

# DNA Sequencing of *Toxoplasma gondii* in Slaughtered Animals (Cattle, Sheep and Goat) in Wasit Province - Iraq

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**Abstract:** DNA of three samples (S1) skeletal muscle of cattle, (S2) brain of sheep, (S3) skeletal muscle of goat was extracted by Genomic DNA purification kit and tested using polymerase chain reaction and specific set of primers with probe to detect B1 gene of *Toxoplasma gondii*, the results of phylogenetic tree refers to corresponding between DNA sequencing of sample one (cattle muscle) and third sample (goat muscle) with main branch of phylogenetic tree under sequence KC607824.1, whereas the DNA sequencing of sample two (sheep brain) correspond with main branch of phylogenetic tree under sequence DQ779196.1. The analysis of the phylogenetic tree refers to close correlation between DNA of samples of the same organs of different animals.

**Keywords:** DNA sequence Toxoplasma cattle sheep goat Real time PCR IRAQ

## 1. Introduction

Toxoplasmosis, zoonotic diseases that are one of the most important public health difficulties in many nations, the pathological agent was intracellular parasite with a global spread called *T. gondii* [1]. It is one of the most widespread apicomplexan parasites found in different animal types and human [2]. It is well-identified that the advance and severity of disease rely on the immunological status of the host, but new studies propose that the genetics of the parasite can as well play a role. Diagnosis established on clinical appearance and serology is not always easy. However, molecular methods do not based on an immune response, and let direct detection of the parasite in biological samples, therefore they can be used to found a diagnosis when serological tests are not final. Multicopy sequences specific for *Toxoplasma gondii*, e.g., the 529-bp or the B1 gene sequence, are especially useful in molecular tests [3]. Molecular epidemiology studies foremost depend on restriction fragment length polymorphism (RFLP) method shown that three main types are prevailing in North America and Europe, whereas other different genotypes are found in other parts of the world [4]. The occurrence of three clonal types (type I, II and III) and very small discrimination between clonal lineages which is why it was decided that *T. gondii* has a clonal population installing. Comparison sequence analysis of individual genes illustrates extremely low allelic variety within the clonal lines, and only 1% divergence at the DNA level. In addition, specific genetic variety between and within clonal lines show that they have quite newly evolved from a common ancestor, 10,000 years ago at the most [5]. In animals, most isolates of *T. gondii* were type II or type III irrespective of clinical status [6, 7]. Along with the phylogenetic study of *T. gondii*, there is advanced research aimed at accounts the possible link between the different genotypes and clinical forms of the disease; although of the results indicating absence of connection, or a much more complex one than some studies show, there are reported findings on population structure of *T. gondii* that are possible to have important clinical implications [8]. In spite of this significant new looking at, the clinical related of the infecting genotypes is an issue that

will remains to intrigue researchers in the coming years, Insight into the global population structure of *T. gondii* and its clinical implications, complex by the increase rate of human migrations among continents, will needs wide research attempt based on more regular protocols, and should include not only clinically apparent cases, but also individuals without symptoms infection [9].

The aim of this study is sequencing of DNA fragment of B1 gene of *T. gondii* to determine the genetic relationship between local isolates and others that world prevalence according to gene structure of parasite, and phylogenetic tree and comparative firstly according to the host species in which the parasite isolates (goat, sheep and cattle) and secondly host body location.

## 2. Materials and Methods

### Samples Collection

Three samples (100 g weight) were collected from randomly from cattle, sheep and goat include skeletal muscle of cattle (S1), brain of sheep (S2) and skeletal muscle of goat (S3) from different location of Wasit province - Iraq, tissue samples were grind using liquid nitrogen and grinder to prepare tissue powder.

### DNA purification

The DNA of samples was extracted by Genomic DNA purification kit (Promega, USA) according to the manufacturer's instructions. Extracted DNA samples were stored frozen at -20°C till used for molecular analysis.

### Conventional polymerase chain reaction (PCR)

Amplified DNA fragment of B1 gene of *T. gondii* was performed according to [10]. The two pairs of Primers, TOX4 (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and TOX5 (5'-CGCTGCAGACACAGTGCATCTGGATT-3') were used, and these flanked a 529bp fragment of *T. gondii* DNA. PCR reaction was performed in a mixture containing 2.5 µl of DNA template, 1.25 µl of each primer and 20 µl of mastermix containing 100mM dNTP (Invitrogen), 60mM

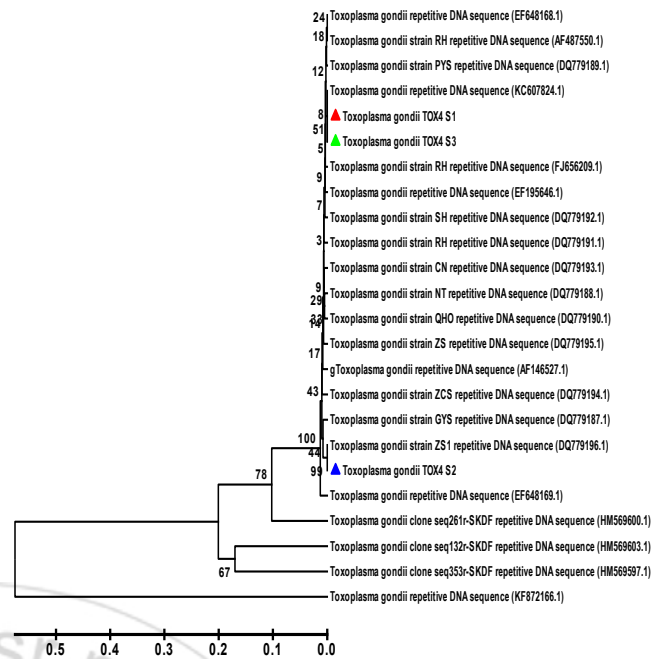
Tris±HCl (PH9.0), 15mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2mM MgCl<sub>2</sub>, 0.5U TaqDNA polymerase (Gibco-BRL) the final volume of reaction mixture was 25 µl. The PCR reaction was performed over 35 cycles in Eppendorf Master cycler Gradient, using the following cycling conditions: 7min at 94°C for denaturation in cycle one, followed by 33 cycles on 60s at 94°C for denaturation, 60s at 55°C for annealing and 60s at 72°C for extension, cycle 35 was followed by a final extension of 10min at 72°C. Aliquot of each PCR product was electrophoresed on 1% agarose gel and ethidium bromide staining.

PCR product of repetitive DNA sequence of *Toxoplasma gondii* was purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). After that, the purified PCR products samples were sent to Macrogen Company in Korea for performed the DNA sequencing by AB DNA sequencing system.

### 3. Results and Discussion

The current study of DNA sequencing of *Toxoplasma gondii* is the first in Iraq and elucidate the genetic closeness among themselves and between world strains depending on the genetic tree where it was identified DNA sequencing of the parasite *Toxoplasma gondii* and knowledge of the relationship between the samples taken from various host ;the results analysis of the sequences has shown that most of the studied samples were identical with what is registered in National Center for Biotechnology Information( NCBI) at a rate of 100%.

The DNA's nucleotides sequences of three samples that obtained in this study with Gene Bank accession numbers (KX963353), (KX963354), (KX963355) were compared with the sequences of the same parasite recorded in NCBI, As well as the use of genetic analysis of the phylogenetic tree Unweighted Pair Group Method with Arithmetic Mean (UPGMA Tree test) and the program of Molecular Evolutionary Genetics Analysis( MEGA 6),the tree was drawn according to the samples that have been detected in this study and analysis the results showed an identical between our samples with others world registered (figure 1).Since the first sample S1 and third S3 were close related to NCBI-BLAST *Toxoplasma gondii* global strain (KC607824.1) at 100% identity, this result was identical with[11]where are the samples of spinal cord of dogs in the United States recorded a correspond with (KC607824.1) at 100% identity. While the second sample S2 was closely related to NCBI-BLAST (DQ779196.1) at 100% identity, this result was identical with[12]in whichcorrespond5 positive samples of goat milk with (DQ779196.1) at 99.9% identity by polymerase chain reaction in Northeastern Brazil. The same researcher also got the same result butthe samples taken from the ovaries of sheep in Brazil [13],that closely related to NCBI-BLAST (DQ779196.1) at 99.9% identity using the same technique. In Scotland there are6 Samples of water also closely related to NCBI-BLAST (DQ779196.1) at 99-100% identity [14].On the other hand the results of 12 brain samples of wild birds (Magpies) closely related to NCBI-BLAST (DQ779196.1) at 95% identity by polymerase chain reaction [15].



**Figure1:** Phylogenetic tree analysis based on repetitive DNA sequence partial sequence that used for confirmative identification of Local *Toxoplasma gondii* isolates.

The phylogenetic tree was constructed using (UPGMA tree) in (MEGA 6.0 version), the local *Toxoplasma gondii* TOX4-S1 and TOX4-S3 were close related to NCBI-BLAST *Toxoplasma gondii* global strain (KC607824.1). Whereas, The Local *Toxoplasma gondii* TOX4-S2 was close related to NCBI-BLAST *Toxoplasma gondii* global strain (DQ779196.1).

Toxoplasma gondii microsatellite sequence; and hypothetical protein gene, complete cds  
 Sequence ID: [gb|KC607824.1](http://gb|KC607824.1) Length: 532 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
972 bits(526)	0.0	526/526(100%)	0/526(0%)	Plus/Plus
Query 1	TGCAGGGGAGGAAAGACGAAAAGTTGTTTTTAAATTTTTTCTTTTCTTCTGATTTT	68		
Sbjct 4	TGCAGGGGAGGAAAGACGAAAAGTTGTTTTTAAATTTTTTCTTTTCTTCTGATTTT	63		
Query 61	tgttttttttGACTCCGGCCAGCTGCCTCTGTGGGATGAGACGCCGGAGCCGAAGTGC	120		
Sbjct 64	TGTTTTTTTGTACTCCGGCCAGCTGCCTCTGTGGGATGAGACGCCGGAGCCGAAGTGC	123		
Query 121	GttttcttttttgatTTTTTTGTTTTTTCACAGGGGAGCTCGCTGTGCTTGGAGCC	180		
Sbjct 124	GTTTTCTTTTTTGATTTTTTTGTTTTTTCACAGGGGAGCTCGCTGTGCTTGGAGCC	183		
Query 181	ACAGAAAGGACAGAAAGTCAAGGGGACTACAGACGCAGTCCCTCCTCAGCGCTCTTG	240		
Sbjct 184	ACAGAAAGGACAGAAAGTCAAGGGGACTACAGACGCAGTCCCTCCTCAGCGCTCTTG	243		
Query 241	GAGGAGAGATATCAGGACTGTAGATGAAGGGGAGGGGTGAGGATGAGGGGGTGGCTGGT	300		
Sbjct 244	GAGGAGAGATATCAGGACTGTAGATGAAGGGGAGGGGTGAGGATGAGGGGGTGGCTGGT	303		
Query 301	GGGAAGCGACGAGAGTCCGGAGGGGGAAGATGTTTCCGGCTTGGCTGTTTTCTGGAG	360		
Sbjct 304	GGGAAGCGACGAGAGTCCGGAGGGGGAAGATGTTTCCGGCTTGGCTGTTTTCTGGAG	363		
Query 361	GGTGGAAAAGAGACCCGGAATGCGATCTAGACGAGACGACGCTTCTCTGTTGGTATGG	420		
Sbjct 364	GGTGGAAAAGAGACCCGGAATGCGATCTAGACGAGACGACGCTTCTCTGTTGGTATGG	423		
Query 421	CGGAGAGAATTGAAGAGTGGGAGAGGGGATCCAGATGCACCTGTGTGCA 526	480		
Sbjct 424	CGGAGAGAATTGAAGAGTGGGAGAGGGGATCCAGATGCACCTGTGTGCA 483			
Query 481	GGGAGGAGGCGGTAGGAGGGAATCCAGATGCACCTGTGTGCA 529			
Sbjct 484	GGGAGGAGGCGGTAGGAGGGAATCCAGATGCACCTGTGTGCA 529			

**Figure 2:** Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S1 with NCBI-BLAST *Toxoplasma gondii* (KC607824.1) at 100% identity



Toxoplasma gondii microsatellite sequence; and hypothetical protein gene, complete cds  
Sequence ID: [gb|KC607824.1](#) Length: 532 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
972 bits(526)	0.0	526/526(100%)	0/526(0%)	Plus/Plus

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Range 1: 4 to 529 GenBank Graphics
Query 1  TGCAGGGAGGAAAGCAAAAGTTGTTTTTAAATTTTTTCTTTTGTGTTCTGATTT 60
Sbjct 4  TGCAGGGAGGAAAGCAAAAGTTGTTTTTAAATTTTTTCTTTTGTGTTCTGATTT 63
Query 61  TGTTTTTTTTGAATCCGCCCCAGCTGCTCTGTGCGGATGAGACCCGCGAGCCGAAGTGC 120
Sbjct 64  TGTTTTTTTTGAATCCGCCCCAGCTGCTCTGTGCGGATGAGACCCGCGAGCCGAAGTGC 123
Query 121  GTTTTTTTTTGATTTTTTTTTGTTTTTACACAGCCGAGCTGCTGCTTGGAGCC 180
Sbjct 124  GTTTTCTTTTTTGAATTTTTTTGTTTTTACACAGCCGAGCTGCTGCTTGGAGCC 183
Query 181  ACAGAAGGGACAGAAAGTGAAGGGGACTACAGACGCGATGCCGCTCTCCAGCCGCTTGG 240
Sbjct 184  ACAGAAGGGACAGAAAGTGAAGGGGACTACAGACGCGATGCCGCTCTCCAGCCGCTTGG 243
Query 241  GAGGAGAGATATCAGGACTGTAGATGAAGGCCGAGGGTGAAGATGAGGGGGTGGCTGGTT 300
Sbjct 244  GAGGAGAGATATCAGGACTGTAGATGAAGGCCGAGGGTGAAGATGAGGGGGTGGCTGGTT 303
Query 301  GGGAAAGCGACGAGAGTCCGAGAGGGGAAGATGTTCCCGCTTGGCTGCTTTCTCGAG 360
Sbjct 304  GGGAAAGCGACGAGAGTCCGAGAGGGGAAGATGTTCCCGCTTGGCTGCTTTCTCGAG 363
Query 361  GGTGAAAAGAGACACCGAATGCGATCTAGACGAGACGACCTTCTCGTGGTATGG 420
Sbjct 364  GGTGAAAAGAGACACCGAATGCGATCTAGACGAGACGACCTTCTCGTGGTATGG 423
Query 421  CGGAGAGAAATTAAGAGTGGGAGAGGGGCGAGGGAGACAGAGTCCGAGGCTTGGAGAA 480
Sbjct 424  CGGAGAGAAATTAAGAGTGGGAGAGGGGCGAGGGAGACAGAGTCCGAGGCTTGGAGAA 483
Query 481  GGGAGAGGAGGCGTGGAGAGGAATCCAGATGCACCTGTGTCTGCA 526
Sbjct 484  GGGAGAGGAGGCGTGGAGAGGAATCCAGATGCACCTGTGTCTGCA 529
    
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Figure 3: Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S2 with NCBI-BLAST *Toxoplasma gondii* (DQ779196.1) at 100% identity

Toxoplasma gondii microsatellite sequence; and hypothetical protein gene, complete cds  
Sequence ID: [gb|KC607824.1](#) Length: 532 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
972 bits(526)	0.0	526/526(100%)	0/526(0%)	Plus/Plus

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Range 1: 4 to 529 GenBank Graphics
Query 1  TGCAGGGAGGAAAGCAAAAGTTGTTTTTAAATTTTTTCTTTTGTGTTCTGATTT 60
Sbjct 4  TGCAGGGAGGAAAGCAAAAGTTGTTTTTAAATTTTTTCTTTTGTGTTCTGATTT 63
Query 61  TGTTTTTTTTGAATCCGCCCCAGCTGCTCTGTGCGGATGAGACCCGCGAGCCGAAGTGC 120
Sbjct 64  TGTTTTTTTTGAATCCGCCCCAGCTGCTCTGTGCGGATGAGACCCGCGAGCCGAAGTGC 123
Query 121  GTTTTTTTTTGATTTTTTTTTGTTTTTACACAGCCGAGCTGCTGCTTGGAGCC 180
Sbjct 124  GTTTTCTTTTTTGAATTTTTTTGTTTTTACACAGCCGAGCTGCTGCTTGGAGCC 183
Query 181  ACAGAAGGGACAGAAAGTGAAGGGGACTACAGACGCGATGCCGCTCTCCAGCCGCTTGG 240
Sbjct 184  ACAGAAGGGACAGAAAGTGAAGGGGACTACAGACGCGATGCCGCTCTCCAGCCGCTTGG 243
Query 241  GAGGAGAGATATCAGGACTGTAGATGAAGGCCGAGGGTGAAGATGAGGGGGTGGCTGGTT 300
Sbjct 244  GAGGAGAGATATCAGGACTGTAGATGAAGGCCGAGGGTGAAGATGAGGGGGTGGCTGGTT 303
Query 301  GGGAAAGCGACGAGAGTCCGAGAGGGGAAGATGTTCCCGCTTGGCTGCTTTCTCGAG 360
Sbjct 304  GGGAAAGCGACGAGAGTCCGAGAGGGGAAGATGTTCCCGCTTGGCTGCTTTCTCGAG 363
Query 361  GGTGAAAAGAGACACCGAATGCGATCTAGACGAGACGACCTTCTCGTGGTATGG 420
Sbjct 364  GGTGAAAAGAGACACCGAATGCGATCTAGACGAGACGACCTTCTCGTGGTATGG 423
Query 421  CGGAGAGAAATTAAGAGTGGGAGAGGGGCGAGGGAGACAGAGTCCGAGGCTTGGAGAA 480
Sbjct 424  CGGAGAGAAATTAAGAGTGGGAGAGGGGCGAGGGAGACAGAGTCCGAGGCTTGGAGAA 483
Query 481  GGGAGAGGAGGCGTGGAGAGGAATCCAGATGCACCTGTGTCTGCA 526
Sbjct 484  GGGAGAGGAGGCGTGGAGAGGAATCCAGATGCACCTGTGTCTGCA 529
    
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Figure 4: Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S3 with NCBI-BLAST *Toxoplasma gondii* (KC607824.1) at 100% identity

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