmann-Witmer coefficient to diagnosis of atypical toxoplasma retinochoroiditis. J Clin Microbial 2009; 7:2131-5.

34. Thomas M, Aubert D, Escott-Binet S, Durand B, Robert C, et al. Anatomical distribution of Toxoplasma gondii in naturally and experimentally infected lambs. Parasite. 2022; 29:3

35. VIDAS® TOXO IgG II (TXG), 30 210-01 13687 D- end- 2015/06. Durham, North Carolina: bioMérieux, Inc.; 2015.

36. Wilking H, Thamm M, Stark K, Aebischer T, Seeber F. Prevalence, incidence estimations, and risk factors of Toxoplasma gondii infection in Germany: a representative, cross-sectional, serological study. Sci Rep. 2016; 6:22551.