How to Cite:
Al-Sudani, G. D. Y., & Al-Amery, A. M. (2022). Molecular detection and genotyping of Taenia hydatigena between intermediate (sheep and goats) and final hosts (stray dogs) in Baghdad city. *International Journal of Health Sciences*, *6*(S3), 630–642.
https://doi.org/10.53730/ijhs.v6nS3.5402

Molecular detection and genotyping of Taenia hydatigena between intermediate (sheep and goats) and final hosts (stray dogs) in Baghdad city

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***Abstract*---**Taenia hydatigena cestode parasite found in the intestine of dogs and larval stage Cysticercus tenuicollis located in visceral organs of intermediate hosts (sheep and goats). In this study, investigate the genetic variation among the recovered isolates from sheep, goats, and stray dogs in Baghdad city, based on the mitochondrial cox1 gene. After DNA extraction, the PCR that was used indicated the specificity at the band (450bp) on the agarose gel. Sequencing and phylogenetic results were divided into three groups depending on the closed relationship between them and to NCBI-Blast T. hydatigena.

***Keywords*---**cysticercus tenuicollis, taenia hydatigena, sheep, goats, prevalence.

Introduction

Adult parasites pathogenicity was not high for definitive hosts while cysticerci were confined in the omentum, mesentery, brain, instinctive surface of the liver (known as hepatitis cysticercosa), subcutaneous tissue, skeletal muscle, eyes, lungs, and heart and they become pathogenic with the measurement of 6–8 cm (Kilinc *et al.,* 2019). The diagnosis could be made in an insightful research facility with PCR and sequencing strategies (Saari *et al.,* 2019). The identification of DNA taeniid cestodes has used specific sequences from both nuclear that was much larger nuclear genome located on chromosomes in the nucleus and mitochondrial genomes that were located in mitochondria that were small and circular (generally less than 20,000 bp in metazoans), multi-copied in the cell (McManus 2006). The

aims of the study were molecular for Genotyping *Taenia hydatigena* adult and *Cysticercus tenuicollis* by sequences and phylogenetic tree for identity.

Materials and Methods

Metacestodes (*C. tenuicollis*) was collected from a total of 200 (100 sheep and 100 goats) carcasses slaughtered in Al-shua'la abattoir at point (33°21'49.84" N 44°14'51.14" E in the northeast region of Baghdad governate during the period from the beginning of December 2020 to the end of August 2021 and adult *T. hydatigena* from 50 stray dogs were killed by shooting with the police municipality from the team of stray dogs control program in Baghdad governate, and these killed dogs were transferred to Baghdad veterinary hospital and examined according to WHO/OIE (2001) figure (1) the worms and their metacestodes will be collected in a petri dish and rinsed several times with physiological normal saline and kept in 70% ethyl alcohol for DNA extraction by gSYAN DNA extraction kit (Tissue protocol) Geneaid. USA, according to the manufacturer's instructions and Genomic DNA estimation for purity and concentration by using a Nanodrop spectrophotometer (Thermo Scientific. UK), the absorbance was read at (260 /280 nm). One specific primer was used in PCR amplification for identification of *Taenia hydatigena* based on sequencing of the mitochondrial gene (Cytochrome c oxidase subunit 1) COX1 gene according to (Liu *et al.,* 2010). Primers were provided by a Scientific Researcher. Co. Ltd, Iraq as the following table. The forward (JB3: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′), reverse (JB4.5:5′TAAAGAAAGAACATAATGAAAATG-3′) sequence, and expected amplicon size (450bp) of the gene and then identification by PCR to study genotypic diversity of *T. hydatigena* isolates from stray dogs and *C. tenuicollis* isolates from sheep and goats by sequencing and constriction of the phylogenetic tree.

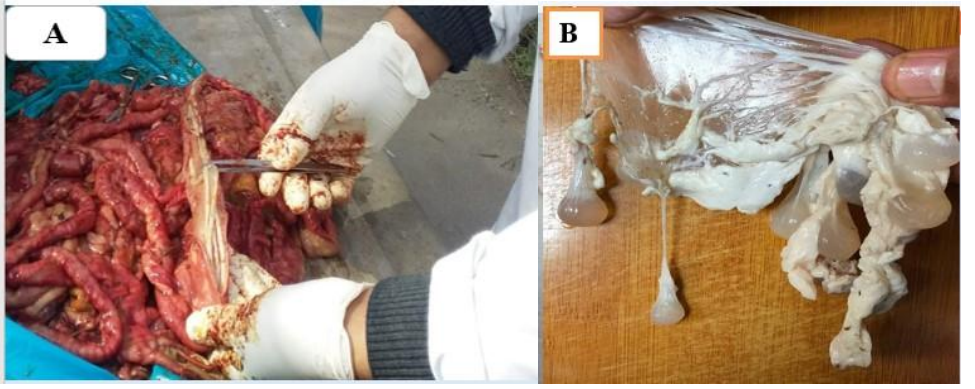

Figure (1). A. Shown sampling adult parasite from dog's intestine B. Shown *C. tenuicollis* from sheep and goat's omentum.

PCR components

Eighty-seven DNA samples were amplified using conventional PCR and the same forward and reverse primer were used by utilizing (GoTaq™ Green PCR Master Mix) (Promega / USA). PCR reaction was prepared according to company instructions 12.5 µl of PCR master mix, 2 µl of each primer, and 3 µl of the DNA template, and PCR water (free nuclease water) to make a total volume of 25 µl.

PCR cycling conditions

Thermo cycler optimal conditions include: The first initial denaturation cycle was performed at 95°C for 5 min., followed by 30 cycles of denaturation at 95°C for 30 sec., annealing at 58°C for 30 sec., extension at 72°C for 45 sec., then the final extension steps were at 72°C of 5min. After completion of the PCR reaction, amplified products were confirmed and analyzed by 1 % agarose gel electrophoresis. Any nonspecific or difference in the size of band observed by running the 100 bp DNA ladder along with PCR product.

Results

Extraction of the DNA parasites, which was directed on 21 samples from sheep, 35 samples from goats, and 31 samples from stray dogs was affirmed typical values of the concentration DNA ranged from 13.2 to 1801 ng\ µl and the purity was ranged between 1.38 to 4.86. Were visualized by electrophoresis using gel concentration of 0.8 % for the best separation of large molecular weight DNA figure (2) showed a good qualification of extracted genomic DNA.

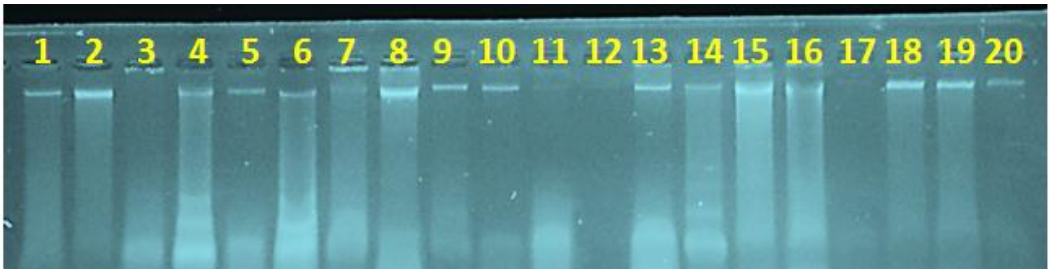

Figure 2: Agarose gel electrophoresis image that showed the Genomic DNA extraction from worm tissue sheep, goat, and dog samples.

Where The lane (1-5) showed Genomic DNA from sheep samples, the lane (6-10) showed Genomic DNA from goat's samples and the lane (11-20) showed Genomic DNA from dog's samples.

Amplification of Sheep samples by PCR

The results of conventional PCR exhibited that DNA sample of *Cysticercus* isolates from sheep as shown in the figure, (3) were successfully amplified individual amplicon represented single band (450bp) on agarose gel indicating the specificity of the PCR that was used. These results were in agreement with many references by using a specific primer (450bp) COX1 like Al-Hamzawi and Al-Mayali, 2020 in Al-Diwaniyah province, Iraq, Hama-Soor *et al.,* 2021 in Kalar, Iraq, Jia *et al.,* (2010), in China, Raissi *et al.,* (2021), in Chabahar, Sistan and Baluchestan Province, Iran.

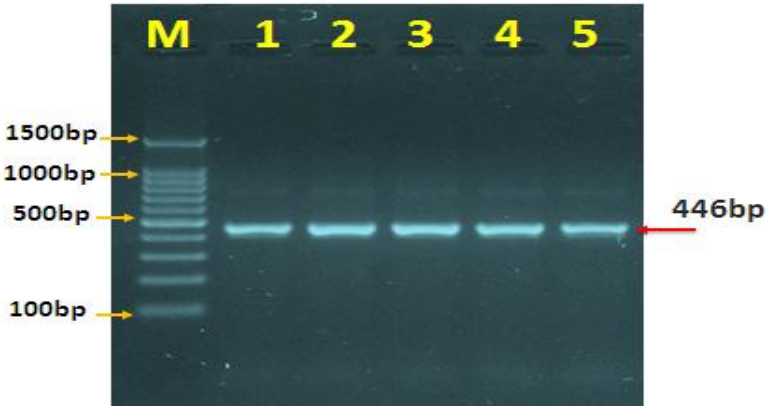

Figure 3. Agarose gel electrophoresis image that showed the PCR product analysis of *Taenia* sp. mitochondrial cytochrome oxidase subunit 1 (COX1) gene from extracted DNA of worm tissue sheep samples. Where M: marker (1500-100bp)

The lane (1-5) showed a positive *Taenia* sp. mitochondrial (COX1) gene from sheep samples at (446bp) PCR product.

Amplification of Goats samples by PCR

The results of PCR displayed that DNA extracted from *Cysticercus* isolates from goats, as displayed in figure (4) were effectively intensified individual amplicon addressed single band (450bp) on agarose gel showing the particularity of the PCR that utilized. These results were agreed with many references like Al-Hamzawi and Al-Mayali, 2020 in Al-Diwaniyah province, Iraq, Hao *et al.,* (2012) in china, Cengiz *et al.,* (2019) in Turkey, Alvi *et al.,* (2020) in Pakistan, and Moudgil, and Moudgil, (2021) in India.

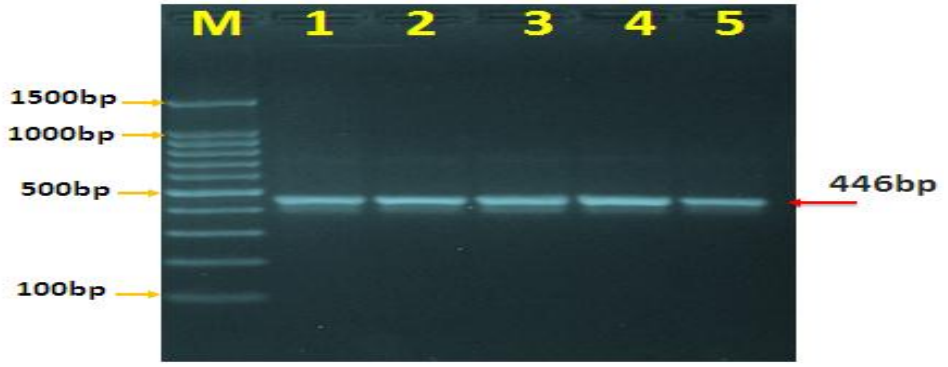

Figure 4. Agarose gel electrophoresis image that showed the PCR product analysis of Taenia sp. mitochondrial cytochrome oxidase subunit 1 (COX1) gene from extracted DNA of worm tissue goat samples

Where M: marker (1500-100bp). The lane (1-5) showed a positive *Taenia* sp. mitochondrial (COX1) gene from goat samples at (446bp) PCR product.

Amplification of dogs' samples by PCR

Although conventional PCR results of extracted DNA of adult worm *T. hydatigena* isolates from stray dogs exhibited that were successfully amplified individual amplicon represented single band on agarose gel indicating the specificity of the PCR was showed a positive *Taenia* sp. mitochondrial (COX1) gene from dogs' samples at (446bp) of PCR product and the conditions used as shown in figure (5). this agreement with Cengiz *et al.,* (2019), in Turkey, Ohiolei *et al.,* 2019, Lu *et al.,* (2020), in China, Narankhajid *et al.,* (2013), in Mongolia that the identification of *Taenia* in species and interspecies was the better effect of the COX1 gene.

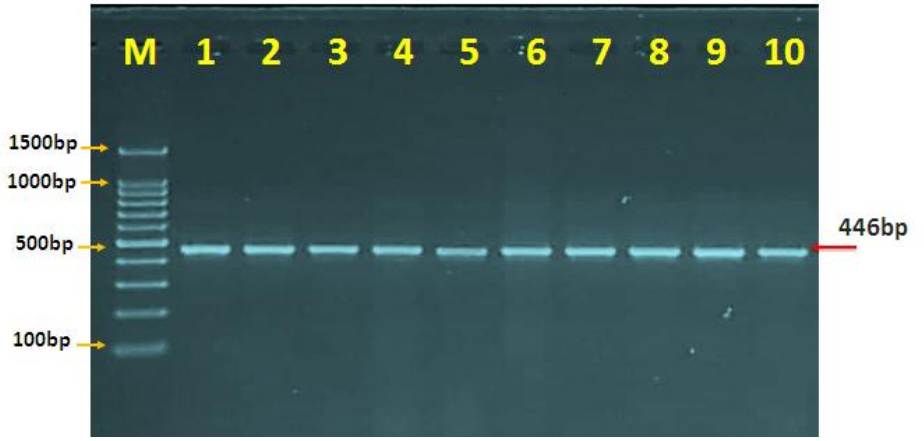

Figure 5. Agarose gel electrophoresis image that showed the PCR product analysis of Taenia sp. mitochondrial cytochrome oxidase subunit 1 (COX1) gene from extracted DNA of worm tissue dog samples

Where M: marker (1500-100bp). The lane (1-10) showed a positive Taenia sp. mitochondrial (COX1) gene from dogs' samples at (446bp) PCR product.

The results were received by email then analyzed using genious software after being sent to Bioneer company in Korea that Sequencing Technology, Sanger dideoxy sequencing, automated DNA sequences, and then results were checked and confirmed by using National Center for Biotechnology Information (NCBI) for published in GenBank. The phylogenetic relationship was analyzed for local *Taenia* sp. isolate using the MEGA 6.0 version. Sequencing and phylogenetic results were divided into three groups depending on the closed relationship between them and to NCBI-Blast *T. hydatigena*. Three groups of *T. hydatigena* isolates from Sheep were five samples, whereas the second group included five isolates from Goats and the third group included ten isolates from stray dogs the alignment of the sequences of nitrogenous bases which carries the serial numbers accession number shown in Table (1).

Table 1
The NCBI-BLAST Homology Sequence identity percentage between local *Taenia* isolates and NCBI-BLAST *Taenia hydatigena* isolate

No.	local *Taenia* sp. isolate	Accession number	Homology sequence identity (%)
1	*Taenia*_sp. Sheep_isolate_No.1	MZ313974	100.00%
2	*Taenia*_sp. Sheep_isolate_No.2	MZ313975	100.00%
3	*Taenia*_sp. Sheep_isolate_No.3	MZ313976	99.50%
4	*Taenia*_sp. Sheep_isolate_No.4	MZ313977	99.24%
5	*Taenia*_sp. Sheep_isolate_No.5	MZ572973	100.00%
6	*Taenia*_sp. Goat_isolate_No.1	MZ313978	99.28%
7	*Taenia*_sp. Goat_isolate_No.2	MZ313979	100.00%
8	*Taenia*_sp. Goat_isolate_No.3	MZ313980	100.00%
9	*Taenia*_sp. Goat_isolate_No.4	MZ313981	99.75%
10	*Taenia*_sp. Goat_isolate_No.5	MZ572974	100.00%
11	*Taenia*_sp Dog_isolate_No.1	MZ313982	99.74%
12	*Taenia*_sp Dog_isolate_No.2	MZ313983	99.75%
13	*Taenia*_sp Dog_isolate_No.3	MZ313984	99.74%
14	*Taenia*_sp Dog_isolate_No.4	MZ313985	99.75%
15	*Taenia*_sp Dog_isolate_No.5	MZ313986	99.50%
16	*Taenia*_sp Dog_isolate_No.6	MZ313987	99.75%
17	*Taenia*_sp Dog_isolate_No.7	MZ572975	99.75%
18	*Taenia*_sp Dog_isolate_No.8	MZ572976	99.74%
19	*Taenia*_sp Dog_isolate_No.9	MZ572977	99.22%
20	*Taenia*_sp Dog_isolate_No.10	MZ572978	99.21%

Discussion

Sheep

Phylogenetic analysis of five products of Sheep isolates revealed that *Taenia hydatigena* infect sheep in Iraq and the identity between related country isolates and local *Taenia hydatigena* isolates found that the concordance ranged between (100%, 99.50%, 99.24%) in sheep isolates. The local *C. tenuicollis* Sheep isolates number (1,2 and 5) MZ313974, MZ313975 and MZ572973 respectively had a close relation with NCBI-Blast *Taenia hydatigena* of India LC617413.2 100% identify. Also, were less related with 99.50% and 99.24% identity with NCBI-Blast *Taenia hydatigena* of India LC617413.2, isolate numbers 3 and 4, that had Accession number MZ313976 and MZ313977 respectively table, (4) The same causes could be the reasons for the similarity in the current study with the Iranian and Turkish dynasties because of Animal trade from the neighboring country across aboard to Kurdistan region and from there to the remaining Iraqi governorates or through direct animal trade with those countries and the remaining governorates of Iraq, including Baghdad. The major movement of domestic animals was imported to Kurdistan-Iraq from Turkey and Iran (Hama,

2018). The similarity with the breeds of other countries such as India, China, Ghana, and Zambia, might be the strain of *T. hydatigena* with Global spread in a group of breeds of sheep.

In the phylogenetic tree, all the local sheep *T. hydatigena* isolates were clustered in one clade, along with isolates from India (LC617413.2), which explain the parasite had a wide distribution of Taeniidae species among sheep and dogs across the world by people migration and relocation from region to another and transportation of animal throughout a significant time. A serious transmission of the parasite among a scope of intermediate host species could expand the chance of genetic changeability inside various populaces of the parasite in the world (Rostami *et al.,* 2013).

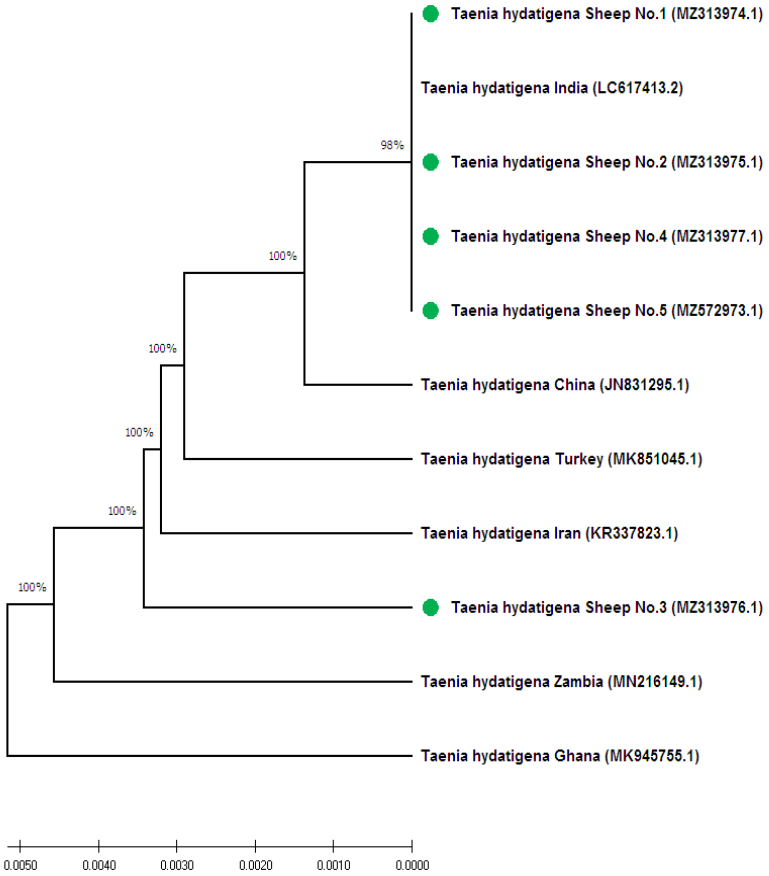

Figure 6. Phylogenetic tree analysis-based cytochrome c oxidase subunit I (COX1) gene partial sequence in local *Taenia hydatigena* sheep isolates that used for genetic relationship analysis

The phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Taenia hydatigena* sheep isolates (No.1 into No.5) were shown more closed related to

NCBI-BLAST *Taenia hydatigena* India isolate (LC617413.2) at total genetic changes (0.0050-0.0010%).

Table 2 The NCBI-BLAST Homology Sequence identity percentage between local *Taenia* hydatigena sheep isolates and NCBI-BLAST country related *Taenia hydatigena* isolate

local *Taenia hydatigena* isolate	Accession number	Homology sequence identity (%)		
		Related country isolate	Accession number	identity (%)
Taenia hydatigena sheep isolate No.1	MZ313974	India	LC617413.2	100.00%
Taenia hydatigena sheep isolate No.2	MZ313975	India	LC617413.2	100.00%
Taenia hydatigena sheep isolate No.3	MZ313976	India	LC617413.2	99.50%
Taenia hydatigena sheep isolate No.4	MZ313977	India	LC617413.2	99.24%
Taenia hydatigena sheep isolate No.5	MZ572973	India	LC617413.2	100.00%

Goats

Phylogenetic analysis of five products of Goats isolates revealed that *Taenia hydatigena* infect goats in Iraq. The local larval stage of *Taenia hydatigena* goats isolates number (2,3 and 5) MZ313979, MZ313980 and MZ572974 respectively had a close relation with NCBI-Blast *Taenia hydatigena* of India LC617413.2 100% identify. Additionally, were less related with 99.28% and 99.75% identity with NCBI-Blast *Taenia hydatigena* of Turkey, MK851045.1, and China JN831295 isolates number 1 and 4, that had Accession number MZ313978 and MZ313981 respectively. The local *Taenia hydatigena* goat isolate (No.1) was shown more closed related to NCBI-BLAST *Taenia hydatigena* Turkey isolate (MK851045.1) and the local *Taenia hydatigena* goat isolates (No.2, No3, and No.5) were shown more closed related to NCBI-BLAST *Taenia hydatigena* India isolate (LC617413.2), whereas, the local *Taenia hydatigena* goat isolate (No.4) was shown more closed related to NCBI-BLAST *Taenia hydatigena* China isolates (JN831295.1), at total genetic changes (0.0050-0.0010%) table, (6), figure, (7). Identity between related country isolate and local *Taenia hydatigena* isolates found that the concordance ranged between (100%, 99.28%, 99.75%) in goats isolates. The same causes could be the reasons for the similarity in the current study with the Iranian and Turkish dynasties because of Animal trade from Kurdistan and from there to the remaining Iraqi governorates or through direct animal trade with Those countries and the remaining governorates of Iraq, including Baghdad. Is the similarity with the breeds of other countries such as China and Ghana, especially in goats, that may be a group of breeds with Global spread due to the origins of goats found in Iraq besides to activity and main rule of that act in transmission and dissemination of the parasite across the fields that contact directly through rural dogs and indirect through stray dogs.

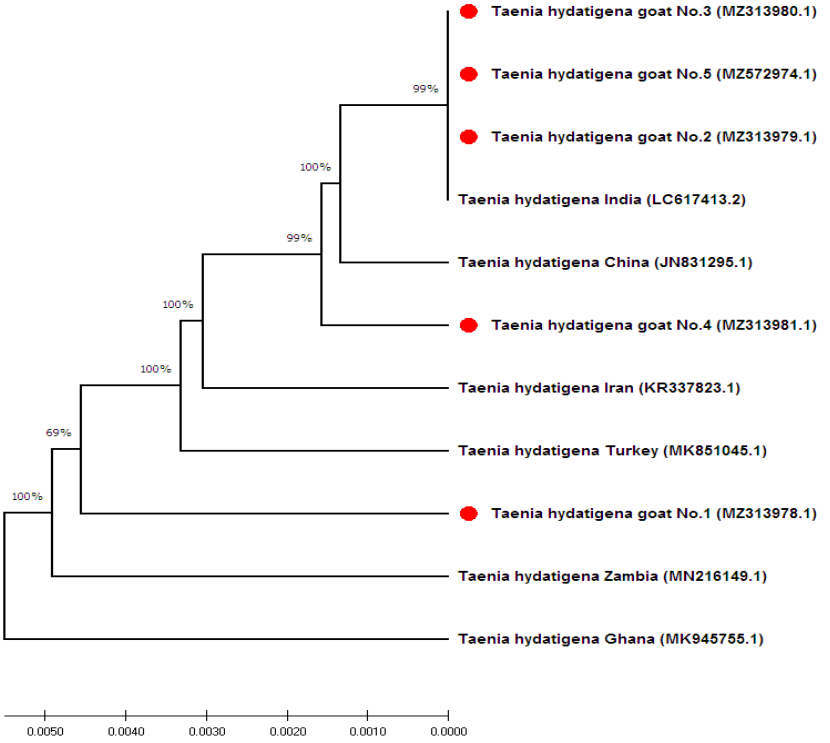

Figure 7. Phylogenetic tree analysis-based cytochrome c oxidase subunit I (COX1) gene partial sequence in local *Taenia hydatigena* goat isolates that used for genetic relationship analysis

The phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Taenia hydatigena* goat isolate (No.1) was shown more closed related to NCBI-BLAST *Taenia hydatigena* Turkey isolate (MK851045.1) and the local *Taenia hydatigena* goat isolates (No.2, No3, and No.5) were shown more closed related to NCBI-BLAST *Taenia hydatigena* India isolate (LC617413.2), whereas, the local *Taenia hydatigena* goat isolate (No.4) was shown more closed related to NCBI-BLAST *Taenia hydatigena* China isolates (JN831295.1), at total genetic changes (0.0050-0.0010%).

Table 3
The NCBI-BLAST Homology Sequence identity percentage between local *Taenia hydatigena* goats isolates and NCBI-BLAST country related *Taenia hydatigena* isolate

local *Taenia hydatigena* isolate	Accession number	Homology sequence identity (%)		
		Related country isolate	Accession number	identity (%)
Taenia hydatigena goat isolate No.1	MZ313978	Turkey	MK851045.1	99.28%

Taenia hydatigena goat isolate No.2	MZ313979	India	LC617413.2	100.00%
Taenia hydatigena goat isolate No.3	MZ313980	India	LC617413.2	100.00%
Taenia hydatigena goat isolate No.4	MZ313981	China	JN831295	99.75%
Taenia hydatigena goat isolate No.5	MZ572974	India	LC617413.2	100.00%

Dogs

Phylogenetic analysis of 10 products of dog isolates revealed that *Taenia hydatigena* infect dogs in Iraq. The local *Taenia hydatigena* dog isolate (No.1, No.5, No.6, No.8, and No.9) that had accession number MZ313982, MZ313986, MZ313987, MZ572976 and MZ572977 respectively were showed more closed related to NCBI-BLAST *Taenia hydatigena* China isolates (JN831295.1) with homology sequence identity was (99.74%, 99.50%, 99.75%,99.74% and 99.22%) respectively, also the local *Taenia hydatigena* dog isolates (No.2, and No.10) that had accession number (MZ313983, and MZ572978) were showed more closed related to NCBI-BLAST *Taenia hydatigena* India isolate (LC617413.2) with homology sequence identity was (99.75% and 99.21%) respectively whereas, the local *Taenia hydatigena* dog isolate (No.3, No.4, and No.7) that had accession number MZ313984, MZ313985, and MZ572975 respectively were showed more closed related to NCBI-BLAST *Taenia hydatigena* Iran isolate (KR337823.1) with homology sequence identity was (99.74%, 99.75% and 99.75%) respectively, at total genetic changes (0.0050-0.0010%) as shown in figure (4-38).

In the phylogenetic tree, all the local dog *T. hydatigena* isolates were clustered in one clade, along with isolates from India (LC617413.2), Iran isolates (KR337823.1), and China isolates (JN831295.1) which mean the local dog *T. hydatigena* isolates were globally strain (India isolates LC617413.2 and China isolates JN831295.1), in addition, that Iran isolates (KR337823.1) was clustered to local dogs isolates due to wide border between country and the trade animals between two country because explain the parasite had a wide distribution of *Taenia hydatigena* among ruminants and dogs across the world by trading and transportation of animals besides through people migration and relocation from region to another throughout a significant time. Baghdad governorate that made freely motion of dogs from neighboring provinces also Iraq one of the countries that have open boarder that let nomadic people and their animals and guards' dog that dogs without veterinarian care which harbor the parasites and spread the infection between sheep and goats and when infected offal consume again to the dog and another than would contamination the food and water of animals later become infected. In addition, dogs were characterized by general behavior that overpopulation and migration for seeking for feed and breeding that traits were explained that male of dogs has an activity for roaming animal slaughter waste from home-slaughter, and illegal slaughter out of abattoir especially in the high number of festivals or occasions and weddings these things favor spreading the infection in rural dogs or stray dogs that maintain the infection rates in dogs and farms animal. A serious transmission of the parasite among a scope of

intermediate host species could expand the chance of genetic changeability inside various populaces of the parasite in the world (Rostami *et al.*, 2013).

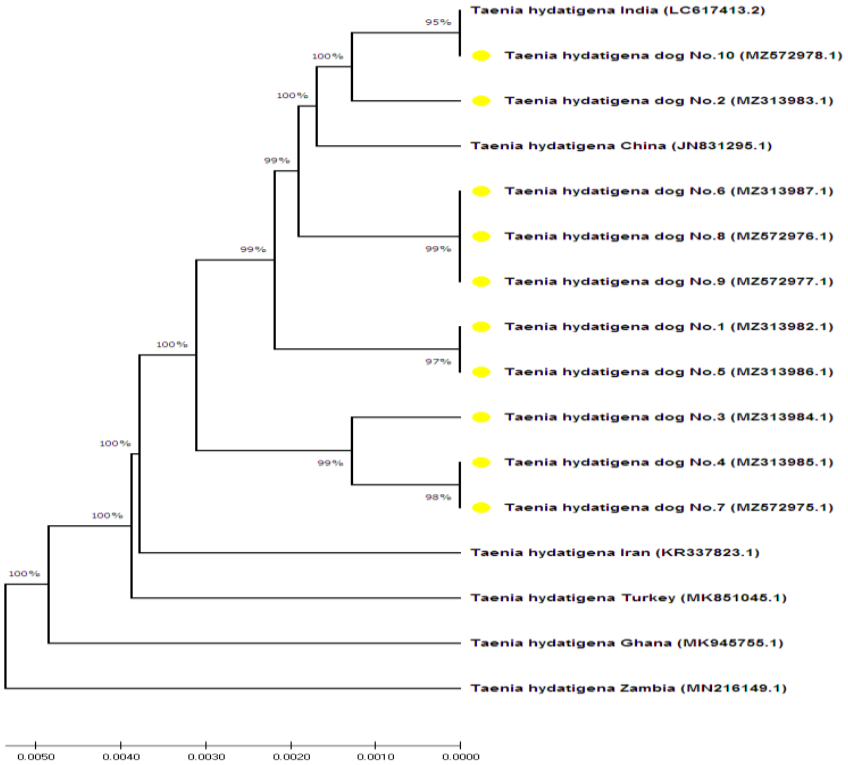

Figure 8. Phylogenetic tree analysis-based cytochrome c oxidase subunit I (COX1) gene partial sequence in local *Taenia hydatigena* dog isolates that used for genetic relationship analysis

The phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Taenia hydatigena* dog isolate (No.1, No.5, No.6, No.8, and No.9) were showed more closed related to NCBI-BLAST *Taenia hydatigena* China isolates (JN831295.1) and the local *Taenia hydatigena* dog isolates (No.2, and No.10) were showed more closed related to NCBI-BLAST *Taenia hydatigena* India isolate (LC617413.2), whereas, the local *Taenia hydatigena* dog isolate (No.3, No.4, and No.7) were shown more closed related to NCBI-BLAST *Taenia hydatigena* Iran isolate (KR337823.1), at total genetic changes (0.0050-0.0010%).

Table 4
The NCBI-BLAST Homology Sequence identity percentage between local *Taenia hydatigena* dog isolates and NCBI-BLAST country related *Taenia hydatigena* isolate

local *Taenia hydatigena* isolate	Accession number	Homology sequence identity (%)		
		Related country isolate	Accession number	identity (%)
Taenia hydatigena dog isolate No.1	MZ313982	China	JN831295	99.74%
Taenia hydatigena dog isolate No.2	MZ313983	India	LC617413.2	99.75%
Taenia hydatigena dog isolate No.3	MZ313984	Iran	KR337823.1	99.74%
Taenia hydatigena dog isolate No.4	MZ313985	Iran	KR337823.1	99.75%
Taenia hydatigena dog isolate No.5	MZ313986	China	JN831295	99.50%
Taenia hydatigena dog isolate No.6	MZ313987	China	JN831295	99.75%
Taenia hydatigena dog isolate No.7	MZ572975	Iran	KR337823.1	99.75%
Taenia hydatigena dog isolate No.8	MZ572976	China	JN831295	99.74%
Taenia hydatigena dog isolate No.9	MZ572977	China	JN831295	99.22%
Taenia hydatigena dog isolate No.10	MZ572978	India	LC617413.2	99.21%

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