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Molecular Characterization, Genotypes and Phenotypes of *Cysticercus Tenuicollis* (*Taenia Hydatigena*) From Naturally Infected Goats Slaughtered in Al-Muthana and Al-Diwaniyah Province

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cytb gene

ABSTRACT:

The current study included the phenotypic and genetic characteristics of Cysticercus.tenuicollis, the thin-necked tapeworm in goats. 3145 samples of goats from animals slaughtered in slaughterhouses were examined for the period from April 2022 to July 2022 in the Diwaniyah and Muthanna governorates. The results of the study showed that the infection rate in Goats: The infected animals amounted to 672 heads, at a rate of 21.36%. The current study aimed to study the morphological characteristics of the sacs, heads, and snout spines of larvae isolated from goats, molecular diagnosis, and identification of genetic mutations of the parasite at the level of DNA and amino acids. In this study, the phenotypic characteristics of The larval stage of C. tenuicollis were determined in terms of the number of sacs and their sizes, and the number of galls and their lengths. It was found that the highest percentage of cysts in goats was 1-2, at a rate of 90.92%. their sizes ranged between 1-12 cm. the liver cysts were 1-2 cm in size, while in the peritoneal cavity, it was 1-12 cm. The number of large and small hooks surrounding the rostellum was calculated, and The total number was 30 hook,which were equal in number and an average of 15 for each. The average length of the large hooks was 192.48±3.04 microns, and the small hooks were 121.25±2.56 microns.

The results of the molecular study of the Polymerase Chain Reaction (PCR) technique using three mitochondrial genes: Cytochrome c oxidase subunit 1 gene (COX1), Mitochondrial cytochrome b gene (Cytb) and NADH dehydrogenase subunit 4 gene (Nd4) showed that the amplification results of DNA extracted from the scolex and germinal layer of *C. tenuicollis* cyst samples were positive at the molecular sizes of mitochondrial genes (444, 568, 680 bp, in all samples respectively).

The results of the genetic sequencing of the larval stage of *C. tenuicollis* showed that all local samples studied were identical to the global isolates, Turkey (MK851045), Iran (KR337823.1) and Egypt (OQ317833 which registered in the National Center for Biotechnology Information (NCBI), and a comparison of the alignment of the sequences of the nitrogenous bases showed For local isolates the match rate of 100%, and as for the Saudi isolates (MZ277312), the match rate was 99.74%.The genetic sequencing of the mt-Cytb and mt-Nad4gene genes, all isolates were identical to the Chinese isolates, and the percentage of identity ranged between 97.62-99.27% and 98.58-98.90%, respectively.Nucleotides bases sequence were converted to the amino acid sequence using the EXpasy software program. The results of the sequence showed the amino acids of the parasite strains in local samples,similar to global isolates which were recorded in the International Gen

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Bank, such as the Iranian isolate (AFL69973.1) was 96.15%, and the Turkish isolate (AFA53641.1) had a match rate of 97.85%. As for the Cytb gene,Mache with Chinese isolate (ACN72662.1)the similarity ranged between 90.21-96.36% , and the NAD4 gene ranged between 89.67-90.78% with the Chinese isolates (UXP71673.1 and AGJ83395.1), respectively. The results of the current study indicated the presence of mutations among the local isolates at the acid sequence level. amino acids, as 14 basic mutations were found in the COX1 gene, 13 basic mutations in the Cytb gene, which included the presence of a deletion mutation, that is, deletion of a complete amino acid, and 13 basic mutations in the Nad4 gene, which also included the presence of a deletion mutation.

Introduction

The thin-necked tapeworm, Taenia hydatigena, is one of the oldest existing species that belongs to the Taeniidae family. It has a wide global distribution. Adult worms infect the ultimate host represented by the Canidae family, such as dogs, lynxes, wolves, jackals, and foxes (Sulieman et al., 2020) Gori et al., 2015; Lesniak et al., 2017;). The larval stage of the worm T. hydatigena is Cysticercus tenuicollis (Omar et al., 2016), and this larvae is one of the largest larvae of the genus Taenia. It is recognized by its shape, size, and site of parasitism (OIE, 2008).), as it is characterized by its spherical shape, white in color and tending to yellow, and sometimes it is surrounded by a membrane of semi-transparent fibrous tissue emerging from the host's body (Pathak and Gaur, 1982). The larvae have a long and slender neck approximately 3-5 mm long that connects to the head of the larvae, which It may be dented inward or not, and the larva completely occupies the inner space of the bag (Jenson and Pierson, 1975). The larvae has a distinctive head located at the top of the neck that can be seen through the wall of the bag in the form of a white spot, and it is folded with the neck to the back of the larval body (Murai and Saquar, 1979). The size of the cyst varies depending on its location in the host's central body, as its diameter ranges between 7-8 cm in the omentum area and 2-5 mm in the liver (Jenson and Pierson, 1975; Hall, 1986).

Materials and methods

Sample collection

Samples of (3,145) samples were collected from slaughtered goats in the governorates of Muthanna and Diwaniyah, for the period from April 2022 to July 2022, from the organs of infected animals (omentum, liver, mesentery, and peritoneal cavity), which are the sources of C. tenucollis larvae in our current study. The samples were collected in refrigerated and sterile plastic boxes to be transported to the laboratory of the biology department in the College of Education to complete the next steps. Bladder cysts were isolated from infected organs in the laboratory, such as the liver, mesentery, omentum, and peritoneal cavity, and washed with normal saline solution, then sterilized with 70% ethanol before opening them. The sizes and numbers of cysts and larvae were then measured using a ruler or tape measure. If the bladder sacs are attached, they are counted externally, but if they are inside the organ, the number of cysts present in it is explained and calculated. The heads of the bladder sacs were preserved in a solution of Polyvinyl Lactophenol and examined under a microscope, to measure the lengths of the spines, and by measuring the total length of the spine, the length of the small blades, and The large one is found in each snout of the study specimens (Zar, 1984).

Molecular study

PCR method was used for mitochondrial genes (COX1 gene, Nad4gene, Cytbgene); To detect the genetic structure of the T. hydatigena parasite in isolates from infected animals, this technique was used by (Nikmanesh et al., 2014), and mtDNA was extracted from bladder cyst samples using the DNA Extraction Kit GSYAN (Taiwan). Genend) by the manufacturer's instructions, in this study, PCR primers were used to identify the genotype of cystic cysts, as well as to determine the genotypes of the parasite in naturally infected sheep and goats, by sequencing three mitochondrial genes (COX1 gene, Nd4gene, and

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Cytbgene) that were amplified using the technique Regular PCR (Liu et al., 2012, these primers were designed by IDT) according to the Canada method, as in Table (1-1).

(1-1) Primers used in study with nucleotide sequences.

Primer	Sequence 5'-3'		Amplicon	Reference
cox1 gene	F	TTTTTTGGGCATCCTGAGGTTTAT	**444bp**	**liu *et al*.,2012**
	R	TAAAGAAAGAACATAATGAAAATG		
Cytb gene	F	GTCAGATGTCTTATTGGGCTGC	**568bp**	
	R	TCTGGGTGACACCCACCTAAATA		
Nd4 **gene**	F	GAGTCTCCTTATTCTGAGCG	**680bp**	
	R	ATAGTAGTAGGAAATGAACA		

R=Revers Primer Primer F=Forward

The Polymerase chain reaction product of the mitochondrial genes (COX1 gene, Nad4gene, Cytbgene) was electrophoresed using an agarose gel electrophoresis device.

The DNA sequences of the studied mitochondrial genes (COX1 gene, Nd4gene, Cytbgene) were determined for the larval stage of the parasite from samples isolated from goats. The PCR results for the mitochondrial genes (COX1 gene, Nd4gene, Cytbgene) were sequenced by Microgen Company in South Korea, and the samples were sent via the Iraqi Biotechnology Company (Iraq Biotechnology). According to the company's requirements, the sample must include 15 µl for each of the forward and reverse PCR products, and 50 µl for each of the forward and reverse primers. Each sample must have its own number and name recorded on it, and the samples must be sent in refrigerated boxes containing cool gel packs, and the genetic tree was analyzed using the (Phylogenetic tree) program (MEGA 11.1) for molecular genetic analysis, and then the evolutionary distances were calculated using the maximum likelihood method (UPGMA), and the identified parasite isolates were submitted to (NCBI-Gen Bank) to obtain Registration number: accession numbers. A comparison was made between the study isolates and the parasite isolates at (NCBI) to identify local strains by analyzing genetic trees on site.

Nucleotide sequence results for the three mitochondrial genes (COX1gene, Nd4gene, Cytbgene) generated by the forward and reverse primers were identical using the BLAST N algorithm at the NCBI website. Then the sequence data from the file that was uploaded in the Blast Nucleotide Sequence program dialog box was processed, and then the type of parasite will appear directly with the percentage of sequence identity with other isolates. The BLASTN results showed that the uploaded DNA sequence was identical (98-100%). the isolates recorded globally on the site, all belong to the T. hydatigenia parasite.

At the level of proteins, the sequence was converted to amino acids using the BLAST P algorithm, and then the results were read. Finally, the results were registered in the NCBI Global Gen Bank and the registration number for each sample was obtained. Accession numbers. We made a comparison between the sequences of the amino acids in the study isolates. Likewise, with the international isolates registered on the site, and using the EXpasy program, the nucleotides were converted to amino acids and compared with the international isolates.

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Phenotypic study of bladder cysts:

Phenotypic study

Bladder cysts were collected after examining 3,145 goats slaughtered in the governorates of Muthanna and Diwaniyah, and the infection rate was 21.36%. The number of bladder cysts in one animal ranged between 1-12 cysts in the affected organs, and the largest percentage was 1-2 cysts. It reached 90.92%, and the lowest percentage was with the number of bags 7-9, as in Table (1-2).

able (1-2): Number of C.tenuicollis cysts in infected goats in the study animals.

Number of bags	No.	Percentage %
1-2	611	%90.92
3-6	52	%7.73
7-9	9	%1.33
total	672	100%

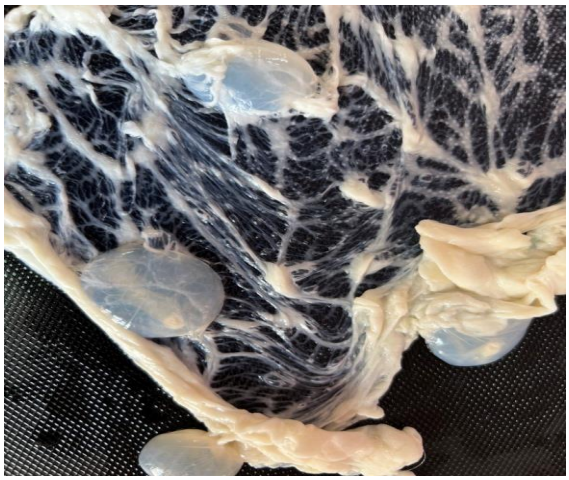

Figure (1-1) Cystic cysts in the peritoneal cavity and shows the initial heads of the larvae inward

Color, shape, and size of the vesical sacs. After examining the affected animals, the vesical sacs were found in various organs of these animals. It was found that most of the cysts in the goats were of a milky white color, and the smaller percentage had a yellowish color. Also, the sacs were very thin and transparent, sticking to or hanging on the surface. The surface of the organ resembles a balloon or bubble, and contains a watery, tissue-like fluid that is transparent and similar to water, but with a higher viscosity. As for the sizes of these cysts, their diameters ranged between 1-12 cm, depending on the affected organ, the duration of the infection, and the age of the animal. It was found that the liver cysts were the size of (1 -2) cm, while in the peritoneal cavity it was (1-21) cm. As in the figure (1-2).

Fig (1-2) Sizes of some isolated bladder bags.

Numbers and measurements of spines in the snout of larvae:

The diameter of the larvae's snout was measured and was 500 microns (µm), and then the number of large and small spines surrounding the snout was calculated in 10 samples of goats. The total number of spines was also 30, distributed evenly as well. The total length of the large spines was measured, and its average length was 192.48 µm, the length of the large fork blade was measured, and it ranged between 95-80 µm, as well as the total length of the small spine, whose average length was 121.25 µm, and the length of the small fork blade, which ranged between 45-75 µm. As shown in Table (1-3) and Figure (1-3).

Figure (3-1) upper section showing the snout, the sockets, and the arrangement of the small and large

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spines and their number around the snout in a goat (magnification x 100).

Table (1-3) Numbers and measurements of spines in the snouts of C.tenuicollis larvae in infected and slaughtered goats in Diwaniyah and Muthanna governorates during the study period

No.	measurements	number
1	No. of hooks (NH	30
2	No.of Large hooks(NLH)	15
3	No.of Small hooks(NSH)	15
4	Total length (LHTL)	175-205 µm
5	Blade of Large length hooks (LHBL)	80-95 µm
6	Average length of large hooks (ALLH)	48-192 µm
7	Total length (SHTL)	115 -140 µm
8	Blade of Small length (SHBL)	45 -75 µm
9	Average length of small hooks (ALSH)	121.25 µm

Molecular study

The study relied on DNA amplification processes for the Cytochrome C oxidase subunit 1 gene Mitochondrial extracted from samples of C. tenuicollis cysts after performing agarose gel electrophoresis.It showed that all 5 samples were positive, as the diagnostic gene 1genexco for the parasite appears. At a molecular weight of 444 base pairs bp. The study relied on DNA amplification processes for the Mitochondrial cytochrome b gene extracted from samples of C. tenuicollis cysts from goats after performing electrophoresis on an agarose gel. It showed that all 5 samples were positive, as the cytb gene of the parasite appeared. At a molecular weight of 568 base pairs bp. The study relied on DNA amplification processes for the Nd4 gene, NADH dehydrogenase subunit 4 gene, extracted from samples of C. tenuicollis cysts from goats. After performing electrophoresis on an agarose gel, it showed that all 5 samples were positive, as the Nd4 gene appeared. gene of the parasite with a molecular weight of 680 base pairs bP

Results of polymerase chain reaction technology for the COX1 gene and genetic variation

The results showed, as shown in Figure (4-1), that the results of the DNA amplification processes for the gene (1genecox) for all samples, namely 5 samples, were positive after performing the electrophoresis process on an agarose gel, as it showed the presence of the diagnostic gene 1genecox for the parasite at the molecular weight. 444 base pairs bp.

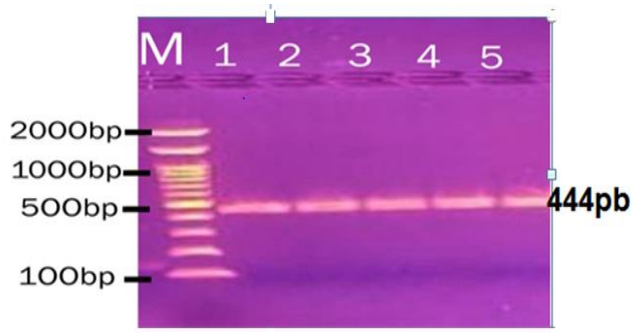

Figure (1-4): Electrophoresis image on an agarose gel of the gene1cox gene and its products of 444 base pairs bp

cytb gene

the results showed that DNA amplification process for the cytb gene for all 5 samples was positive after performing the electrophoresis process on an agarose gel, as it showed the presence of the cytb gene of the parasite at the molecular weight. 568 base pairs bp as shown in Figure (1-5).

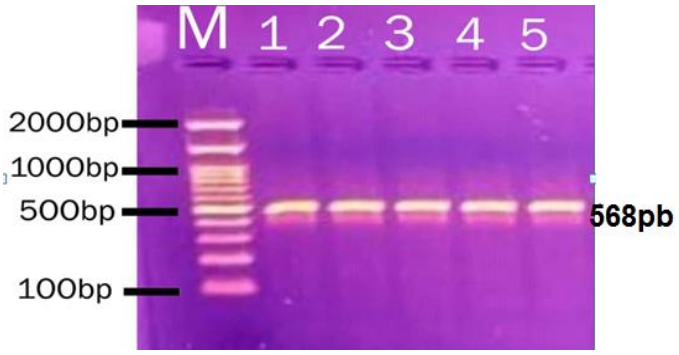

Figure (1-5): Electrophoresis image on an agarose gel of the gene Cytb and its products of 568 base pairs bp

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Nad4

The results showed DNA amplification process for the Nd4 gene (for all 5 samples and the extract from the goat bladder sacs) was positive after performing the electrophoresis process on an agarose gel, as it showed the presence of the gene. Nd4 gene of the parasite has a molecular weight of 680 base pairs bp as shown in Figure (4-3).

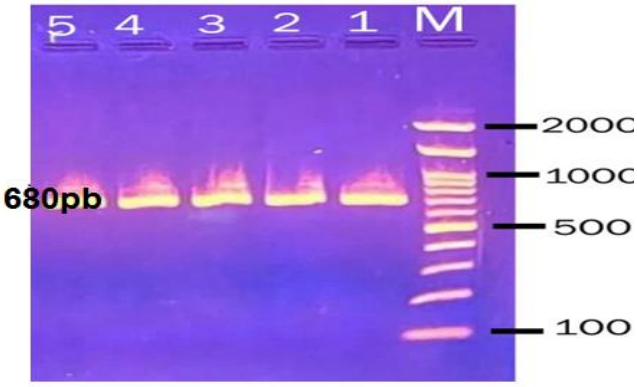

Figure (1-6): Electrophoresis image on an agarose gel of the Nd4 gene and its product of 680 base pairs bp.

Proteins

The genetic tree was designed using the Unweighted Pair Group method with Arithmetic Mean (UPGMA) and using (MEGA 11.1) program to draw the genetic tree of the larval stage of C. tenuicollis of the parasite Taenia hydatigena

Cox1 gene

Using the above-mentioned program, the genetic tree of the Cox1 gene was drawn to determine the sequence of amino acids in the protein (Amino acid analysis) and to determine the genetic relationship between the local isolates of the parasite isolated from Iraqi goats (IQ-Goat isolates) slaughtered in the Muthanna and Diwaniyah governorates with the international isolates of the parasite recorded in International Gene Bank (NCBI-Taenia hydatigena) and compared with the same gene encoding the same amino acids, as shown in Table (1-4).

Genetic tree analysis of the local isolates of the C.tenuicollis parasite bearing the serial numbers (OQ748014, OQ748015, OQ748016, OQ748017, OQ748018) showed that the local isolates of the parasite were all identical to the Iranian isolates.

AFL69973.1, QPA13915.1, the Italian isolate ALE99370.1, the Egyptian isolate BBN66044.1, and the Turkish isolate AFA53641.1, with percentages ranging between 96.15-97.96%.

Table (4-1) Percentage of similarity in amino acid sequence (%) Homology Sequence Identity between the local Cysticercus tenuicollis isolates of goats in the cities of Diwaniyah and Muthanna and the global isolates of the Taenia hydatigena parasite registered in the International Gene Bank (NCBI-BLAST) for the COX1 gene.

Cysticercustenicollis No.	**NCBI-BLAST Homology Sequence identity (%)**			
	Genbank Accession number	**Genetic related Country**	**Genbank Accession number**	**Identity (%)**
Cysticercustenuicollis IQ- goat No.1	OQ748014	Iran	AFL69973.1	96.15%
Cysticercustenuicollis IQ-goat No.2	OQ748015	Iran	QPA13915.1	97.69%
Cysticercustenuicollis IQ-goat No.3	OQ748016	Italy	ALE99370.1	97.35%
Cysticercustenuicollis IQ-goat No.4	OQ748017	Turkey	AFA53641.1	97.85%
Cysticercustenuicollis IQ- goat No.5	OQ748018	Egypt	BBN66044.1	97.96%

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Cytb gene

Using the Cytb gene to determine the amino acid sequence and determine the genetic relationship between local isolates of the parasite isolated from Iraqi goats slaughtered in the governorates of Muthanna and Diwaniyah with global isolates of the parasite registered in the International Gene Bank (NCBI-Taenia hydatigena) and using the same gene.The genetic tree analysis of the local isolation of the Ebeti C.tenuicollis that bears the serial numbers (OQ506453, OQ506454, OQ506455, OQ506456, OQ506457) showed that local defaults came all identical to Chinese insulation (ACN72662.1, qsv08807.1) and with a proportions ranging between 90.21- 96.36%.

Table (5-1): Percentage of similarity in amino acid sequences (%) Homology Sequence identity between local isolates of Cysticercus tenuicollis goats in the cities of Diwaniyah and Muthanna and global isolates of the parasite Taenia hydatigena registered in the International Gene Bank NCBI-BLAST for the Cytb gene.

Cysticercustenicollis No.	**NCBI-BLAST Homology Sequence identity (%)**			
	Genbank Accession number	**Genetic related Country**	**Genbank Accession number**	**Identity (%)**
Cysticercustenuicollis IQ-goat No.1	OQ506453	China	QSV08807.1	93.44
Cysticercustenuicollis IQ-goat No.2	OQ506454	China	ACN72662.1	92.06
Cysticercustenuicollis IQ-goat No.3	OQ506455	China	ACN72662.1	90.21
Cysticercustenuicollis IQ- goat No.4	OQ506456	China	ACN72662.1	96.36
Cysticercustenuicollis IQ- goat No.5	OQ506457	China	ACN72662.1	92.70

NAD4 gene

A comparison showed the alignment of amino acid sequences of local isolates of the T parasite. hydatigena for the gene (mt-ND4) bearing the serial numbers shown in Table (4-6) with global isolates of the same parasite and for the same gene in the NCBI Global Gene Bank, the match rate ranged between 89.67% - 90.78%, and the alignment results for the local goat isolates in The current study, which was registered in the International GenBank under Accession numbers: All of the studied isolates from local goats were identical to the Chinese isolates (UXP71673.1 and AGJ83395.1) with percentages ranging between 89.67-90.78%, as shown in Table (1-6).

Table (1-6) Percentage of similarity in amino acid sequences (%) Homology Sequence identity between local isolates of Cysticercus tenuicollis goats in the cities of Diwaniyah and Muthanna and global isolates of the parasite Taenia hydatigena registered in the International Gene Bank (NCBI-BLAST) for the NAD4 gene.

Cysticercustenicollis No.	**NCBI-BLAST Homology Sequence identity (%)**			
	Genbank Accession number	**Genetic related Country**	**Genbank Accession number**	**Identity (%)**

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Cysticercustenuicollis IQ-goat No.1	OQ506463	China	UXP71673.1	89.67%
Cysticercustenuicollis IQ-goat No.2	OQ506464	China	UXP71673.1	89.67%
Cysticercustenuicollis IQ-goat No.3	OQ506465	China	UXP71673.1	89.67%
Cysticercustenuicollis IQ-goat No.4	OQ506466	China	UXP71673.1	89.67%
Cysticercustenuicollis IQ-goat No.5	OQ506467	China	AGJ83395.1	90.78%

Identification of genetic mutations on local isolates using the COX1 gene

The results of comparison between the sequences of pairs of nucleotides indicated the presence of genetic mutations between the samples in the amino acid sequences using the COX1 gene, as in Table (1-7).

Table (1-7) positions of mutations in the protein sequences of the cox1 gene among isolates from the same study.

No.	Isolation number	Mutation place	Type	Total
1	OQ748015	7	R instead of S	4
		32	R instead of S	
		112	R instead of S	
		113	R instead of S	
2	OQ748017	7	R instead of S	4
		32	R instead of S	
		112	R instead of S	
		113	R instead of S	
3	OQ748016	7	R instead of S	3
		32	R instead of S	
		113	R instead of S	

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4	OQ748014	7	R instead of S	3
		32		
		113		

Identification of genetic mutations on local isolates using the Cytb gene

The results of comparison between the sequences of pairs of nucleotides indicated the presence of genetic mutations between the samples in the amino acid sequences using the Cytb gene, as in Table (1-8).

Table (1-8) positions of mutations in the protein sequences of the Cytb gene among the isolates of the same study

No.	Isolation number	Mutation place	Type	Total
1	Q506454	37	S instead of G I instead of V V instead of E	3
		45		
		104		
2	Q506457	44	M instead of T V instead of L W instead of S V instead of L	4
		46		
		108		
		145		
3	Q506453	82	G instead of V Deletion mutation	3
		90		
		128		

Identification of genetic mutations in local isolates using the Nad4 gene

The results of comparison between the sequences of pairs of nucleotides indicated the presence of genetic mutations between the samples in the amino acid sequences using the Nad4 gene, as in Table (1-9).

Table (1-9) positions of mutations in the protein sequences of the Nad4 gene among isolates from the same study

No.	Isolation number	Mutation place	Type	Total
1	Q506463	74	L instead of F G instead of V Deletion	3
		82		
		120		
2	Q506464	29	S instead of G I instead of V	3
		37		

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		96	V instead of E	
6	Q506465	36	V instead of L W instead of S P instead of L	4
		38		
		100		
		137		

Discussion

Bladder cysts were collected from 672 samples from sheep slaughtered in Diwaniyah and Muthanna governorates. The number of vesical cysts in one animal ranged between 1-12 cysts in the affected organs, and the largest percentage was 1-2 cysts, reaching 90.92%, and the lowest percentage was 7 cysts. -9. The current study agreed with a study by Mohammed and Kadir (2019), conducted in the city of Sulaymaniyah in Iraq. The reason may be due to the duration of the infection and the number of eggs that infect the organs. If the new infection and the number of eggs is small, the number of cysts decreases and vice versa (Miller et al., 2012).

Bladder cyst shapes and sizes

The current results showed that the shapes of vesical cysts in goats were milky white in color, and a smaller percentage had a yellowish color. The cysts were also very thin and transparent, attached or hanging to the surface of the organ, resembling a balloon or bubble, and containing a similar transparent watery fluid. It is similar to water, but its viscosity is higher. The tissue fluid contains average high concentrations of glucose in goats (Mokhtaria et al., 2018). As for the sizes of these cysts, their diameters ranged between 1-12 cm, depending on the affected organ, the duration of the infection, and the age of the animal. It was found that the liver cysts were Its size was 1-2 cm, while in the peritoneal cavity it was 1-12 cm.

The study agreed with Al-Hamzawi's study (2020) in Diwaniyah Governorate and (2022). Dey et al in Bangladesh in the shape, color and size of the cysts, and the reasons for the differences in the lengths and sizes of the cysts are due to the duration of the infection, the age of the cyst, and the locations of the cyst (Miller et al., 2012; Jayousi et al., 2014).

Numbers and measurements of hooks in the snout of larvae:

The results of the current study showed that the snout measurement of larvae in sheep samples reached 500 micrometers (mμ), and this was confirmed by Al-Hamzawi and Al-Mayali (2020) when measuring the snout of five larvae from sheep samples, as it reached 500 mμ. As for the gnats, their surrounding numbers were calculated. With the snout in 10 samples. The length of the blades of the large hooks was measured and their average length was 0.82±80.35 microns, while the total length of the small hooks was 115-140 μm and the average total length of the small hooks was 2.56±121.25 microns, and their average length was 175-205 μm. Blades Small hooks 2.03±55.72 microns. The results of the current study agreed with a study in India (Singh et al., 2015), and the results of the current study agreed with a study in Diwaniyah Governorate (Al-Hamzawi and Al-Mayali, 2020).

Molecular study

The PCR method was used for three mitochondrial genes (COX1, Cytb, Nad4), and primers carrying molecular weights (bp, 568bp, 444bp 680), respectively (Liu et al., 2012), and the results of the genetic sequences of the local strains of the parasite were shown in 5 samples in the current study. Those recorded in the International GenBank showed similarity to the samples registered in the International GenBank, with similarity rates ranging between 99-100%.

The results of the genetic sequencing of the cytochrome oxidase gene (COx1gene) for the local strains of the parasite in the current study were 100% similar to the Turkish strain (MK851045), the Iranian strain (KR337823.1), and the Egyptian strain (OQ317833), while it was 99.74% identical to the Saudi

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strain (MZ277312), and the Indian strain (LC708281), and this is consistent with the study of Hama-Soor et al, (2021) in Iraqi Kurdistan. And (Yaseen, (2022) in Baghdad, and Al-Hamzawi (2020) in Diwaniyah.

The results of the genetic sequencing of the cytochrome b gene (Cytb gene) and the Nad4 gene NADH dehydrogenase subunit 4 for the local strains of the parasite in 5 samples were similar to the Chinese isolates (QSV08807.1, MT784875.1) in the Cytb gene, with percentages ranging between 97.62-99.27%, respectively. As for the Nad4 gene, the isolates were similar to the Chinese isolate (KC794831.1, ON379164.1), with percentages ranging from 98.90 - 98.58%, respectively.

Bowles et al., (1992) pointed out that the COX1 gene of tapeworms is the gene responsible for classifying the internal and external variables of these worms. Saarma et al., (2009) explained that one of the most important features of the COX1 gene is its presence in more than one copy per cell, and it has a high diagnostic value, especially when samples are broken. In addition, the COX1 gene contains a high rate of mutations and is subject to very little or no recombination (that is, mutations gradually accumulate), and the Nad gene, which contains subunits. (Nad2, Nad3, Nad5, Nad6) may be useful in molecular studies and genetic differences of Taenia species (Jia et al., 2010).

Analysis of nucleotide and protein sequence results

Sequencing results showed that the amino acids of the parasite strains in 5 samples of the current study, which were registered in the International GenBank, ranged between 96.15-97.85% for the COX1 gene, and the match rate with the Iranian isolate (AFL69973.1) recorded in Iran was 96.15%, and the Turkish isolate (AFA53641). 1) The match rate recorded in Turkey was 97.85%. As for the Cytb gene, the results of the amino acid sequences of the parasite strains showed that the percentage of similarity between the highest and lowest similarity percentage ranged between 90.21-96.36% with the Chinese isolate (ACN72662.1), and for the NAD4 gene the percentage ranged between 89.67-90.78% with Chinese isolates (UXP71673.1 and AGJ83395.1), respectively.

The reason for the conformity of the isolates of the current study with the Iranian and Turkish strains may be due to the close borders between these countries and the exchange of animal trade with the Kurdistan region or with other governorates or between Kurdistan and the rest of the Iraqi governorates, including Al-Muthanna and Diwaniyah governorates.

As for the similarity with Chinese breeds, these breeds may have a global spread, and this is confirmed by previous studies in Iraq and outside Iraq mentioned previously, or because of the origins of the goats found in Iraq, or the spread of the aforementioned genetic studies in China.

Identification of genetic mutations on local isolates

The results of the current study showed the presence of mutations among the local isolates at the amino acid sequence level, as 14 basic mutations were found in the amino acid sequence in the COX1 gene, while in the Cytb gene, 13 basic mutations occurred, in different locations, and a deletion mutation appeared, and as for the Nad4 gene, it occurred. Also 13 mutations, with different locations and the appearance of a deletion mutation.

Ohiolei et al., (2019) in Nigeria, confirmed the study of three types of mitochondrial genes (cox1, Nad1, Nad5) in sheep and goat samples to describe and compare the genetic diversity of the T. hydatigena parasite in different regions of Nigeria and noted the presence of mutations in the amino acid sites. Goat isolates are genetically distinct from sheep isolates, meaning that the genetic diversity is higher for goat isolates than sheep isolates. The results of the current study indicate that there are genetic differences between the studied isolates in sheep and goats, and the reason may be due to the presence of new and different strains among the study samples belonging to the same T parasite. hydatigena. This confirms the presence of phenotypic differences in the cysts in terms of shapes and sizes, as well as the number and size of thorns, and this is consistent with what was indicated by the study of Singh et al., (2015) in India, which indicated that the difference in phenotypic characteristics may be due to the presence of two strains that infect goats and sheep. Phenotypic differences can be used as a means of distinguishing between T. hydatigena parasite cysts.

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It is consistent with the study of Varcasia et al., (2012) in the United Arab Emirates, which indicated that differences in the sequence of nitrogenous bases help parasites adapt quickly to new intermediate host types and infection sites occupied by larval stages.

References

1. Al-Hamzawi, Ihab Ghanem Mutashar (2020). Study of the genetic sequence of the parasite Taenia hydatigena and the pathological and epidemiological changes of the larval stage in sheep and goats. Master's thesis, College of Education/Life Sciences - Al-Qadisiyah University, number of pages: 92.
2. Gori F, Armua-Fernandez MT, Milanesi P, Serafini M, Magi M, Deplazes P, et al. (2015). The occurrence of taeniids of wolves in Liguria (northern Italy). International Journal for Parasitology: Parasites and Wildlife 4:252–255.
3. Sulieman, Y., Zakaria, M. A., and Pengsakul, T. (2020). Prevalence of intestinal helminth parasites of stray dogs in Shendi area, Sudan. Annals of Parasitology, 66(1), 115-118.
4. Lesniak, I., Heckmann, I., Heitlinger, E., Szentiks, C. A., Nowak, C., Harms, V., ... and Krone, O. (2017). Population expansion and individual age affect endoparasite richness and diversity in a recolonising large carnivore population. Scientific Reports, 7(1), 1-14.
5. Omar, M.A.E., Elmajdoub, L.O., Al-Aboody, M.S., Elsify, A.M., Elkhtam, A.O. and Hussien, A.A. (2016). Molecular characterization of *Cysticercus tenuicollis* of slaughtered livestock in upper Egypt governorates. Asian Pac. J. Trop. Biomed., 6: 706–708.
6. OIE, Terrestrial Manual. (2008). Chap. 2.1.4In: Echinococcosis / Hydatidosis , Paris, pp 175–189.
7. Pathak, K. M. L. and Gaur, S. N. S. (1982). The incidence of adult and larval stage *Taenia hydatigena* in Uttar Pradesh (India). Vet. Parasitol., 10(1):91–95.
8. Jensen, R., and Pierson, R. E. (1975). Cysticercosis from *Taenia hydatigena* in feedlot lambs. Journal of the American Veterinary Medical Association, 166(12), 1183-1186.
9. Murai, É., and Sugár, L. (1979). Taeniid species in Hungary (Cestoda, Taeniidae). I. Cysticercosis, coenurosis and hydatidosis of wild ungulates. Parasitologia Hungarica, 12, 41-52.
10. Hall, M. (1986). Disease and parasites of livestock in the tropics, Economic and zoonotic importance. The huge, martins.
11. Zar, J. H. (1984). "Multisample hypotheses: the analysis of variance." Biostatistical analysis 3: 179-210.
12. Nikmanesh, B., Mirhendi, H., Ghalavand, Z., Alebouyeh, M., Sharbatkhori, M., Kia, E., ... and Rokni, M. B. (2014). Genotyping of Echinococcus granulosus isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. Iranian journal of parasitology, 9(1), 20.
13. Liu, G. H., Li, B., Li, J. Y., Song, H. Q., Lin, R. Q., Cai, X. Q., ... and Zhu, X. Q. (2012). Genetic variation among Clonorchis sinensis isolates from different geographic regions in China revealed by sequence analyses of four mitochondrial genes. *Journal of helminthology*, *86*(4), 479-484.
14. Mohammed, A. and M. Kadir. (2019). Frequency of *Taenia hydatigena* cysts in slaughtered sheep in Sulaymaniyah province/Iraq. Revue Méd. Vét.,170. 144-148.
15. Miller, J. E., Kaplan, R. M., and Pugh, D. G. (2012). Internal parasites. Sheep and goat medicine, 106.
16. Mokhtaria, K., Fadela, S., Ammar, S. S. M., Belcacem, B. T., Ammar, A. A., Ameur, A. S., and Abdelkader, B. (2018). *Cysticercus tenuicollis* in small ruminants of Algeria: abattoir survey, biochemical and morphological characterizations. Bulgarian Journal of Agricultural Science, 24(4), 698-703.
17. Dey, A. R., Hasan, M., Hoque, M. R., Siddiqui, T. R., and Alam, M. Z. (2022). Morphometry and genetic diversity pattern of *Cysticercus tenuicollis*, an important food-borne taeniid metacestode in goats in

Bangladesh. Infection, Genetics and Evolution, 105, 105364

18. Jayousi, A.A.Y. (2014). Prevalence and molecular characterization of *Cysticercus tenuicollis* cysts in sheep slaughtered in Palestine. M.Sc. Thesis. An-Najah University.Pp.77.
19. Al-Hamzawi, E. G., and Al-Mayali, H. M. H. (2020). Morphological characterizations of *Cysticercus tenuicollis* of Taenia hydatigenia isolated from sheep and goats slaughered in Al-Diwaniyah Province Abattoirs, Iraq. EurAsian Journal of BioSciences, 14(1), 2261-2266.
20. Hama-Soor, T. A., Mohammed, A. A., and Weli, S. (2021). Novel isolate of *Cysticercus tenuicollis*-Kalar isolate has been revealed in Kalar, Iraq. J. Infect. Dev. Ctries., 15(10): 1532–1538.
21. Yaseen,G.D.(2022). Prevalence, Morphological and Molecular Identification of *Taenia hydatigena* in Baghdad City. Council of the College of Veterinary Medicine/ University of Baghdad.
22. Bowles, J., Blair, D. and McManus, D.P. (1992): Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol., 54(2): 165 – 173.
23. Saarma, U., Jõgisalu, I., Moks, E., Varcasia, A., Lavikainen, A., Oksanen, A., ... and Maravilla, P. (2009). A novel phylogeny for the genus Echinococcus, based on nuclear data, challenges relationships based on mitochondrial evidence. Parasitology, 136(3), 317-328.
24. Jia, W. Z., Yan, H. B., Guo, A. J., Zhu, X. Q., Wang, Y. C., Shi, W. G., ... and Cai, X. P. (2010). Complete mitochondrial genomes of Taenia multiceps, T. hydatigena and T. pisiformis: additional molecular markers for a tapeworm genus of human and animal health significance. *BMC genomics*, *11*, 1-13.
25. Ohiolei, J. A., Luka, J., Zhu, G. Q., Yan, H. B., Li, L., Magaji, A. A., Alvi, M. A. Wu, Y. T., Li, J. Q., Fu, B. Q. and Jia, W. Z. (2019). First molecular description, phylogeny and genetic variation of *Taenia hydatigena* from Nigerian sheep and goats based on three mitochondrial genes. Parasite Vector, 12(1):520
26. Singh, B. B., Sharma, R., Gill, J. P. S., and Sharma, J. K. (2015). Prevalence and morphological characterisation of *Cysticercus tenuicollis* (*Taenia hydatigena* cysts) in sheep and goat from north India. *Journal of Parasitic Diseases*, *39*, 80-84.
27. Varcasia A, Jia WZ, Yan HB, Manunta ML, Pipia AP, Garippa G, Scala A, Schuster RK. 2012. Molecular characterization of subcutaneous and muscular coenurosis of goats in United Arab Emirates. *Veterinary Parasitology*, 190, 604–607.