# Phylogenic study of toxoplasmosis in aborted women in Al- Diwnaniya province

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## Abstract

The current study included the detection of *Toxoplasma gondii* from aborted women attented to the Maternity and Childhood Teaching Hospital to detected the rate of abortion due to toxoplasmosis using PCR technique, the samples which was used placenta and fetus, this study also identify the differentiation between it by phylogenic with SAG3 gene.

A total of 60 ( placenta and fetus) samples were collected randomly from aborted women who received to the Maternity and Childhood Teaching Hospital.

The present study include molecular method to detected small subunit ribosomal RNA ( ssRNA) using Nested Polymerase Chain Reaction ( nPCR) ,the results of the nPCR for ssRNA gene, the result showed that the rate of toxoplasmosis in the aborted women was Positive 60%. (There is a statistically significant between species in P <0.05).

The present study also detected for Surface Antigen (SAG3) gene to conform the nPCP result and to send the samples for sequences.

The SAGA gene showed 100% positive results according to the ssRNA gene .

T.gondii isolates surface Ag SAG3 gene with NCBI BLAST *Toxoplasma gondii* isolates, and the DNA nucleotides sequencing analysis of surface Ag SAG3 complete gene was show clear gentic variation between isolates from different host.

**Keyword** ; T.gondii , human ,camel and goat , SAG3 , Npcr

## Introduction

*T.gondii* is a single-celled protozoan parasite of which felids are the definitive host (Dubey *et al.*, 1970).

Dubey and Beattie (1988 ) explaned that the *T.gondii* has three infectious stages, including sexually produced oocyst, rapidly dividing tachyzoites and slow multiplying bradyzoites in tissue cysts.

Charles Nicolle showed the occurrence of a banana -shaped life form in the tissues during studied the infectious diseases of the desert rodent (Nicolle, 1907). In 1907, at the city in Tunis, the initial description of this discovery was published by Nicolle and his collaborator, Louis Manceaux (Nicolle, 1908). In Brazil, identified the same microbe as a parasite of rabbits (Tait and Hunter , 2009).

Since *T. gondii*'s discovery, its clinical importance has influenced the research groups investigating the immunobiology of toxoplasmosis, Today, *T. gondii* is recognized as an important opportunistic parasite of fetuses, newborns and patients with a variety of primary genetic and acquired immunodeficiencies (Petersen and Dubey, 2001).The majority of immunocompromised patients that develop clinical disease have defects in T cell function, highlighting the

importance of lymphocytes in controlling this persistent infection, Consequently, there has been a focus on understanding how T cells provide protection against disease and how cytokines modulate T cell responses ,This theory was provided an overview of the events, consider how the study of *T. gondii* has had a significant impact on the field of immunology and discuss future studies that may provide new insights into the mechanisms necessary for the control of this pathogen(Lieberman and Hunter, 2002).

## Materials and Methods

The Nested PCR primers for detection small subunit ribosomal RNA gene in *Toxoplasma gondii* were design by pervious study (Vitale et al., 2008). These primers was provided from Macrogen company, Korea as following

| Primer | Sequence | | Amplicon |
|---|---|---|---|
| 18SrRNA gene First round PCR Primers | F | TGCGGAAGGATCATTCACACG | 530bp |
| | R | CCGTTACTAAGGGAATCATAGTT | |
| 18SrRNA gene Second round Nested PCR Primers | F | GATTTGCATTCAAGAAGCTGATAGTAT | 313bp |
| | R | AGTTAGGAAGCAATCTGAAAGCACATC | |
| DNA sequence Toxo-SAG3primers | F | ATGCAGCTGTGGCGGCGCAG | 1158bp |
| | R | TTAGGCAGCCACATGCACAAG | |

The study was carried out during the beginning of November 2017 until the end of febrewary2018 in AL-Qadisya provenance.

The present study include 60 human samples from aborted women ( placenta and fetus ) who attended to the Maternity and Childhood Teaching Hospital .The samples were collected randomly and using two clean Container containing and the then put in deep freezeunder for DNA extraction as a target for PCR amplification.

Tissue Genomic DNA from tissue samples were extracted by using DNA extraction kit (Tissue DNA protocol) Geneaid. USA and the extraction was done according to company instructions kit.

Nested Polymerase chain reaction (nPCR)

The nested PCR technique was performed for detection *Toxoplasma gondii* based small subunit ribosomal RNA gene from all tissue samples, The method was carried out according to method described by (Vitale *et al*., 2008)

**DNA sequence method**

DNA sequencing method was performed for study of genetic relationship between *Toxoplasma gondii* isolates from different

organs. The genetic analysis done by phylogenetic tree analysis between local *Toxoplasma gondii* isolates and NCBI-Blast submission *Toxoplasma gondii*. Then the identification *Toxoplasma gondii* isolates were submitted into of NCBI-GenBank. The PCR SAG3 genes positive products were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

The DNA sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis of the partial SAG3 gene based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

**Results and discussion:**

**Detection of *Toxoplasma gondii* using Molecular methods.**

The results of PCR amplification which was performed on the DNA extracted of ss RNAgene of *Toxopalasma gondii*, the 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 313 pb band product size .

The result of nPCR in present study was 60%

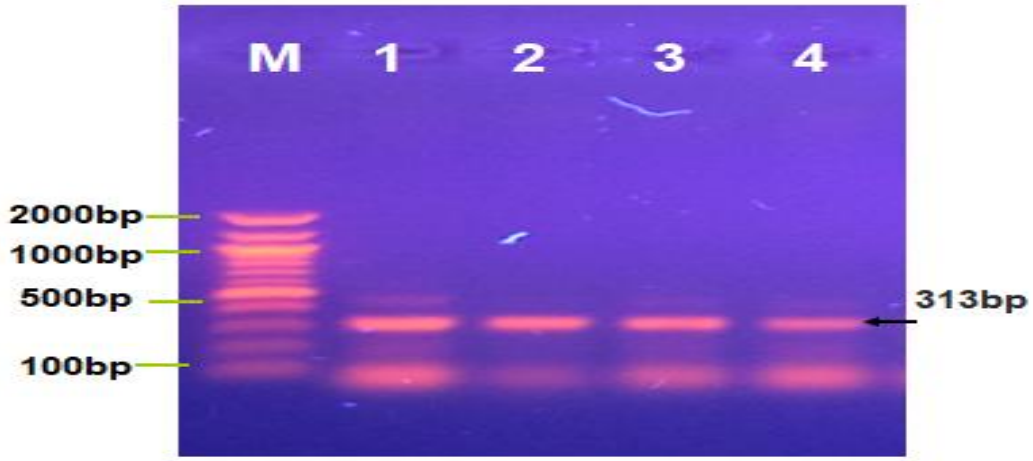
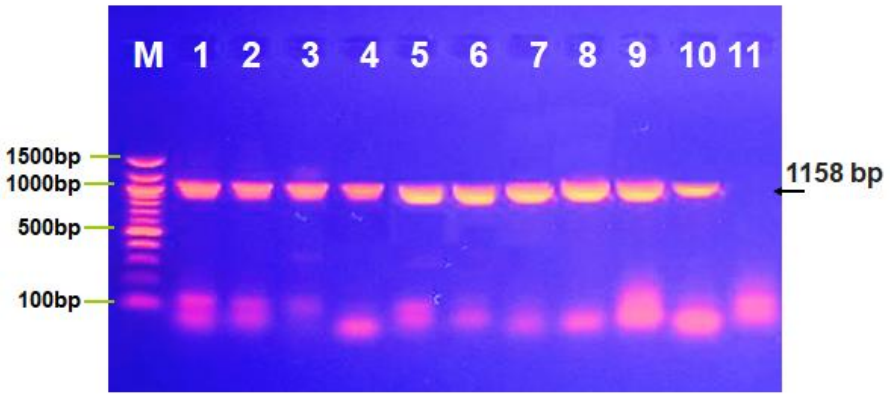



**Figure (1) Agarose gel electrophoresis image that show the NestedPCR product analysis of small subunit ribosomal RNA gene in *Toxoplasma gondii*. Where Marker ladder (2000-100bp), (1-4) some positive *Toxoplasma gondii* from placenta and fetus samples at 313bp PCR product size.**

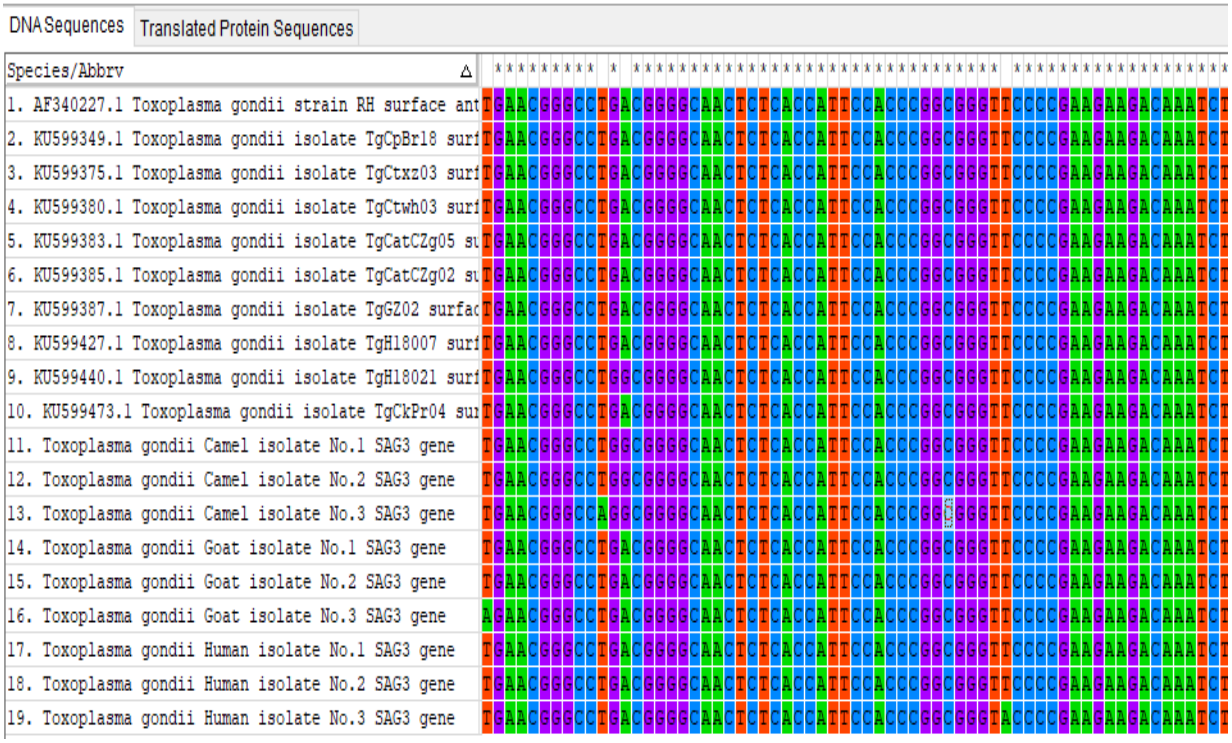
The present study found 60% in the human, while the result of Al- Kalaby, (2008) who recorded that 83.3% of tested samples from Iraq women was positive by PCR technique using *B1* gene, on the other hand the results of Okay *et al.*, (2009) who reported that 17.65%, the result of present study agreed with the results of Al-Addlan, (2007) who reported that 63.49% .

**SAG3 gene for DNA sequences:** All of samples which were selected positive in both nPCR and SAG3 gene positive result (100%).

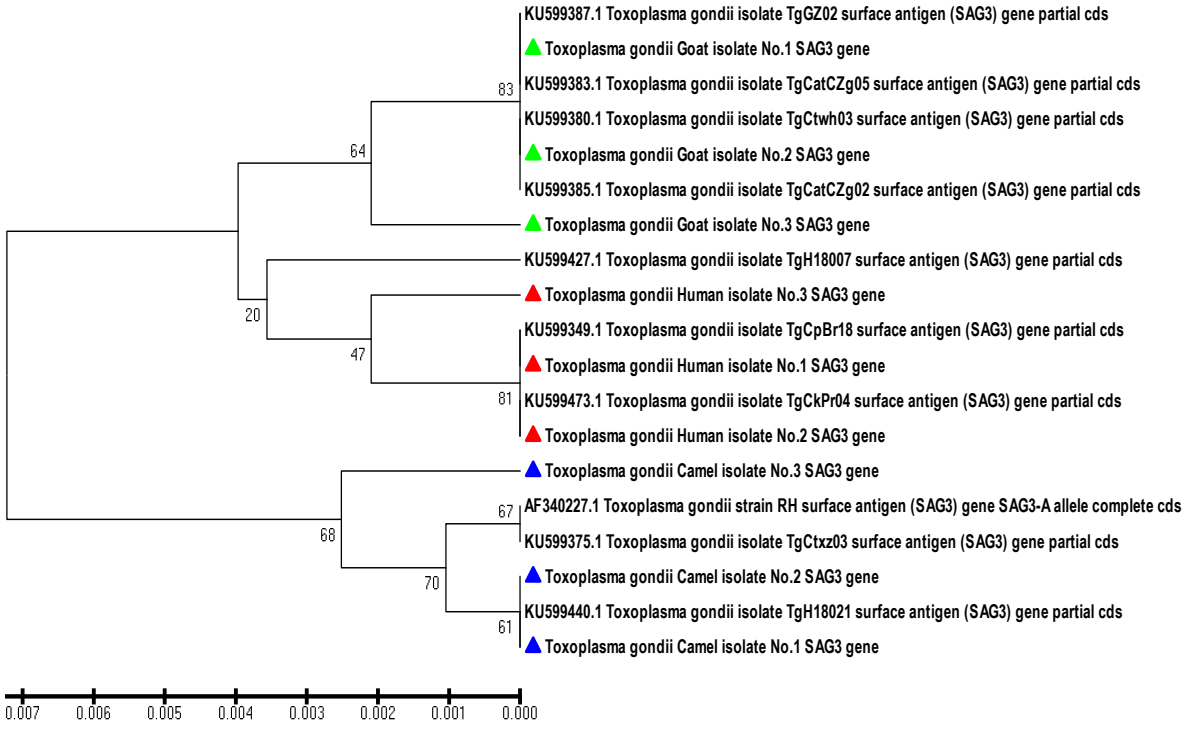



Detection of SAG3 gene in the present study was done for to select the samples sequences,

**Figure (2): Agarose gel electrophoresis image that show the PCR product analysis of SAG3gene in *Toxoplasma gondii*. Where Marker ladder (2000-100bp), (1-10)SAG3 positive *Toxoplasma gondii* from camel samples at 1158bp PCR product size.**




**Figure(3): Multiple sequence alignment analysis of surface antigen (SAG3) gene partial sequence in local *Toxoplasma gondii* isolates and different NCBI-Genbank *Toxoplasma gondii* based ClustalW alignment analysis by using (MEGA 6.0, multiple alignment analysis tool). The multiple alignment analysis similarity (*) and differences in surface antigen (SAG) gene nucleotide**




**Figure(4) Phylogenetic tree analysis based on the partial sequence of surface antigen (SAG3) gene in local *Toxoplasma gondii* isolates that used genetic relationship analysis. The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (MEGA 6.0 version)**

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