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Use molecular technique to detect toxoplasmosis in placenta and aborted fetus in women in Al-Diwaniyah province

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***Abstract*---**The current study included the detection of Toxoplasma gondii from aborted women from Maternity and Childhood Teaching Hospital. It drew attention to the human infection rate in order to detect the differentiation them with SAG3 gene. The samples were collected during the period from October, 2021 until the end of March 2022 in AL-Qadisyah province. Total of 100 samples (placenta and fetus) were collected and send to the laboratory of veterinary medicine AL- Qadisiyah University in order to detect women with toxoplasmosis by impression method all of are positive. The present study included a molecular method to detect surface antigen 3 (SAG3) using Nested Polymerase Chain Reaction (nPCR), the results of the nPCR for SAG3 gene showed that 18/24 (75 %) were positive in the aborted woman. the NCBI-BLAST Homology Sequence identity (%) between local T. gondii isolated from women cases that have been deposited in the NCBI under the following accession numbers (ON240954, ON240955, ON240956, ON240957, ON240958, ON240959, ON240960, ON240961, ON240962, and ON240963) and NCBI-BLAST deposited other global strains.

Keywords*---Toxoplasma gondii*, PCR, SAG3 gene, Sequence analyzing.

Introduction

Toxoplasma gondii is an obligatory intracellular protozoan parasite that causes serious disease in humans, small ruminants, other warm-blooded mammals, and birds (Yektaeian *et al.*,2021). It is distributed all around the world (Woyesa *et al.*,2021), *Toxoplasma gondii* has been isolated from a variety of geographical regions (Yang *et al.*,2021). According to estimates, this parasite threatens one-

third of the world's population (Bandelj *et al.*,2021). The parasite which is common in both humans and animals, causes the disease depending on where they reside, 15 to 85 percent of the human population is asymptomatically infected (Mose *et al.*,2020). except for feline species, which acts as a definitive and intermediate host, all animal species act as intermediate hosts) Elsheikha *et al.*,2021). The apicomplexan zoonotic parasite *Toxoplasma gondii* has three infective stages: sporozoites in sporulated oocysts, which are shed in unsporulated form into the environment by infected felids; tissue cysts containing bradyzoites, and fast replicating tachyzoites that are responsible for acute toxoplasmosis (García *et al.*,2021). It has a complex life cycle and infected people with a range of diseases (Overstreet,2021). The disease is transmitted in humans by mother-to-child transmission, undercooked meat with latent cysts, contaminated water containing sporulated oocysts, and consumed contaminated food (Al-Malki *et al.*,2021). Although the infection is usually asymptomatic in immunocompetent people, there were a few instances of ocular symptoms such retinochoroiditis and retinitis as a result of acquired toxoplasmosis in humans, both initial infection and reactivated toxoplasmosis put immunocompromised persons at risk for severe disease (Fabiani *et al.*,2022). If a primary infection occurs during pregnancy, immunocompetent women may develop fetal infection, which can be transmitted to the fetus (de Lima Bessa *et al.*,2021).

Aim of Study

The main purpose of the current study was Detection of toxoplasmosis in aborted woman infected in AL-Qadisiyah province and the genetic tree of types by Molecular method.

Materials and Methods

Collection of human samples

The study was carried out during the beginning of October,2021 until the end of March 2022 in AL-Qadisyah province, included 100 samples from aborted woman (placenta and fetus) who have toxoplasmosis history, their ages range from 15 to 45 years, who attended to the Maternity and Childhood Teaching Hospital and Al-Fourat Al-Awsat National Hospital, as well as private the samples divided two for and impression method molecular

Microscopic Examination (by stamp method or impression method)
A primary diagnostic method by noting the parasite stages in prepared stamp the tissue on the glass slid and left in air to dray and methanol alcohol 90% was added for ten minute to fixating then was stained with Giemsa stain for 15 mints and was washed by tab water then left to dray, after drying they were examined under the oil immersion lens of light microscope (Zhang *et al.*,2019).

DNA extraction
The aim of using a primer is to amplify a specific gene segment SAG3, to detect *Toxoplasma gondii* in different samples. These primers were designed by (Grigg *et al.*,2001).), The primers were synthesized by (Macrogen company, Korea).

Primer

First round	Sequence 5-----3	annealing	Amplicon size
SAG3ExF	CAACTCTCACCATTCCACCC	55 C	450 bp
SAG3ExR	GCGCGTTGTTAGACAAGACA		
Second round	Sequence 5-----3	annealing	Amplicon size
SAG3InF	TCTTGTCGGGTGTTCACTCA	56 C	225 bp
SAG3InR	CACAAGGAGACCGAGAAGGA		

Statistical Analysis

Use the percentage to analyze the results that were reached in the current study to prove the existence of a relationship between toxoplasmosis infection in aborted women AL-Diwanyiah province.

Results

Prevalence of *toxoplasma gondii* by impression smear.

The results presented in this study of infection in the placenta and fetus (100) samples of aborted women, who have toxoplasmosis history, tissue cyst clear in all of smear(fig. 1).

Table (1) show distribution of *Toxoplasma gondii* according to the type of sample of infection in human

Organ	No.	(+ve)	%
Placenta	50	50	50%
Fetus	50	50	50%
Total	100	100	100%

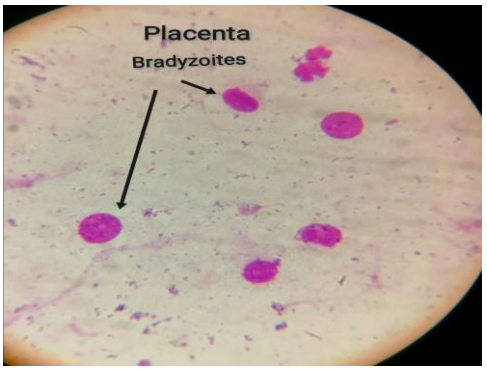

Figure (1): Microscopic image of tissue cysts of impression smears of placenta stained with Giemsa stain (100x).

Percentage of *Toxoplasma gondii* according to the age in aborted woman have toxoplasmosis

A total of 100 samples (50 placenta & 50 fetus) for woman have toxoplasmosis history and positive to impression methods by found tissue cyst of parasite in a smear with giemsa stain according to the age (15-45) the parasitic infection is identified in all of them. The highly percentage recorded in age (25-35) years 40(40%) then 30(30%) in (35-45) years and the lowest 30(30%) in (15-25) years, Table (2).

Table (2) The percentage of toxoplasmosis in human accorded to the age

Age of patients	No	%
15-25	30	30%
25-35	40	40%
35-45	30	30%
Total	100	100%

Distribution of *Toxoplasma gondii* according to the region of infection.
The results showed that the infection rate in the city was 60%, which was higher than in the countryside, which amounted to 40%. Table (3).

Table (3) distribution of *T.gondii* according to the region in human

region of infection	No.	%
City	60	60%
Rural	40	40%
Total	100	100%

Distribution of *Toxoplasma gondii* according to the trimesters of pregnancy

The results showed that the incidence of toxoplasmosis for women by smear impression, that Toxoplasma transmission rate through placenta in the first, second, and third trimesters is 40, 25, and 35%, The first trimester is the most dangerous for the fetus, respectively, and early diagnosis and specific treatment of mothers can reduce the risk of fetal infection up to 50%, how have toxoplasmosis history included 100 human samples from aborted woman.

Table (4) distribution of *T.gondii* according to the trimesters of pregnancy

Trimesters pregnancy	NO	(+ve)	%
First	40	40	40%
Second	25	25	25%
Third	35	35	35%
Total	100	100	100%

Detection of *T. gondii* using Molecular methods

The results of PCR amplification which performed on the DNA extracted of SAG3 gene of *Toxopalasma gondii*, the samples were taken from 100 samples which were positive result with impression method as (placenta 50 and fetus 50), respectively the studied isolates confirmed by electrophoresis analysis.

Nested polymerase chain reaction (nPCR) targeting a specific region within to SAG3 gene

The results of PCR amplification which was performed on the DNA extracted of SAG3 gene of *Toxopalasma gondii*, the samples were taken from 100 samples which were positive result with impression method as (placenta 50 and fetus 50), respectively the studied isolates confirmed by electrophoresis analysis. Analysis the strands of DNA which are resulted from the successful binding between specific primers and extracted DNA of isolates, these successful binding appear as single band together with the 225 pb band product size. The result of nPCR in present study of human was 18/24 (75 %) from positive samples to impression method .Fig(2).

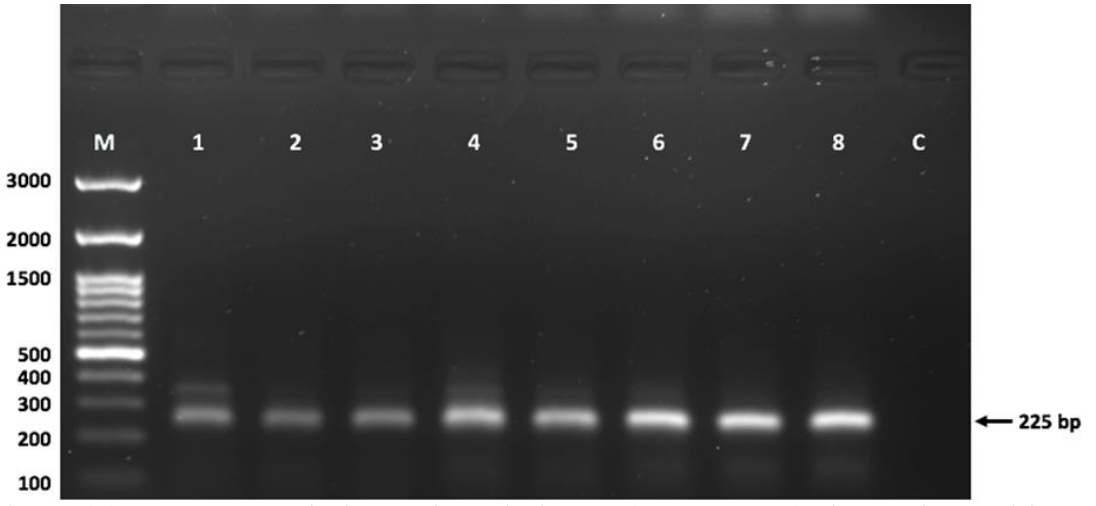

Figure (2): - Agarose gel electrophoresis image (2 %Agarose) shows the positive amplicons (1-8) of *Toxoplasma gondii* targeting SAG3 gene (size= 225 bp) originated from testing human samples. C is control negative in which similar PCR reaction mixture was used except H2O was added instead of DNA. M is molecular marker from Genedirex, Korea.

DNA sequencing, genetic tree analysis and genotyping (SAG3)

Toxoplasmosis isolates number (1-10) taken from aborted women who had a genetically related disease history with NCBI-BLAST showed Toxoplasmosis species (4,5,7) (MT358429, AF340227.1, MH744782), match ratio (99.45-100%), Whereas, isolates (1, 2, 3, 6, 8, 9 and 10) showed a close relationship with NCBI-BLAST of the parasite *Toxoplasma gondii* (AF340227.1, AF340227.1, AF340227.1,

AF340227.1, AF340227.1, AF340227.1 and AF340227.1), 100% match as shown in the tables (5).

Table (5): - the NCBI-BLAST Homology Sequence identity (%) between local *Toxoplasma gondii* isolated from human cases that have been deposited in the NCBI under the following accession numbers (ON240954, ON240955, ON240956, ON240957, ON240958, ON240959, ON240960, ON240961, ON240962, and ON240963) and NCBI-BLAST deposited other global strains

Toxoplasma gondii sequence	Obtained Accession Number	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	Genbank Accession number	Country	Identity (%)
1	ON240954	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
2	ON240955	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
3	ON240956	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
4	ON240957	*Toxoplasma gondii*	MT358429	Spain	99.45
5	ON240958	*Toxoplasma gondii* strain A allele	AF340227.1	USA	98.91
6	ON240959	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
7	ON240960	*Toxoplasma gondii*	MH744782	USA	99.45
8	ON240961	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
9	ON240962	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100
10	ON240963	*Toxoplasma gondii* strain A allele	AF340227.1	USA	100

Results identification of genetic polymorphisms and Multiple alignment analysis of the identified *T. gondii* amino acids sequences (SAG3 gene) for infected human

After the gene region is amplified in SAG3 by PCR technique we observed some genetic variation in the SAGE region for infected human, by resequencing DNA samples (10 infected), that were below are the numbers installed in the Genbank accession number (10 humans) for all samples according to their approval relative to the MegaX polymorphism for SAG3 gene. Results of this study after making the alignment of all samples it shows has five-position polymorphism at site with accession number in the strain USA (AF340228.1) *T.gondii* statin E allele (42) C/T, (62) G/A, (66) A/G, (114) G/C, (122) C/G, (137) A/G. As for the human being (98) T/C ON240957and ON240960, (107) G/A ON240958, (130) G/C ON240958. While results multiple alignment analysis of the identified *T. gondii* amino acids sequences(SAG3) in human, there was there was a substitution of a single nucleotide of T /C at position (98) ON240957 of the SAG3 of *T. gondii*. The nucleotide substitution resulted in change of a single nucleotide residue in the

deduced amino acid sequence at position 33 as Leucine (L) instead of Serine (S) obtained accession numbers (ON240957) and (ON240960), and G/A at the position (107) substitute to amino acid sequence at position (36) as Glycine(G) instead of Glutamic acid (E) obtained accession numbers (ON240958), and G/C at the position (130) substitute to amino acid sequence at position (44) as Glycine(G) instead of Arginine (R) obtained accession numbers (ON240958).Fig.(3)

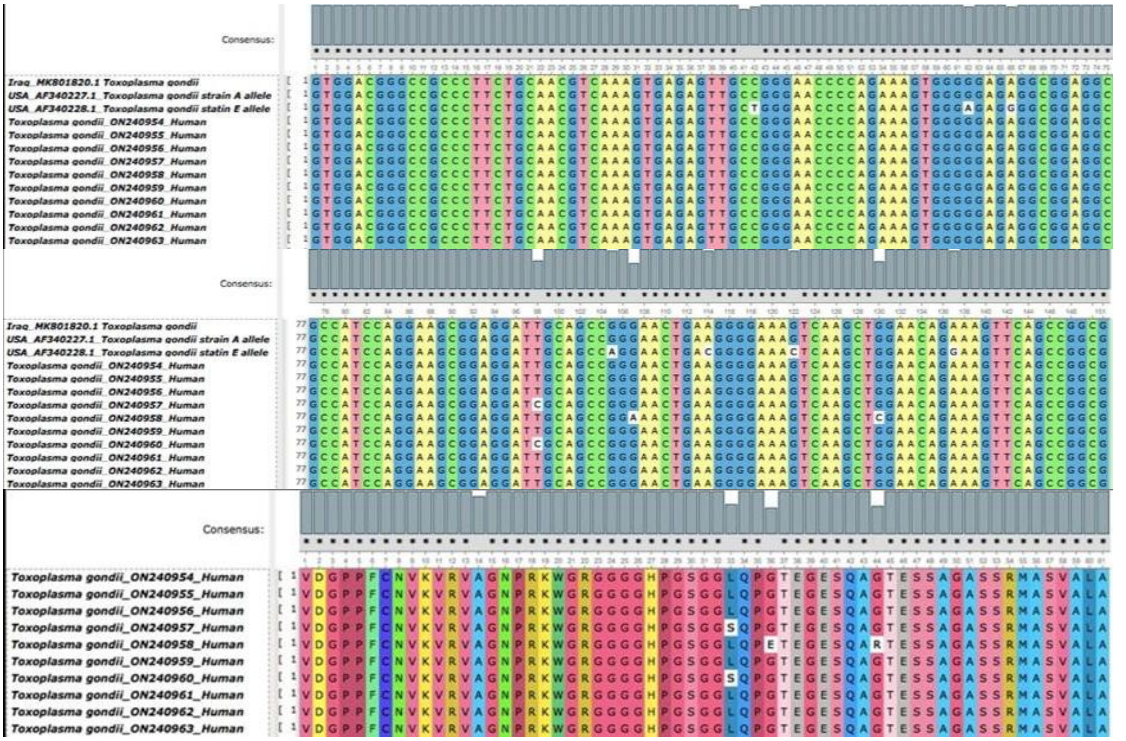

Figure (3): -Multiple alignment analysis of the identified *Toxoplasma gondii* sequences and amino acids sequences (SAG3 gene) followed by the obtained accession numbers and the host from which originated (human) that shows the similarity and differences among these sequences which compared with global deposited sequences. This was carried out by MegaX software

Discussion

The current study, by means of the impression smear, to detected toxoplasmosis bradyzoite in the placenta and fetus who have toxoplasmosis history woman who attended to the maternity and childhood hospital in Al-Diawanyia province, 100 sample positive that meanwhile the parasite pass from the uterus to the placenta and fetus my study agree with Guyton and Hall (2006) they says that the parasite can pass from placenta to the fetus. Different study were recorded the parasite in many diagnosis method or the result of placental and fetus tissues agreed with (Abid and mohammed, 2013) who showed 78% positive in placentas in salah Al-Din province and 70% in Anbar province. While the results showed that compared to other humans in terms of age, the frequency of *T. gondii* was significant in these samples, depending on the host's age. In the group between (15 and 45

years old), A higher prevalence was observed between (20-35) with a percentage of 65%, while the prevalence was observed between (15-19), (36-45) and the lowest percentage was 30%. The positive rates for the other age groups varied, and this was consistent with what was indicated by many researchers in different regions of the world, as the infection rate was high in the age group (20-35 years) (Kalantari et al.,2021; Mostafavi et al.,2012). The age of activity of women to pregnant. Whilst the result of nPCR in present study of human was 18/24 (75 %) from positive samples, the present study found 75% in the human, the result of who recorded that 83.3% of tested samples from Iraq women was positive by PCR technique, this result is consistent with the current results on the other hand the results of (Al-Sray *et al.,*2019) who reported that 17.65%, the result of present study agreed with the results of (ALSaady *et al.*,2021) who reported that 63.49%. The differences among percentages that, recorded in the current study and other studies. *T. gondii* seroprevalence varies substantially both within and between regions. While results DNA sequencing, genetic tree analysis, identification of genetic genotyping (SAG3) for infected human, results showed the DNA sequence analysis of the products of the Nested polymerase chain reaction of the SAG3 gene, Clear genetic diversity among *T. gondii* isolates, according to a genetic tree analysis as in the Figure (2) that analyzed Toxoplasma isolates from human with standard *T. gondii* isolates in NCBI, most of the studied samples were identical to what was recorded in the National Center for Biotechnology Information, With a matching rate of (99.36-100%). That SAG3 is important for the parasite adhesion to host cells (Dzierszinski et al.,2000). Toxoplasmosis isolates number (1-10) taken from aborted women as in the table (5) who had a genetically related disease history with NCBI-BLAST showed Toxoplasmosis species (4,5,7) (MT358429, AF340227.1, MH744782), match ratio (99.45-100%), Whereas, isolates (1, 2, 3, 6, 8, 9 and 10) showed a close relationship with NCBI-BLAST of the parasite *T. gondii* (AF340227.1, AF340227.1, AF340227.1, AF340227.1, AF340227.1, AF340227.1 and AF340227.1), 100% match. Our findings reveal that molecular characterization of the SAG3 gene in *T. gondii* isolates from various hosts exhibits genetic diversity. This finding was agreement with previously study by (Gelaye *et al.*, 2016). Who revealed that that SAG3 gene in *T. gondii* is considered significant homologous variations between the different loci of various strains of parasites.

Conclusions

PCR assay can serve as a sensitive tool to diagnosis *T.gondii*. Therefore, evolution and sequencing method confirmed *T.gondii* in human after comparing the related isolates in the bank gene and indicated the importance of SAG3 gene in diagnosis of species.

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