REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 4 (2022)
http://www.veterinaria.org
Article Received: 12 August 2022; Revised: 30 October 2022; Accepted: 21 November 2022

Molecular Characterization of *Cysticercus tenicollis* from Slaughtered Sheep and Goats in Basrah Province, Iraq

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Abstract
Taenia hydatigenia refers as cestoda in carnivorous species, whereas *Cysticercus tenicollis* is a common larval stage found in herbivorous animals, ruminants and swine. An investigation into molecular identification of *C. tenicollis* from slaughtered sheep and goats was carried out by creating Cysticercus species-specific and Cysticercus specific primers in Basrah governorate, southern Iraq. Study the molecular characterization of *C. tenicollis* and *C. ovis* from sheep and goats, by designing primer to *Cysticercus* spp. and specific primers to detect *C. tenicollis* and *C. ovis.* a total of 23 sheep (7 males, 16 females) and 23 goats (19 males, 4 females) were inspected. This investigation took place in Basrah province from November 2020 to November 2021, and the animal isolates were gathered throughout that time. The primers were developed as universal for the identification of *Cysticercus* species; they can amplify a portion of (ND1) and (COX1) genes of *C. tenicollis* and *C. ovis*, respectively. In general, Each cyst was identified as belonging to the genus *T. hydatigenia* by its unique mt-CO1 sequence, which yielded 267 bp product. This strain of *T. hydatigenia* is 100% identical to the Iraqi strain first isolated from Southern Iraq and entered into the Gene Bank under the designation "Iraqi strain," as shown by multiple sequence alignment of the nucleotide with previously published references (OK3556791, OK356792, OK356793, and OK356794).

Keywords: Sheep and goats, *Cysticercus tenuicollis*, *Taenia hydatigenia*, Gene Bank.

Introduction

A global frequent parasite, *Taenia hydatigenia*, is detected in carnivorous animals as the ultimate host, but *Cysticercus tenicollis*, the larval stage, is commonly found in herbivorous animals, such as ruminants and pigs (1).The percentage of *T. hydatigenia* infection in stray dogs varied with time, location, and monthly variation, and the proportion of *T. hydatigenia* infection in stray dogs in Basrah city, for example, was determined to be 54.2% by (2), 7.62% by(3), and 83.8% by (4). And Due to (5), after reaching a diameter of 6–8 cm, larvae are often found on internal visceral surfaces such as the liver, omentum, mesenterium or abdominal serous surfaces and become infectious(6) successfully transmitted *T. hydatigenia* ova to laboratory mice, where the larvae grew and developed normally. Furthermore, clinical symptoms in *C. tenicollis*-infected intermediate hosts vary with infection intensity, and mature cysticercus larvae are less damaging in the peritoneal space than in the liver (7).When a high number of larvae migrate into the liver during a severe infection, hepatitis cysticercosa may occur (8).Economic losses due to lamb fatalities and liver degeneration are also a result of these migrations, which produce hemorrhagic-fibrotic lesions in the liver (9).(10) observed histopathological changes in mice after feeding them the gravid segment of *T. hydatigenia*. Infiltration of inflammatory cells, simple vacuolation of hepatocytes with limited diffuse

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vacuolation, barnachymal focus of mononuclear cells, conspicuous white palp lymphoid tissues coupled with lympho folical development in the spleen, while, serological tests and screening are used to identify the illness, but they aren't conclusive. Cysts after butchering establish ultimate diagnosis. According to (11), taeniasis Proper anthelmintics for final hosts can limit parasite transmission, but the natural cycle and stray dogs make this challenging. Phylogenetic studies of Taeniid cestodes, such as *T. hydatigenia*, and other related species have been conducted. However, few research have examined variation within and among individuals of the same species. Understanding this economically significant parasite's genetic diversity within the same species is vital for epidemiological research and management programmers.

This study examined *C. tenicollis* from sheep and goats killed in southern Iraq's Basrah governorate. Prior to this study, there had been no molecular studies conducted on this parasite in southern Iraq, and in order to accomplish this goal, primers unique to *C. tenicollis* as well as general primers for *Cysticercus* spp. were created.

Materials And Methods

Collection of Samples

In the Basrah province slaughterhouse, a total of 23 sheep (7 males, 16 females) and a total of 23 goats (19 males, 4 females) were inspected. This investigation took place in Basrah province from November 2020 to November 2021, and the animal isolates were gathered throughout that time. The infected organs from the slain animals were sent to the laboratory where they underwent a thorough examination. When cysts were found, a sample was taken and stored in clean containers containing 70% ethyl alcohol for further study.

Preserving Samples

Isolated cysts were washed multiple times with distal water, and to remove scolex and fluids, the liquid was placed in a tube using a sterile medical blade, cut open the cyst and deposited it in an alcohol tube with 70% concentration for PCR analysis (12).

Extracting DNA

The Wizard ® Genomic DNA Purification Kit was employed to extract DNA from the scolex and liquid, in accordance with the manufacturer's instructions (Promega, USA). A temperature of -20°C was used to keep the DNA.

PCR amplification of gene fragment:

The amplification was done with primers. Table 1 shows the *Cysticercus* spp. of the following animals. *C. tenuicollis* and *C. ovis* were identified using three primers. *Cysticercus* spp. DNA was amplified using PCR (Promega GoTaq Hot Start Green Master Mix).The species *C. tenuicollis* and *C. ovis* were identified using three sets of primers. Initial denaturation at 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 58°C for 30

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seconds, and 72°C for 1 minute. After 5 minutes at 72°C, the reaction was cooled to 4°C.On ethidium bromide-stained agaros gel. Table 2 shows the amount of PCR cycled.

DNA sequencing and analysis of sequences

The PCR production was delivered to Macrogen, a Korean business, to be sequenced. Then, the Parbi-Doua and NCBIBLAST programmers were used to fix the sequences and put them in the right order. Gene Bank's ExPASY programme, which is available online at the NCBI, may be compared to your results. The NCBI software was used for phylogenetic analysis.

Table 1.*Cysticercus* spp. PCR detection primers

Species	Gene	Primer name	5'-3'	PCR product	Ta (ºC)	Ref
Cysticercus spp.	**12S rRNA**	CYS-F	CGATTCTTTTTAGGGGAAGGTGTG	267 bp	58	1
		CYS-R	GCGGTGTGTACATGAGCTAAAC			
C. tenuicollis	NADH dehydrogenase subunit 1 (**ND1**)	CYTE-F	AGATTCGTAAGGGGCCTAATA	471 bp	52	2
		CYTE-R	ACCACTAACTAATTCACTTTC			
C. ovis	Cytochrome c oxidase subunit 1 (**COX1**)	CYOV-F	ATGAATATTAAAACTTTATTAAGTTGGA	1620 bp	50	3
		CYOV-R	TTAAACTAAAAAACCACGGGCA			

Table 2 . Traditional PCR conditions

Phase	Ta (ºC)	Time	Cycles
Initial denaturation	94ºC	5 min	1X
Denaturation	94ºC	30 sec.	35X
Annealing	58ºC	30 sec.	
Extension	72ºC	1 min	

Results

To prepare for PCR, a Wizard® DNA Purification Kit was used to purify the DNA of cysts obtained from infected animal samples. A total of 44 samples from sheep and goats were examined in this study (See Figures 1,2).

Figures 1,2.*Cysticercus* spp.

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This study's primers are capable of amplifying (ND1) from *C. tenicollis* and (COX1) from *C. ovis*. Each cyst was *T. hydatigenia*, and the mt-CO1 PCR included 267 base pairs. (See Figures 3,4).

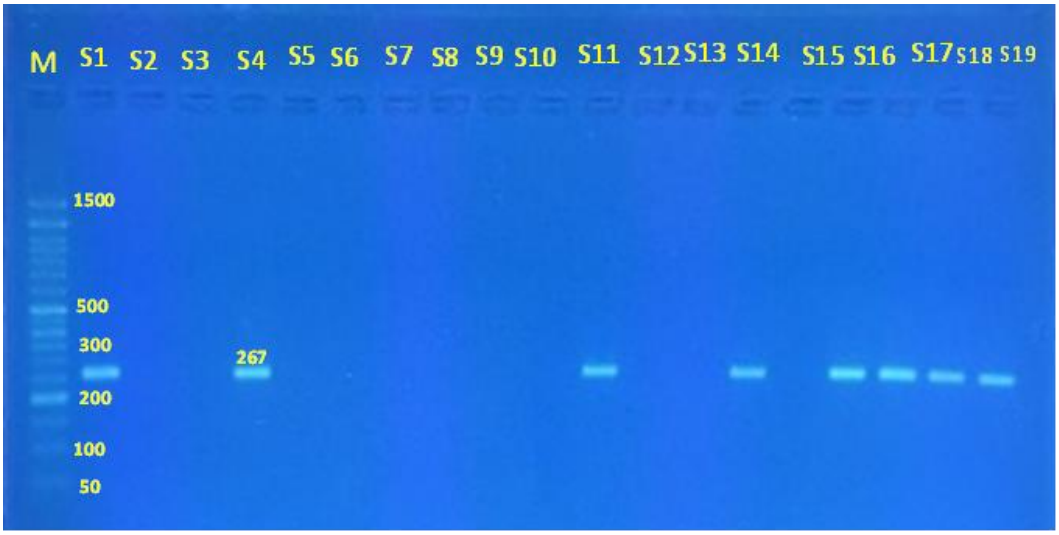

Figure 3.TraditionalPCR for *Cysticercus* spp. 12S rRNA gene

There were 19 samples taken from sheep that were examined. Each band that is generated by PCR has a size of 267 bp. The red dye used for the DNA is completely safe, and the concentration of the gel is 1.5%. (Intron, Korea). V represents 90, Time is 40 minutes and M stands for DNA ladder.

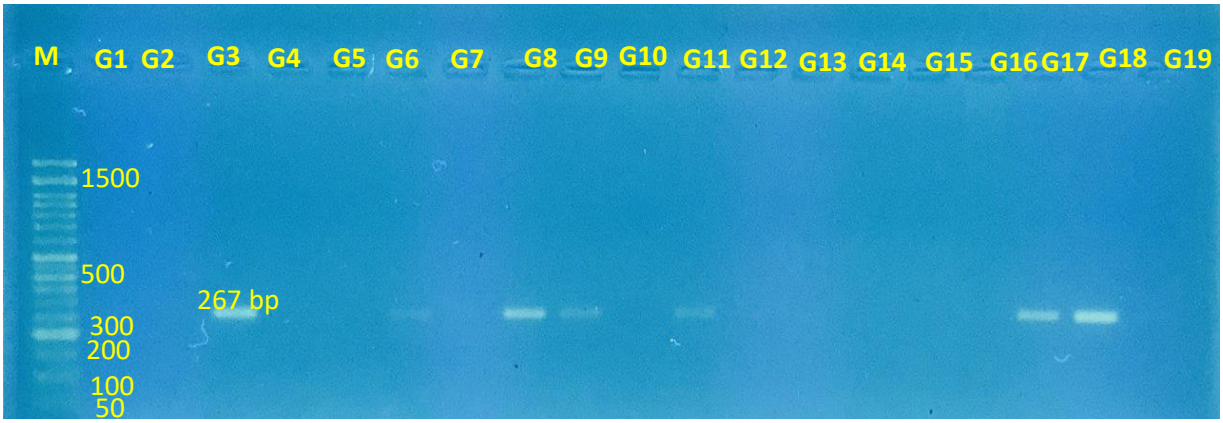

Figure 4.Traditional PCR for *Cysticercus* spp. 12S rRNA gene

Tests were done on 19 samples from goats. The width of the PCR bands is 267 bp. The gel's concentration was 1.5%, and the DNA dye's name is Red Safe (Intron, Korea). V is 90, Time is 40 minutes and M stands for DNA ladder.

Sequencing

The (mt-CO1) gene's partial nucleotide sequence was submitted to the Gene Bank and assigned the accession number (SUB10462553); the outcome was *T. hydatigenia*. The

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outcome of multiple sequence alignment of the nucleotide with published references for *T. hydatigenia* (OK3556791, OK356792, OK356793, and OK356794) demonstrates that our discovery is 100% similar with *T. hydatigenia* strain in Iraq (Table 3).

Recorded in GenBank

In this work, recorded several genes in the Gene Bank, and Table 3 shows the accession results. Because it is a larval stage, the Gene Bank declined to record it as *C. tenicollis* and instead documented it as *T. hydatigenia***.**

Table 3. Registration of sequences in the NCBI database

	Strain	Code by student	Gene (partial)	Accession number
1	HSS01	G3	*12S rRNA*	OK356791.1
2	HSS02	G8	*12S rRNA*	OK356792.1
3	HSS03	G9	*12S rRNA*	OK356793.1
4	HSS04	G18	*12S rRNA*	OK356794.1

Analysis of phylogeny

The NCBI software was used to create the phylogenetic tree (See Figures 5, 6).

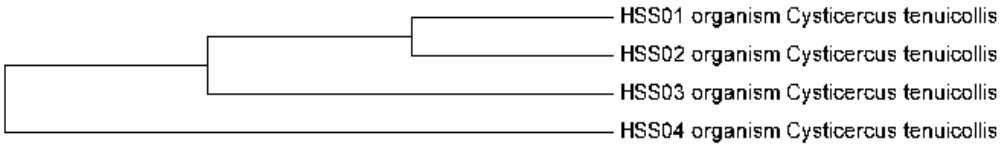

Figure 5.Partial sequencing of the gene 12S rRNA in four goat-isolated local strains of *Cysticercus tenuicollis* reveal evolutionary links.

According to (13), utilizing the Neighbor-Joining approach allowed for the inference of the evolutionary history of the species. The 500-replicate bootstrap consensus tree shows how the species being studied have changed over time (14).The collapse of branches in the tree that corresponded to partitions that were replicated in fewer than 50% of bootstrap replicates was performed. The Jukes-Cantor technique (15) was utilised in the computation of the evolutionary distances, and the results are presented in base substitutions per site units. Four different nucleotide sequences were looked at, and there were places for the 1st+2nd+3rd+Noncoding codons. Gaps and missing data were filled in everywhere. The final dataset had 169 locations. In order to conduct an evolution study, MEGA7 was utilised (16).

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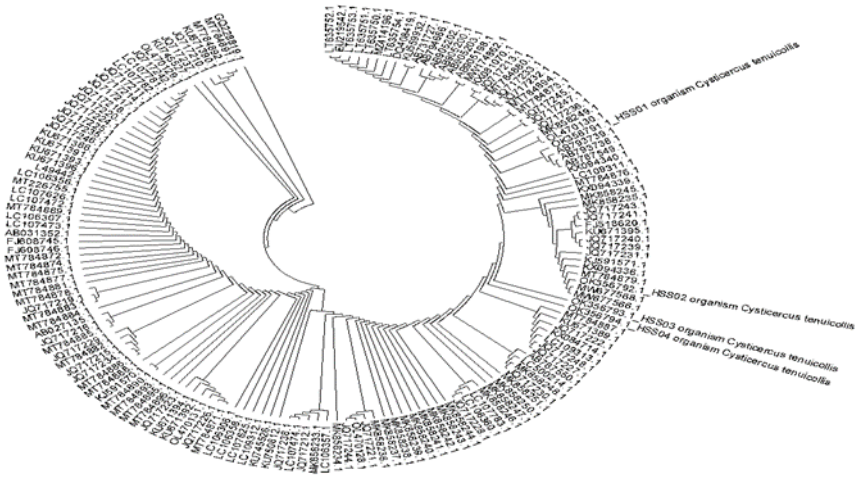

Figure 6.Partial sequences of the gene 12S rRNA from four local strains of *Cysticercus tenuicollis* found in goats and those from the NCBI database

The Neighbor-Joining method was utilized to figure out the history of evolution (13). The 500 iterations of the bootstrap consensus tree show how the taxa studied have changed over time(14).It was decided to remove all branches corresponding to partitions that were not replicated more than 50% of the time in bootstrap tests. The Jukes-Cantor technique was used to calculate the evolutionary distances in base substitutions per site unit(15). A total of 159 nucleotide sequences were examined. T. hydatigenia obtained from sheep (Accession number: GQ228819.1) was used as the ancestral (root) strain in the study, which included 159 nucleotide sequences. The positions of the 1st+2nd+3rd+Noncoding codons were provided. Gaps and missing data were deleted from every spot. There are 128 places in total in the final dataset. The evolutionary study was carried out using MEGA7 (16).

Disussion

As with (17), *C. tenicollis* is a parasite cause a disease that affects herbivores and is resulted from the larval stage of *T. hydatigenia*. It has a global distribution and is important in terms of veterinary medicine, medicine, and economics. Diseased dog faeces pollute water, soil, and plants, spreading sickness. The current study found a low prevalence of *C. tenicollis* among slaughtered sheep and goats (2.6%), compared to prior studies in Iraq; (4) in Basrah detected 40.55 and 26.25 percent infection rates among sheep and goats, respectively. Furthermore,(18) discovered (14.22%) sheep and (16%) goats among slaughtered animals in Baghdad. Cyst identification with molecular-based techniques is not a replacement for visual inspection, but the combination is crucial to un ravelling the origin and development of cysts (19).

Morphological and biochemical indicators can assist identify Taenia spp. species and strains, however DNA markers are more accurate and reliable for molecular epidemiology research (20).DNA markers and molecular characterization are critical for accurately identifying *Taenia* spp. and strains(21; 22). Gene Bank sequences (MH638348) are 100% similar to Iran's (JQ710588) and Palestine's (KM032284), and 99.9% similar to Turkey's (JN827307)

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and Iran's (JQ710627). This may be because the Kurdistan Region of Iraq is close to Iran and Turkey, which have comparable climates, cultures, and lifestyles, and since most domestic animals were imprisoned there (23; 24).

It is possible to identify *Taenia* spp. and strains using a wide range of molecular markers. The most prevalent methods are DNA sequencing, restriction enzyme fragment length, linked restriction fragment length (PCR-RFLP), and Internal transcribed spacers. PCR-RFLP (ITS1 and ITS2) was used to identify two rDNA sequences (ITS1 and ITS2) (25).In this particular piece of research, the mitochondrial DNA component known as the partial cytochrome oxidase gene (CO1) was analyzed. Met-CO1 DNA sequencing helps with evolutionary and relationship study, as well as worm species and strain identification. Mitochondrial DNA sequences are used worldwide to analyses genetic population structure.

Using PCR and partial sequencing on the mt-cox1 gene, the *T. hydatigenia* strain was identified in sheep and goats in Basrah, Iraq. The phylogenetic analysis also revealed that every single *T. hydatigenia* cyst isolate that was investigated came from animals that had been murdered.

According to the results of the phylogenetic analysis, the *T. hydatigenia* strain that was discovered in the Sulaimaniya area of Iraq was closely related to the Th04 strain that was discovered in Mongolia, the Panzhihua 8 strain that was discovered in China, and other *T. hydatigenia* strains that were discovered in other countries, such as Iran and Turkey. Given that the region shares a border with Iran, which has extremely limited genetic variety in compared to the rest of the globe, this is not surprising. Due to commerce between Iran and China and Iran and Iraq, *T. hydatigenia* may have originated in China. This study's findings are crucial for understanding the parasite's epidemiology in Sulaimaniya, Iraq, and devising future control programmers (26).Moreover, due to negative Tajima's D and Fu's F, the trimmed nad1 gene sequence dataset (435 bp) from *T. hydatigenia* isolates from other geographical regions and hosts revealed a population network consistent with population expansion, revealing unusual haplotypes as well as the characteristic star-shaped structure with a central haplotype (27).

Diverse hosts and places contributed to network haplotypes. Several Nigerian sheep haplotypes based on 4083 bp of mitochondrial DNA have merged to generate a single haplotype that spans isolates from many hosts (including Nigerian goat isolates) and geographic areas. *T. hydatigenia* questions the validity of partial gene sequences in determining genetic population organization or species phylogeny. The constraints of using short gene sequences to assess *Echinococcus* spp. (28).

Conclusion

The isolated cysts from infected animals(sheep and goats)at Basrah city was C. tenicollis which was the first record as molecular study and record to NCBI.

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