Molecular Diagnosis for Seropositive and Negative of Toxoplasma Gondii in Aborted Women, Sheep and Goats in Erbil Province

Hamad Mustafa Saleh[1], Zuber Ismael Hassan[2]
[1, 2]Department of Medical Laboratory Technology, Erbil Health and Medical Technical College, Erbil Polytechnic University, Kurdistan Region/Iraq.
Email: hamad.mstafa@epu.edu.iq

Abstract
The overall 85 aborted women anti-*T. gondii* IgG immunoglobulin was detected in 27.5% (22/80) of the women, of which 22.5% (18/80) were positive for only IgG, and 5% (4/80) were positive for IgM antibodies. Furthermore, the total seropositivity of anti-*T. gondii* immunoglobulin among aborted sheep and goats were 15(17.65%) and 9(30%), respectively. however, IgG plus IgM antibodies were found in (23.64%, 3.64%) and (26.67%, 3.33%), respectively. The present study showed that 8 of 165 blood and placental tissue samples were found to be positive for both IgG Cobas e 411 assay and conventional PCR. On the other hand, two blood and tissue specimens were positive for IgM and conventional PCR positive cases, and the remaining five cases of IgM were seronegative, and the remaining IgG cases were seronegative. Conventional PCR detected 12 positive cases from placental tissue that could not be detected by Cobas e 411 assays against IgM and IgG. According to molecular analysis, the specimens examined in this study were shown to share a greater 99.9%–100% agreement with sequences of *T. gondii* (KX270387 and MK704513) in Erbil province. The information is helpful for educational authorities responsible for designing and implementing effective measures to prevent disease.

Keywords: Toxoplasmosis, Seroprevalence, PCR, Genetic analysis

1. Introduction

Toxoplasma gondii, an obligatory intracellular and cyst-forming protozoan able to infect warm-blooded animals, including humans, and is the source of the zoonotic disease, which is widespread throughout most of the world (1). When humans consume Toxoplasma cysts found in raw or uncooked meat, water, food, or soil contaminated by cat feces, they become infected (2). Increase the risk of transmission with the pregnancy age, and the severity of the fetus decrease. In fact, during the first trimester of pregnancy, the placenta barrier is more effective, permitting the entry of parasites in lesser than 10% of infected pregnant women (3). As (4) revealed, as pregnancy progresses, it becomes more permeable, resulting in parasite dissemination in the second and third trimesters in about 30% and 60–70% of infected pregnant women. In addition, infections throughout pregnancy can result in congenital toxoplasmosis in the embryo, which can cause neurological disorders that can be seen during pregnancy or at delivery, as well as spontaneous miscarriage, stillbirth, hydrocephalus, and microcephaly (5, 6). In farm animals, especially sheep and goats, the infection of toxoplasmosis is an economically undependable disease (7). In many cases of acquired toxoplasmosis, there are no symptoms or minor symptoms (8). The serological tests commonly used to diagnose toxoplasmosis include the sabin Feldman dye test, indirect hemagglutination test(IHT), indirect fluorescent antibody test(IFAT), complement fixation test (CFT) and intradermal test(IDT)(9, 10). Besides these tests, PCR is used to identify toxoplasmosis in pregnant women (11) in order to avoid and reduce serious consequences in the fetus or infant and to improve the prognosis of infection, and it also used to detect of congenital toxoplasmosis in the uterine or post birth (5, 12). Maternal serological screening for toxoplasmosis is often essential to avoid fatal injuries by medical treatments or prophylaxis, especially in seroconverting women throughout pregnancy (13). In recent years, DNA-based molecular techniques have better sensitivity and specificity than serological techniques, and it has been used to identify infections more precisely throughout pregnancy. Various targets of the T. gondii genomic have also been researched. For the identification of the T. gondii infection, the PCR method's 200-300-fold repeated 529 bp fragment is a valuable diagnostic target with good Accuracy and sensitivities (14, 15). Additionally, it is believed that the B1 gene for molecular characterization, with the proper rate of variability, is a promising target for T. gondii (16). Microsatellite analyses are divided into type I, type II, or type III clones lineages (17). In Portugal, types II and III of T. gondii strains, which are frequently seen in pigs and fowl (18). Studies conducted in other European nations, such as France, Spain, Switzerland, and Germany, have shown that genotype II is more prevalent in domestic animals (19). The study aim is to detect

doi.org/10.31838/hiv23.01.89 Received: 14.09.22, Revised: 18.10.22, Accepted: 07.12.22

of T. gondii infections in aborted women sheep, and goats by serological and molecular techniques with phylogenic tree in Erbil province.

2. Material and Methods

2.1- The study area

The cross-sectional study was carried out in Erbil province during the period from November 2021 to April 2022. Five ml of blood and placental tissue were collected from 85 aborted animals (55 sheep and 30 goats) and 80 women aged 16 to 48 old that had a spontaneous abortion or were having pregnancy problems at Erbil Maternity Teaching Hospital and Soran Maternity Teaching Hospital. The blood was separated and kept at -20°C until use for Cobas e 411-based Elecsys test. Furthermore, placental tissue biopsies were taken from aborted women, sheep and goats throughout the first and second trimesters and stored in 70% of ethanol. The whole blood and placental tissue are kept for DNA extraction.

2.2. Serological Testing

Cobas e 411-based Elecsys was done by using two kits (Elecsys) Laboratories to recognize IgG and IgM-specific immunoglobulin anti T. gondii antigens in the patient's serum.

2.3. Molecular Analysis

Trypsin was used to degrade tissue samples for two hours at 37 Co. DNA from placenta tissue and blood by using a commercial DNA extraction kit {Bio-Tech Korea} was extracted, and it was amplified using specific primers for Toxoplasma strains, primer forward TOX4 (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and primer reverse TOX5 (5'-CGCTGCAGACACAGTGCATCTGGATT-3') (20). The mixture of amplification consist of 25µl which included (2X) Go-Tag Master Mix (12.5µl), 1.5 µl of each primers (forward and reverse), DNA template (2µl) and nuclease-free water (7.5µl). The amplifying reaction was carried out under the following condition: initially denatured (93oC for 5min), Following, 35 cycles of denaturation (90oC for 60 second), annealing (60oC for 1 min.), elongation (72oC for 1 min), and final extension step (72oC for 5minute). The products of amplification were examined by 1.5% agarose-gel electrophoresis, following by staining with SYBR Safe dyes. (0.5µg/ml) in Tris-Acetate EDTA (TAEbuffer) 1X. DNA ladder (100 bp) makes by Fermentas Life Sciences, Waltham, MA, USA, was used as the marker (molecular weight marker) and photographed under UV trans-illumination. The amplified products of PCR were commercially sequenced in both direction (Macrogen Inc. South Korea).

2.4. Nucleotide sequencing and phylogenetic analysis

The specimens of Toxoplasma were verified by analyzing the nucleotide sequences of the B1 gene. The sequences were aligned through the ClustalW algorithm (21), provided by BioEdit v7.2.5(22), with sequences available in the GenBank (NCBI) database. Nucleotide sequences of the partial B1 gene of the T. gondii from aborted sheep, goats and women were analyzed, and the phylogenetic tree was performed on individual partial gene sequences utilizing MEGA-6 software (Molecular Evolutionary Genetics Analysis) http://www.megasoftware and the neighbour-joining were used to build the tree.

2.5. Statistical Analysis

Scatter plot graphing software (Graph Pad Prism v.7, CA, USA) is used for data analyses. The Chi-square test was used to analyze the data, and a P value ($P<0.05$) is ordered as statistically significant.

3. Result and Discussion

Out of eighty women who have had spontaneous abortions, the median age 28 years (ranging from 16–48 years) in the first and second trimesters, were clinically diagnosed and tested for Toxoplasma infection with Cobas. The rate of infections was 18 (22.5 %) for IgG antibodies and 4 (5%) for IgM Table (1). The result partially agreed with (23) the overall of 110 pregnant women in Baghdad were seroprevalence of T. gondii IgG and IgM immunoglobulin result showed that 40 (63.4 %) of 110 pregnant women were reactive for IgG while revealed that 16 (13.6 %) of 110 pregnant women were reactive for IgM by Cobas and (24) in Iraq which showed that, out of 63 aborted women in Kirkuk reported that 6 (10%) were reactive with both IgG and IgM immunoglobulin, while 6 (10%) reported seropositive for only IgM immunoglobulin, and 19 (30%) reported seropositive for only IgG immunoglobulin. Furthermore, (25) in Tunisia expressed that, Seroprevalence total was 44.4%, of which 352 (44%), 3 (0.4%) for IgG and both of them IgG, IgM anti-T. gondii immunoglobulin, accordingly, was positive. In contrast(26) in Egypt showed that Anti-Toxoplasma IgM seropositivity was found in 10% of the patient's group, while anti-Toxoplasma IgG seropositivity was considerably greater in the patient group (23.3%) comparable to the controls (10%). As well as, (27) reported that IgG immunoglobulin was diagnosed in 28% (14/50) of women, but all species were non-reactive for IgM antibody. in the avidity ELISA test, 26% (13/50) of the specimens had a higher avidity index, indicating latent infectious, while a low avidity index was diagnosed in only one case (2%), which indicated recent infections. In recent infections with T.gondii, the particular IgM immunoglobulin is first recognized and produced after acute T. gondii infection. IgM typically turns negative after a few months. (26, 28). Anti-Toxoplasma IgG immunoglobulin often manifest during the first two weeks of infections, peak after one to two months, and then gradually decrease; however, they can last a lifetime (29).

Table (1) shows the distribution of seropositive by Cobas e 411 in local aborted sheep and goats collected from

different localities in Erbil Governorate. Out of 55 sheep and 30 goat sera, 15 (17.65%) and 9(30%) were seropositive. however IgG plus IgM antibodies were found in (23.64%, 3.64%) and (26.67%, 3.33%), respectively. The result partially agreed (30) in Misan province-Iraq, which expressed that the prevalence of toxoplasmosis was 25% in aborted ewes by using ELISA. According to the findings of the current study, ewes in the Erbil Governorate had a lower seropositivity rate of 17.65% compared to other studies' prevalence rates in various Iraqi provinces. Such as in Mosul by (31), Sulaimani (32) and Duhok (33), in which the rates were 42.7%, 57% and 67.31%, respectively, by utilizing LAT. Also, it is lower than those recorded in Sulimani (32), Ninevah (34) and Duhok (35), in which the seroprevalence was 51.7%, 32.8% and 42.1% by ELISA, respectively. On the other hand,(36) in India revealed that, Among the 72 serum goat samples, 31 (43%) were found negative for the presence of T. gondii immunoglobulin, whereas 13 (18%) were weakly positive, and 28 (38.88%) were detected to be highly positive for the presence of T. gondii specific antibodies. IgM of T.gondii were initially found in animals with recently acquired infections, and in almost cases, these titers disappeared within a few months. Furthermore, in certain animals, a particular titer of positive T.gondii IgM might be seen during the chronic stages of the infections. It has been documented that IgM antibodies might last up to 12 years past the acute infection. The animals should be viewed as chronic infection carriers because the persistent IgM antibodies do not seem to have any clinical significance(33).

Table 1: Seropositivity of T. gondii infections in aborted Women, sheep, and goats in Erbil Province by Cobas e 411.

Species	number of samples	Anti- toxoplasma IgG	Percentage (%)	Anti- Toxoplasma IgM	Percentage (%)	Total	Percentage (%)
aborted Women	80	18	22.5	4	5	22	27.5
Aborted sheep	55	13	23.64	2	3.64	15	17.65
Aborted goats	30	8	26.67	1	3.33	9	30.00

Table 2 revealed that the comparison between conventional PCR and Cobas assays, results indicated that 8 of 165 blood and placental tissue samples were founded to be reactive for both conventional PCR and IgG Cobas assay. Two blood and tissue samples of conventional PCR positive cases were IgM reactive, and the remaining five cases of IgM seronegative with the remaining IgG seronegative (table 3). On the other side, conventional PCR detected 12 positive cases from placental tissue that could not be diagnosed by Cobas assay against IgM and IgG. The result agreed with(37), who reported that Only 60, 60, and 5 specimens from sheep, women, and equines were randomly chosen for PCR detection of the seropositive samples found by ELISA. The results revealed that 13/60 (21.7%), 5/60 (8.3%) and 5/26 (19.2%) of a seropositive specimen of ewes, women and equine were reactive to conventional PCR. The lack of the parasites in the blood at the during of collecting and the parasite's localization as tissue cysts, tachyzoites, and/or bradyzoites inside the body of the host are thought to be the causes of the low positive ratio by PCR compared to the results of the Cobas assay. Additionally, the acute infection may have subsided or changed into a persistent infection

Table 2: The comparison between Cobas e 411 and conventional PCR.

Species	Anti-toxoplasma	No. of Examined Samples (165)	Positive No. by cobas e 411	Percentage (%)	PCR positive No.	Percentage (%)
Aborted Women	IgG	80	18	22.50	3	3.75
	IgM		4	5.00	1	1.25
Aborted Sheep	IgG	55	13	23.64	2	3.64
	IgM		2	3.64	1	1.82
Aborted goats	IgG	30	8	26.67	3	10.00
	IgM		1	3.33	0	0.00

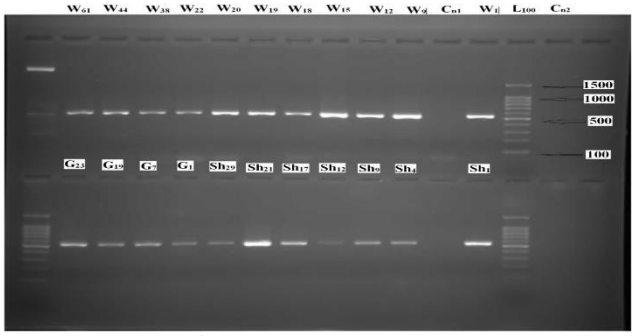

Figure 1: Amplified Tox4 and Tox5 region in agarose gel electrophoresis. Lanes (W1, W9,W12,W15,W18,W20, W22, W38, W44 and W61) denote women samples, (Sh1, Sh4, Sh9, Sh12, Sh17, Sh21 and Sh29) denote sheep and Lanes (G1, G7, G19 and G23) denotes goats samples amplified as a single band of 529 bp; Cn1 denotes DNA extraction negative control; Cn2 denote PCR negative control, and 100bp denote 100bp ladder molecular weight marker.

Figure 1 shows gel electrophoresis after PCR amplification with TOX4 and TOX5 primers. The placental tissue and whole blood with seropositivity by Cobas (18 women, 15 sheep and 9 goats) were tested for targeting the B1 gene by utilizing conventional PCR. The results revealed that 11 women, 7 sheep and 4 goats were positive and obviously showed an amplicon size of 529 bp. The result agreed with(38), which revealed that the seropositive samples of both ewes and horses were randomly selected for PCR to validate the results of ELISA. In the horse, all samples were found to be positive by ELISA, while in the case of sheep, only 60 samples were found positive by ELISA. These samples were randomly selected to be verified by amplification B1 gene using PCR. The PCR result shows two positive samples out of 11 in a horse at a rate (18.2%) and13 positive samples out of 60 were

positive in sheep at the rate (21.7%), clearly showing an amplicon size of 529 bp. On the other hand,(39) reported that T. gondii DNA was diagnosed in 114 females out of 307 placenta tissue samples (37.1%) by conventional PCR based on B1 gene amplification. Furthermore, (40) revealed that 87 tissue samples were examined for T. gondii, which was found through the B1 gene. Seven tissue specimens were positive for T. gondii. Intriguingly, nested PCR results for the heart muscle, liver, brain, and placenta were reactive. In addition, lung samples from this investigation produced negative results. Positive outcomes were more prevalent in the placenta and brain than in the heart muscle. Comparing lung and liver sample results, the lung sample yielded the least adverse findings. The lack of protozoa in the blood at the moment of blood collection and the parasite's localization as tissue cysts, tachyzoites, and/or bradyzoites within the host's body are thought to be the causes of the reduced ratio by PCR compared to the Cobas results. Additionally, the acute infection may have subsided or changed into a persistent infection(41).

Table 3: Presence of T.gondii in tissue placenta based on PCR assay.

Variables	Total Cases	Anti- toxoplasma IgG and IgM	Sero positive and PCR positive	Percentage (%)	Sero negative PCR positive	Percentage (%)	Total Positive PCR
Women Placental tissue	80	22	4	5.00	7	8.75	11
Sheep Placental tissue	55	15	3	5.45	4	7.27	7
Goats Placental tissue	30	9	3	10.00	1	3.33	4

The occurrence amplification of fragments was 100% of the toxoplasma samples. As well, the expected patterns were provided in the samples with T. gondii, as shown in figure 2. The result showed that the Toxoplasma species (Women 1, Sheep 2, and goat 1) was 100% and sheep 12, goat 7 were 99.9% homologous to T. gondii under the accession number (KX270387 and MK704513) due to nucleotide substitution (A → G) at the position of 207 as shown in Figure (2). The nucleotide sequences agreed with the result and were consistent with T. gondii (37, 38, 42-44). Thus, it was found that our isolates were near the type strain (45). The molecular method used for the isolation of Toxoplasma species resolves the time-consuming and difficulties in interpreting some morphological, and physiological patterns and confirmation of strains (19, 46). The different cultures and probably the ethnical, climatic and geographical elements and features of cases are responsible for factors determining this variation (47).

Figure 2: Alignment of a fragment of T. gondii (women, sheep 1, 12 and goats 1, 7) with GenBank accession numbers KX270387 and MK704513.

Toxoplasma species (women, sheep 1,12 and goats 1,7) were compared with reference sequences(MK012093, MN267831, MK012099, MK012098, MK012095, MK012094, MK012091, MK012092, MK704514, AF179871, MK704513, KX270388, KX270365, KX270387, KX270378, KX270367, KY514162, KY514164, MN275919, MN275910, EU348881, MW883447, MW883448, MN275988, MN275908, MN275907, MH744807, KU748884, KU748883, KU748882, KT266792, KT266791, KU672635) in the GenBank database was supported in the species determination by utilizing the BLAST Algorithm (https://blast.ncbi.nlm.nih.gov/). Alignments of the sequences is performed by using Clustal W (version 5.6.1, 2009, CLC bio, Aarhus, Denmark) and the identity matrix options of Bioedit (22, 48), respectively. The neighbouring MEGA Version 7 program was used to build a phylogenetic tree (Figure 3). The result agreed with(16), which revealed

that the phylogenetic analysis by the B1 gene was categorized taxonomically as a single clade. Various geographic conditions, the level of parasite genomic diversity for a given region, the sensitivities and polymorphism of the used genetic markers, and the number of polymorphism loci may all have an impact on the variances in genotype in different parts of the world (49, 50).

Figure 3: Phylogenetic tree based on the B1 gene of T. gondii isolates from naturally infected women, sheep and goats with accession numbers which previously deposited in the GenBank database

4. Conclusion

A higher ratio of toxoplasmosis can be detected by serological testing than by polymerase chain reaction (PCR) in the current investigation. Consequently, Cobas, in combination with the PCR method, is advised as a tool for precise toxoplasmosis diagnostic. The findings clarified the relationship between various *T. gondii* genotypes and abortion in sheep, goats, and women. Low genetic variability was seen when toxoplasma strains were characterized from aborted women, sheep and goats. Sharing databases across medical, agricultural, and veterinary agencies was regarded as a significant step in implementing a strategy for the early identification, control and treatment of diseases.

References

1. Ahmed F, Cappai MG, Morrone S, Cavallo L, Berlinguer F, Dessì G, et al. Raw meat based diet (RMBD) for household pets as potential door opener to parasitic load of domestic and urban environment. Revival of understated zoonotic hazards? A review. 2021;13:100327.
2. Pinto-Ferreira F, Caldart ET, Pasquali AKS, Mitsuka-Breganó R, Freire RL, Navarro ITJEid. Patterns of transmission and sources of infection in outbreaks of human toxoplasmosis. 2019;25(12):2177.
3. Mandelbrot LJPD. Congenital toxoplasmosis: What is the evidence for chemoprophylaxis to prevent fetal infection? 2020;40(13):1693-702.
4. Bollani L, Auriti C, Achille C, Garofoli F, De Rose DU, Meroni V, et al. Congenital Toxoplasmosis: The State of the Art. 2022;10.
5. Saki J, Zamanpour M, Najafian M, Mohammadpour N, Foroutan MJJoPR. Detection of Acute and Chronic Toxoplasma gondii Infection among Women with History of Abortion in the Southwest of Iran. 2021;2021.
6. Verteramo R, Santi E, Ravennati F, Scutiero G, Greco P, Morano DJRM. Ultrasound Findings of Fetal Infections: Current Knowledge. 2022;3(3):201-21.
7. Abdelbaset AE, Mossaad E, Ismail AA, Ibrahim AM, Xuan X, Suganuma K, et al. Seroprevalence of Toxoplasma gondii in farm animals in West Kordofan, and Blue Nile states, Sudan. 2020;30(1-2):31-7.
8. Blaizot R, Nabet C, Laghoe L, Faivre B, Escotte-Binet S, Djossou F, et al. Outbreak of Amazonian Toxoplasmosis: A One Health investigation in a remote Amerindian community. 2020;10:401.
9. Damour NMA. Sero-prevalence of Toxoplasmosis in Cattle in El-Gadarif State: Sudan

University of Science & Technology; 2018.
10. Sigle M, El Atrouni W, Ajlan RSJAJoOCR. Seronegative ocular toxoplasma panuveitis in an immunocompetent patient. 2020;19:100745.
11. Al-Yami FS, Dar FK, Yousef AI, Al-Qurouni BH, Al-Jamea LH, Rabaan AA, et al. A pilot study on screening for gestational/congenital toxoplasmosis of pregnant women at delivery in the Eastern Province of Saudi Arabia. 2021;29(4):343-50.
12. Khan K, Khan WJPI. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. 2018;67(6):715-21.
13. Picone O, Fuchs F, Benoist G, Binquet C, Kieffer F, Wallon M, et al. Toxoplasmosis screening during pregnancy in France: Opinion of an expert panel for the CNGOF. 2020;49(7):101814.
14. Molaei S, Dadkhah M, Fathi FJT. Toxoplasmosis diagnostic techniques: Current developed methods and biosensors. 2022:123828.
15. Galli L, Del Grande C, Rindi L, Mangia C, Mangano V, Schiavi E, et al. lack of circulating Toxoplasma gondii DNA in seropositive patients with bipolar or schizophrenia spectrum disorders. 2019;273:706-11.
16. Arefkhah N, Sarkari B, Asgari Q, Moshfe A, Khalafi MH, Mohammadpour IJIJoP. Molecular genotyping of Toxoplasma gondii in sheep aborted fetuses reveals predominance of type I infection in southwest of Iran. 2020;15(3):374.
17. Deiró AGdJ, do Prado DP, Sousa IP, Rocha DdS, Bezerra RA, Gaiotto FA, et al. presence of atypical genotypes of Toxoplasma gondii isolated from cats in the state of Bahia, Northeast of Brazil. 2021;16(10):e0253630.
18. Uzelac A, Klun I, Ćirković V, Bauman N, Bobić B, Štajner T, et al. Toxoplasma gondii Genotypes Circulating in Serbia—Insight into the Population Structure and Diversity of the Species in Southeastern Europe, a Region of Intercontinental Strain Exchange. 2021;9(12):2526.
19. Fernández-Escobar M, Calero-Bernal R, Benavides J, Regidor-Cerrillo J, Guerrero-Molina MC, Gutiérrez-Expósito D, et al. Isolation and genetic characterization of Toxoplasma gondii in Spanish sheep flocks. 2020;13(1):1-14.
20. Maani S, Rezanezhad H, Solhjoo K, Kalantari M, Erfanian SJMP. Genetic characterization of Toxoplasma gondii isolates from human spontaneous aborted fetuses in Jahrom, southern Iran. 2021;161:105217.
21. Thompson JD, Higgins DG, Gibson TJJNar. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. 1994;22(22):4673-80.
22. Hall TA, editor BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series; 1999: [London]: Information Retrieval Ltd., c1979-c2000.
23. Al-Rawazq HSAMJA-KCMJ. Seroprevalence of immunoglobulin G (IgG) and immunoglobulin M (IgM) and risk factors of toxoplasmosis for a sample of pregnant women in Baghdad. 2017;13(2):40-5.
24. Mahmood AR, Abdulla AK, Hussein NMJPoE, Sciences N. Molecular detection of Toxoplasma gondii specific repeat element in blood of recurrent aborted women by real-time PCR. 2021;9(4):708-14.
25. Lachkhem A, Lahmar I, Galal L, Babba O, Mezhoud H, Hassine M, et al. Seroprevalence of Toxoplasma gondii among healthy blood donors in two locations in Tunisia and associated risk factors. 2020;27.
26. Elzeky SM, Nabih N, Abdel-Magied AA, Abdelmagid DS, Handoussa AE, Hamouda MMJJoTM. Seroprevalence and Genetic Characterization of Toxoplasma gondii among Children with Neurodevelopmental Disorders in Egypt. 2022;2022.
27. Khademi SZ, Ghaffarifar F, Dalimi A, Davoodian P, Abdoli AJJoO, Research G. Spontaneous abortion among Toxoplasma gondii IgG seropositive women: Molecular detection, genotype identification, and serological assessment with conventional ELISA and avidity ELISA. 2022.
28. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz HJJocm. Role of Toxoplasma gondii IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. 2020;58(9):e00505-20.
29. Montoya JGJTJoid. Laboratory diagnosis of Toxoplasma gondii infection and toxoplasmosis. 2002;185(Supplement_1):S73-S82.
30. Khadi J, Thamer M, Al-Amin AJIJoVS. Prevalence of antibodies to Toxoplasma gondii in aborted ewes in south of Iraq. 2009;23(Suppl. 1).
31. Al-Sim'ani RJM, Iraq, University of Mosul. A serological study to diagnose toxoplasmosis in sheep and human in Ninevah governorate [thesis]. 2000.
32. Al-Taie LHJTIJoVM. Seroprevalance of Toxoplasmosis in sheep and goat: Iraq/Sulaimania: Lazem H. Al-Taie, Shadan H. Abdulla. 2011;35(1):16-24.
33. Al-Barwary L, Mikail FJIJoVS. Seroprevalence of toxoplasmosis in aborted ewes by using different immunologic tests in Duhok governorate, Kurdistan region, Iraq. 2014;28(1):11-5.
34. Al-Dabagh I, Jasim B, Jarjees MJIJoVS. Seroprevalence of antibodies to toxoplasmosis, brucellosis and chlamydiosis in abortive sheep in Nineveh governorate, Iraq. 2014;28(1):21-5.
35. Al Hamada A, Habib I, Barnes A, Robertson IJVPRS, Reports. Risk factors associated with seropositivity to Toxoplasma among sheep and goats in Northern Iraq. 2019;15:100264.
36. Devada K, Lakshmanan B, Syamala K, Vijayakumar K, Pooja G, Karthika RJJoV, et al. Seroprevalence of Toxoplasma gondii in aborted goats in Kerala. 2021.
37. Mikaeel F, Al-Saeed AJTIJoAS. Serological and molecular diagnosis of Toxoplasma gondii among ewes and horses in Duhok province-Iraq.

2020;51(4):1212-9.
38. Shaapan R, Toaleb NI, Abdel-Rahman EHJIJoVS. Detection of Toxoplasma gondii-specific immunoglobulin (IgG) antibodies in meat juice of beef. 2021;35(2):319-24.
39. ISSAD NA, Abdelouahed K, Mimoune N, Bekhouche S, Boubeuker R, ADJMI HH, et al. Molecular Detection of Toxoplasma gondii in Ewes Placenta in Northeastern Algeria. 2022;28(2).
40. Hasan T, Mannan A, Hossain D, Rekha A, Hossan MM, Alim MA, et al. Molecular detection of Toxoplasma gondii in aborted fetuses of goats in Chattogram, Bangladesh. 2021;14(9):2386.
41. Lopes AP, Vilares A, Francisco N, Rodrigues A, Martins T, Ferreira I, et al. Genotyping characterization of Toxoplasma gondii in cattle, sheep, goats and swine from the North of Portugal. 2015;10(3):465.
42. Shamaev ND, Shuralev EA, Petrov SV, Kazaryan GG, Aleksandrova NM, Valeeva AR, et al. Seroprevalence and B1 gene genotyping of Toxoplasma gondii in farmed European mink in the Republic of Tatarstan, Russia. 2020;76:102067.
43. Lamy SA, Kawan MH. Seroprevalence of toxoplasmosis in quail birds (Coturnix coturnix) in Baghdad City, Iraq.
44. Martínez-Flores WA, Palma-García JM, Caballero-Ortega H, Del Viento-Camacho A, López-Escamilla E, Martínez-Hernández F, et al. Genotyping Toxoplasma gondii with the B1 gene in naturally infected sheep from an endemic region in the pacific coast of Mexico. 2017;17(7):495-502.
45. Abdul Hafeez M, Mehdi M, Aslam F, Ashraf K, Aleem MT, Khalid AR, et al. Molecular characterization of Toxoplasma gondii in cats and its zoonotic potential for public health significance. 2022;11(4):437.
46. Salehi M, Nezami H, Niazkar HRJVMI. Investigation of Toxoplasma gondii infection in aborted fetuses of sheep using PCR: a study in north Khorasan province, Iran. 2020;2020.
47. Amouei A, Sarvi S, Mizani A, Hashemi-Soteh MB, Salehi S, Javidnia J, et al. Genetic characterization of Toxoplasma gondii in meat-producing animals in Iran. 2022;15(1):1-11.
48. Hall GE, Hord SM, Aguilera R, Zepeda O, von Frank VJTLP. Implementation: Learning builds the bridge between research and practice. 2011;32(4):52.
49. Rouatbi M, Amairia S, Amdouni Y, Boussaadoun MA, Ayadi O, Al-Hosary AAT, et al. Toxoplasma gondii infection and toxoplasmosis in North Africa: a review. 2019;26.
50. Liang W, Zhao S, Wang N, Tang Z, Zhao F, Liu M, et al. Molecular occurrence and risk factors for Toxoplasma gondii infection in equids in Jilin, China. 2022;12(1):1-7.