International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

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

Altamemy[1], Abdulkareem Aakool[2], Suadadbresam Khiry[3]

[1]Wasit University, College of Science, Department of Biology, Iraq

Abstract: ***DNA of three samples (S1) skeletalmuscle of cattle, (S2)brain of sheep, (S3)skeletal muscle of goat was extracted by Genomic DNA purification kit and tested using polymer as echain reaction and specific set of primers with probe to detect B1 gene of Toxoplasmagondii, 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 Toxo plasma cattle sheep goat Real time PCRIRAQ

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 onmore 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 hostspecies 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)

Amplificated 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 2.5µl of DNA temple, 1.25µl of each primer and20µl of mastermix containing 100mM dNTP (Invitrogen), 60mM

Tris±HCl (PH9.0), 15mM $(NH_4)_2SO_4$, 2mM $MgCl_2$, 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 94ºC for denaturation in cycle one, followed by 33 cycles on 60s at 94ºC for denaturation, 60s at 55ºC for annealing and 60s at 72ºC for extension, cycle 35 was followed by a final extension of 10min at 72ºC. 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 but the 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].

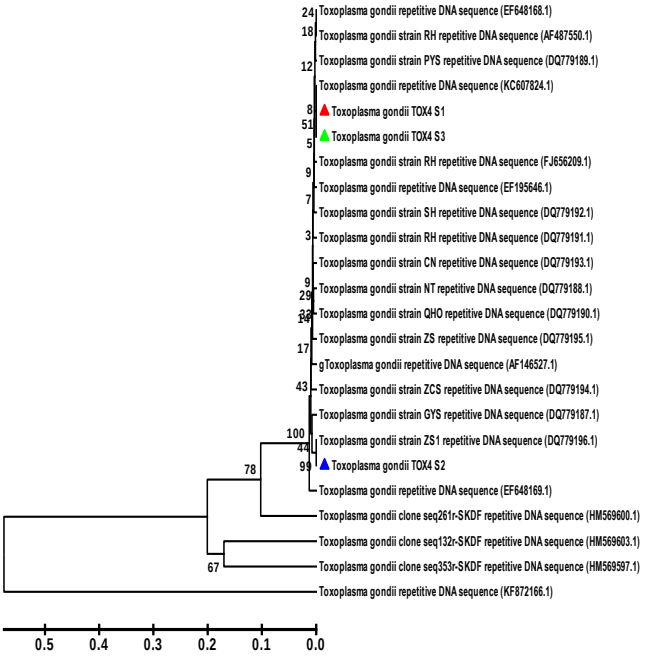

Figure1: Phylogenetic tree analysis based on repetitive DNA sequence partial sequence that used for confirmative identification of Local *Toxoplasma gondii* isolates.

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 gond*i global strain (DQ779196.1).

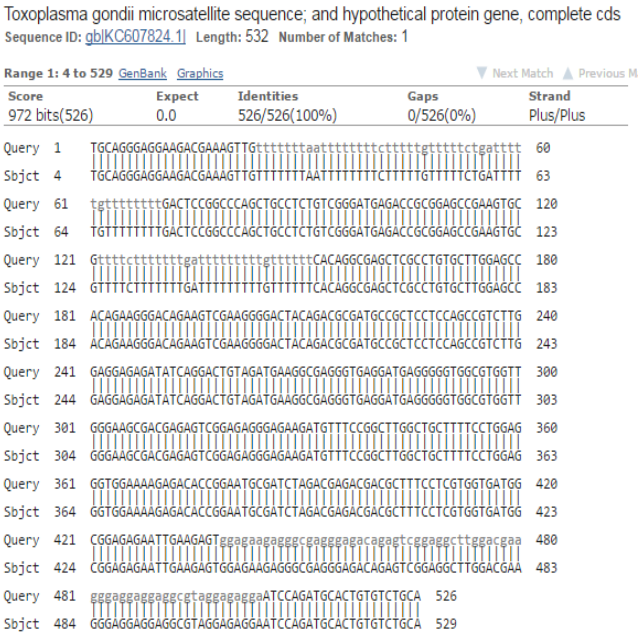

Figure 2: Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S1 with NCBI-BLAST *Toxoplasma gondii* (KC607824.1) at 100% identity

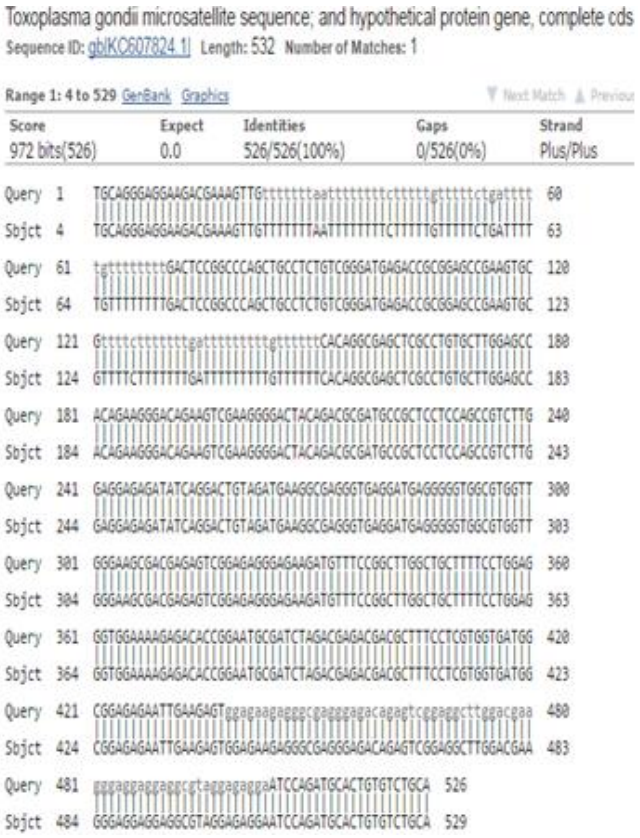

Figure 3: Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S2 with NCBI-BLAST *Toxoplasma gondii* (DQ779196.1) at 100% identity

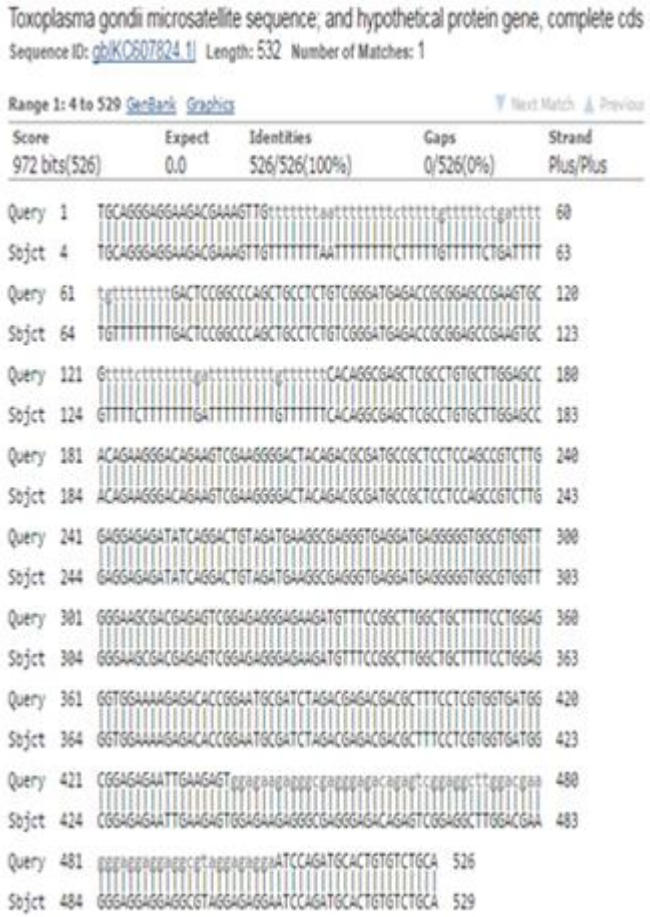

Figure 4: Basic local sequence alignment analysis of *Toxoplasma gondii* TOX4 S3 with NCBI-BLAST *Toxoplasma gondii* (KC607824.1) at 100% identity

References

[1] **Dubey, J.P.(2004) :** Toxoplasmosis - a waterborne zoonosis. Vet Parasitol, 126: 57-72.

[2] **Dubey, J.P.(2010):** Toxoplasmosis of Animals and Humans. 2 nd edition, CRC Press; Boca Raton, FL, USA

[3] Switaj, K.; Master, A.; Skrzypczak, M. and Zaborowski, P.(2005): Recent trends in molecular diagnostics for *Toxoplasma gondii* infections, Jour. ClinMicrobiol Infect; 11:170-176.

[4] **Li, M. ; Xu, W.M. ; Lin, W.; He, C.; Qing, L.L.; Hui, Q.W.; Ai, M.Z.; Jian, D., et al., (2014) :** Phylogeny and virulence divergence analyses of *Toxoplasma gondii* isolates from China, Jour. Parasites and Vectors 7:133.

[5] **Su, C.; Evans, D.; Cole, R.H.; Kissinger, J.C.; Ajioka, J.W. and Sibley, L.D. (2003):** Recent expansion of Toxoplasma through enhanced oral transmission. *Science* 299, 5605: 414-416.

[6] **Dubey, J.P. ; Navarro, I.T.; Graham, D.H.; Dahl, E.; Freire, R.L; Prudencio, L.; Sreekumar, C.; Vianna, M.C. and Lehmann. T. (2003):** Characterization of *Toxoplasma gondii* isolates from free range-chickens from Parana´, Brazil. Veterinary Parasitology 117: 229-234.

[7] **Dubey, J.P.; Parnell, G.; Sreekumar, C.; Vianna, M.C.B.; Deyoung, R.W.; Dahl, E. and Lehmann, T. (2004):** Biologic and molecular characteristics of Toxoplasma gondii isolates from striped skunk (*Mephitis mephitis*), Canada goose *(Brantacanadensis*), blacked wingedlory *(Eos cyanogenia*), and cats (*Feliscatus)*. Journal of Parasitology 90: 1171–1174.

[8] **Darde, M.; Ajzenberg, D. andSmith, J. (2007):** Population Structure and Epidemiology of Toxoplasma gondii. In: Weiss, L.M., Kim, K. (Eds.) Toxoplasma gondii The Model Apicomplexan: Perspectives and Methods. Elsevier, pp. 49-76.

[9] **Lvovic, V.; Marija, V.; Tijana, Z.; Lvana, K. and Olgica, D.(2012) :** Molecular detection and genotyping of *Toxoplasma gondii* from clinical samples, project No : III 41019 From ministry of Education and Science of Serbia, Chapter 5.

[10] **Homan, W.L.; Vercammen, M.; De Braekeleer, J.(2000):** Identification of a 200 to 300 fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int. Jour. Parasitol., v.30, p:69-75.

[11] **Gerhold, R.; Shelley, J.N.; Caroline, M.G.; Amanda, C. ; Amy, H. and Chunlei, S. (2014) :** Acute onset of encephalomyelitis with atypical lesions associated with dual infection of *Sarcocystisneurona and Toxoplasma gondii* in a dog, Jou. Veterinary Parasitology, 205: 697-701.

[12] **Bezerra, M.J.G.; Kim, P.C.P. ; Moraes, E.P.B.; Sa, S.G.; Albuquerque, P.P.F. ; Silva, J.G.; Alves, B.H.L. and Mota, R.A. (2013) :** Detection of *Toxoplasma gondii* in the milk of naturally infected goats in the northeast of Brazil, Jou. Transboundary and Emerging Disease, 62(4) : 421-424.

[13] **Bezerra, M.J.G.; Jefferson, A.L.O.; Eugenio, S.K.; Jose, G.S.; Andre S.S. ; Erica, P.B.X. et al., (2014) :** Occurrence of *Toxoplasma gondii* DNA in sheep naturally infected and slaughtered in abattoirs in Pernambuco Brazil, Pesq. Vet.Bras. 34(4) : 329-331.

[14] **Wells, B.; Hannah, S.; Giles, I.; Stefano, G.; Emily, H.; Maria, P., et al., (2015) :** Molecular detection of *Toxoplasma gondii* in water samples from Scotland and comparison between the 529bp real time PCR and ITS1 nested PCR, Jou. Water Research, 87: 175-181.

[15] **Darwich, L.; Cabezon, O.; Echeverria, I.; Pabon, M.; Marco, I.; Molin, r., et al., (2012) :** Presence of *Toxoplasma gondii* and *Neosporacaninum* DNA in the brain of wild birds, Journal of Veterinary Parasitology, 183:3