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Genetic variation of *Taenia saginata* cyst isolates from Iraq based on mitochondrial COX1 sequences

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Summary

The zoonotic parasite disease of economic and public health relevance is bovine cysticercosis, resulting from the larval stage of *Taenia saginata*. The presented research aims to identify intraspecific variation in *T. saginata* isolated from cattle in Iraq's Sulaymaniyah province using the mitochondrial cytochrome c oxidase subunit 1 (COX1) gene. Sequence analysis of the COX1 gene revealed that five distinct haplotypes were identified in 37 *T. saginata* specimens from Iraq. Four of the five *T. saginata* haplotypes may have been identified for the first time in the world. Phylogenetic research revealed that all *T. saginata* haplotypes had been clustered in a single clade, with Korean and Iranian isolates sharing a high degree of closeness. In addition, individual haplotypes related to COX1 had a pairwise evolutionary divergence of 0.005- 0.013, whereas the overall evolutionary divergence regarding all five haplotypes ranged between 0.000-0.018. It was concluded that added newly recorded data on *T. saginata* genetic variation could have substantial implications for taeniasis epidemiology and control.

Keywords: Genetic diversity; *Taenia saginata*; COX1; cattle; Iraq

Introduction

Cysticercus bovis, the larval stage of *T. saginata*, causes bovine cysticercosis. Cattle get infected with *T. saginata* by ingesting the eggs excreted from humans infected with *T. saginata.* Humans infected by consuming beef infected with larval cysticercus of *T. saginata* (*C. bovis*) (Abusier *et al*., 2007; Ogunremi &Benjamin, 2010). A bovine carcass infected with *C. bovis* might contaminate 8 – 20 individuals (Sato *et al*., 2018). *T. saginata* is found worldwide and affects developing and industrialized countries (Dorny *et al.,* 2000; Silva & Costa-Cruz, 2010). Cysticercosis usually results in few clinical signs or is asymptomatic, especially if the infection is mild. On the other hand, cases are accountable for significant economic losses in the meat industry (WHO, 2005; Torgerson, 2013). Typically, cysticercosis is diagnosed through macroscopic examination throughout carcasses' post-mortem inspections; yet, the approach was criticized for its low sensitivity in the detection of cysticercosis (Geysen *et al*., 2007) and diagnostic competence (Abuseir *et al*., 2006). Molecular methods, like PCR, have excellent specificity and sensitivity, allowing for accurate differentiation and identification of *Taenia* species while overcoming various drawbacks of traditional approaches (Yamasaki *et al.,* 2004; Gonzalez *et al.,* 2004; Sato *et al.,* 2018).

Advanced tools for detecting and researching the relation between taeniid species have been developed thanks to the introduction of molecular genetic approaches. Mitochondrial DNA sequencing has proven helpful in identifying and genetically characterizing such parasites (Bowles & McManus, 1994). Mitochondrial genes,

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Supplementary Table 1. *T. saginata* and other taeniids COX1 nucleotide sequences from GenBank were utilized for genetic diversity and phylogeny.

Taeniid parasites	GenBank accession no.	Origin	Host	Author (reference)
T. saginata	AY684274	Korea	Human	Jeon *et al.,* 2007
T. saginata	AB533173	Thailand	Human	Okamoto, 2016 unpublished
T. saginata	AB465242	Thailand	Human	Okamoto *et al.,* 2010
T. saginata	JQ756969–JQ756979	Iran	Cattle	Rostami *et al.,* 2015
T. asiatica	AB597287	Japan	Human	Yamasaki *et al.,* 2021
T. solium	AB066491	Ecuador	Pig	Nakao *et al.,* 2002
T. multiceps	JX535576	China	Sheep	Li *et al.,* 2013
T. hydatigena	MT784895	China	Dog	Ohiolei *et al.,* 2021
Echinococcus granulosus	MW214711	Iran	Sheep	Babaei *et al.,* 2021

particularly COX1, are widely used markers for the identification of the helminth parasite (Gasser *et al*., 1999). In addition, the parasites' genetic population structure can help with epidemiological investigations and focus on their evolutionary history (Campbell *et al*., 2006; Anantaphruti *et al*., 2013). Knowing the parasite's population variations aids in the analysis of transmission patterns (Pajuelo *et al.,* 2017).

Several studies have looked into human taeniid tapeworm genetic variations, but most studies focused on *T. solium* and human cysticercosis (Rostami *et al.,* 2015). There's a scarcity of knowledge on genetic variation in *T. saginata* from various world regions. In 2007, the whole mitochondrial genome of *T. saginata* was reported (Jeons *et al*., 2007). *T. saginata* was genetically characterized in Ethiopia and Thailand (Okamoto *et al*., 2010; Hailemariam *et al*., 2014). In surrounding countries, Jahed Khaniki *et al.* (2009) recorded a low rate of *C. bovis* infection in Iran (0.25 %), as well as Kus *et al.* (2014) reported that the prevalence of infection in Turkey ranged from 0.3 to 30 %.In Iraq, thorough molecular investigations are necessary to improve our understanding of such species' genetic diversity and to develop an efficient parasite vaccine. In Iraq, epidemiological data are scarce, and the available pieces of literature are few; the prevalence of bovine cysticercosis in cattle, buffaloes, and taeniasis in humans were recorded in Iraqi province (San & Zana, 2017; Al-Jadar & Hayatee 1988; Kadir & Salman, 1999; Musa, 2017; Al-Saqur *et al.,* 2020).Because genetic information on Iraqi *T. saginata* isolates is scarce, this study was performed using COX1 sequences to analyze the intra-specific variation of *T. saginata* isolates acquired from cattle in the Sulaymaniyah province of Iraq.

Materials and Methods

From December 2020 until May 2021, 37 *T. saginata* cysts were obtained from cattle in the Modern Sulaimani Slaughterhouse in the Sulaymaniyah district of Iraq. The specimens were disinfected and preserved using 70 % ethyl alcohol. Following the manufacturer's instructions, genomic DNA was extracted from each scolex using the *EasyPure*™ Genomic DNA kit (Trans Gen Biotech Co., China) and stored at -20°C. JB3 (forward): 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and JB4.5 (reverse):5'-TAAAGAAAGAACATAATGAAAATG-3' were employed for amplifying a 400-bp-long mitochondrial COX1 gene fragment (Bowles *et al*., 1992). The amplification was carried out with *f-Pfu* DNA polymerase (SBS Genetech Co., China) under the same circumstances as Rostami *et al.* (2015).

SiMax PCR Products/Agarose Gel Purification Kit was used to purify the purified DNA fragments from agarose gel (SBS Genetech Co.). An ABI -3730XL capillary machine was used to sequence the purified DNA (Macrogen Inc., South Korea). In BioEdit software, sequences were aligned with ClustalW multiple sequence alignments (Hall, 1999). Also, the representative COX1 nucleotide sequences have been submitted to the NCBI and are accessible in the Gen-Bank database under the accession numbers OK036447 – OK036451.Using the maximum likelihood method, a phylogenic

Supplementary Table 2. Depending on mitochondrial COX1 sequences and accession numbers, the distribution pattern related to *T. saginata* haplotypes in Iraqi cattle.

Taenia saginata haplotypes	No. of samples (n)	GenBank accession no.
IQTS-H1	17	OK036447
IQTS-H2	8	OK036448
IQTS-H3	6	OK036449
IQTS-H4	4	OK036450
IQTS-H5	2	OK036451

Table 1. Pairwise evolutionary divergence in COX1 gene sequences between *T. saginata* and other taeniids.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	IQTS-H1:OK036447																								
2	IQTS-H2:OK036448	0.005																							
3	IQTS-H3:OK036449	0.005	0.011																						
4	IQTS-H4:OK036450	0.005	0.011	0.010																					
5	IQTS-H5:OK036451	0.008	0.013	0.013	0.013																				
6	T.saginata:JQ756969(Iran)	0.000	0.005	0.005	0.005	0.008																			
7	T.saginata:JQ756970(Iran)	0.003	0.008	0.003	0.008	0.011	0.003																		
8	T.saginata:JQ756971(Iran)	0.003	0.003	0.008	0.008	0.011	0.003	0.005																	
9	T.saginata:JQ756972(Iran)	0.003	0.008	0.008	0.003	0.010	0.003	0.005	0.005																
10	T.saginata:JQ756973(Iran)	0.003	0.003	0.008	0.008	0.011	0.003	0.005	0.005	0.005															
11	T.saginata:JQ756974(Iran)	0.003	0.008	0.008	0.003	0.011	0.003	0.005	0.005	0.005	0.005														
12	T.saginata:JQ756975(Iran)	0.011	0.016	0.005	0.016	0.018	0.011	0.008	0.013	0.013	0.013	0.013													
13	T.saginata:JQ756976(Iran)	0.005	0.011	0.011	0.011	0.013	0.005	0.008	0.008	0.008	0.008	0.008	0.016												
14	T.saginata:JQ756977(Iran)	0.008	0.013	0.013	0.013	0.016	0.008	0.010	0.010	0.010	0.010	0.010	0.018	0.013											
15	T.saginata:JQ756978(Iran)	0.013	0.018	0.018	0.018	0.010	0.013	0.016	0.016	0.016	0.016	0.016	0.024	0.018	0.018										
16	T.saginata:JQ756979(Iran)	0.005	0.005	0.011	0.011	0.008	0.005	0.008	0.003	0.008	0.008	0.008	0.016	0.011	0.013	0.018									
17	T.saginata:AB465242(Thailand)	0.003	0.008	0.008	0.008	0.011	0.003	0.005	0.005	0.005	0.005	0.005	0.013	0.008	0.010	0.016	0.008								
18	T.saginata:AB533173(Thailand)	0.003	0.003	0.008	0.008	0.011	0.003	0.005	0.000	0.005	0.005	0.005	0.013	0.008	0.010	0.016	0.003	0.005							
19	T.asiatica:AB597287(Japan)	0.027	0.027	0.032	0.032	0.035	0.027	0.029	0.024	0.029	0.029	0.029	0.038	0.032	0.035	0.038	0.027	0.029	0.024						
20	T.saginata:AY684274(Korea)	0.000	0.005	0.005	0.005	0.008	0.000	0.003	0.003	0.003	0.003	0.003	0.011	0.005	0.008	0.013	0.005	0.003	0.003	0.027					
21	T.solium:AB066491(Ecuador)	0.125	0.119	0.132	0.132	0.128	0.125	0.129	0.122	0.128	0.122	0.129	0.138	0.129	0.135	0.138	0.119	0.129	0.122	0.138	0.125				
22	T.multiceps:JX535576(China)	0.058	0.058	0.063	0.063	0.066	0.058	0.061	0.055	0.060	0.061	0.061	0.069	0.063	0.066	0.072	0.058	0.061	0.055	0.075	0.058	0.107			
23	T.hydatigena:MT784895(China)	0.124	0.127	0.131	0.131	0.134	0.124	0.127	0.127	0.127	0.124	0.127	0.137	0.124	0.134	0.137	0.131	0.127	0.127	0.143	0.124	0.146	0.134		
24	E.granulosus:MW214711(Iran)	0.216	0.212	0.223	0.219	0.219	0.216	0.219	0.212	0.219	0.216	0.216	0.230	0.219	0.219	0.230	0.209	0.212	0.212	0.234	0.216	0.212	0.237	0.189	

tree was generated based on the reference sequences of *T. saginata* and related species (Supplementary Table 1). Genetic distances were estimated using Kimura's 2-parameter model, and the tree topology's robustness was assessed using a bootstrap value of 1000 repetitions using datasets available in MEGA 7 version 7. (Kumar *et al.,* 2016). The maximal composite possibility model has been utilized to calculate the evolutionary divergence regarding nucleotide sequences of *T. saginata* from the present and previously published studies for further analysis (Kumar *et al.,* 2016).

Ethical Approval and/or Informed Consent

The Ethical Committee approved the study protocol of the University of Sulaimani's Veterinary Medicine College. All the samples used in this study were taken post-mortem from discarded infected carcasses unfit for human consumption. No animals were killed in the course of this investigation.

Results

The COX1 gene was successfully amplified in all 37 samples. A 384-bp fragment was obtained after the sequences' trimming and editing. The COX1 gene sequence analysis revealed five distinct haplotypes were identified, designated as IQTS-H1 (n=17), IQTS-H2 (n=8), IQTS-H3 (n=6), IQTS-H4 (n=4), and IQTS-H5 (n=2) (Supplementary Table 2). The pairwise evolutionary divergence between different COX1 haplotypes has been found to range between 0.005 – 0.013, whereas the available nucleotide variation among all five haplotypes is 0.000 – 0.018 (Table 1). In total, nine mutations in the COX1 gene were found in nine segregation sites. The Maximum likelihood approach was used to create a phylogram based on COXI gene sequences. Phylogenetic relationships revealed that all *T. saginata* haplotypes had been clustered in a single clade, with Korean and Iranian isolates sharing a high degree of closeness (Fig. 1).

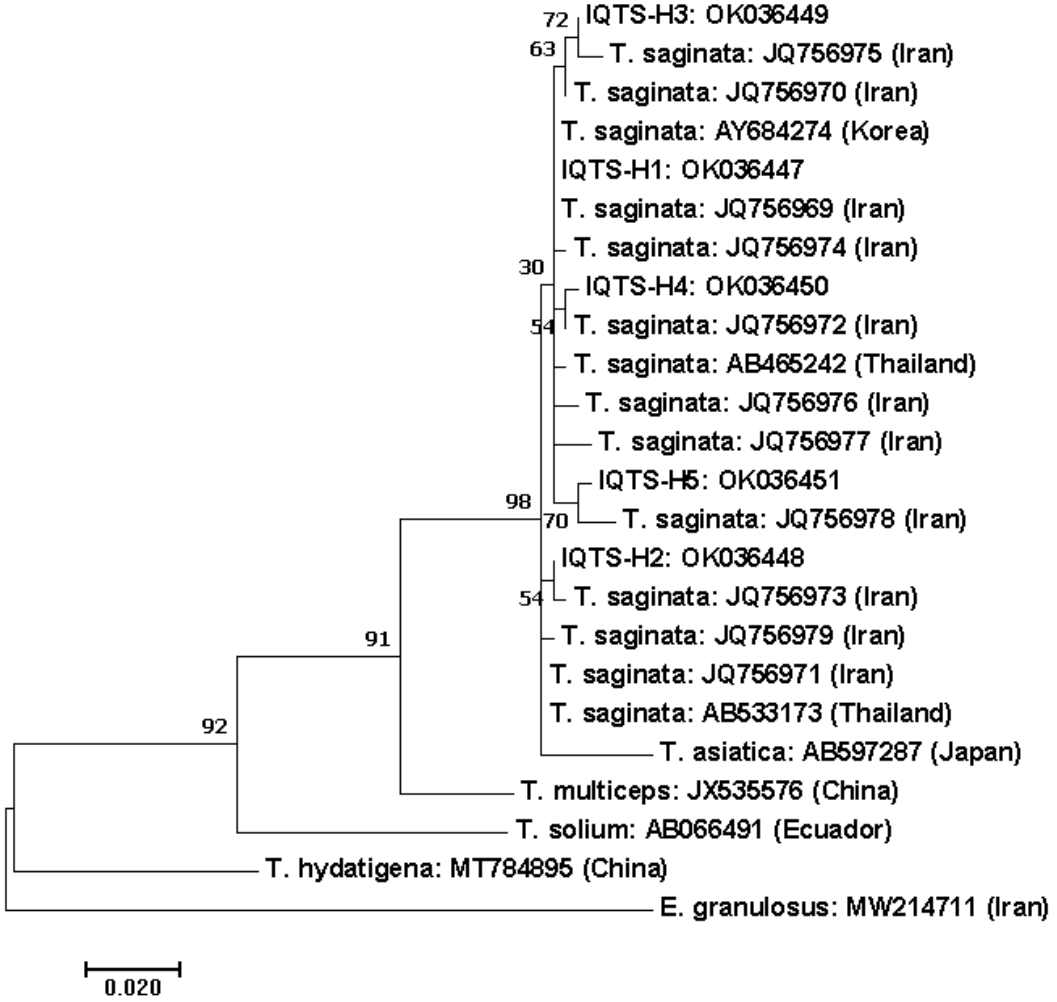

Fig.1. Phylogenetic relation of *T. saginata* from the present study and other taeniids. Phylogenetic tree was constructed using maximum likelihood method (Kimura's 2-parameter model) based on partial COX1 sequences. Sequences reported in the present study are shown as IQTS-H1– IQTS-H5 with accession numbers (OK036447– OK036451), respectively.

Table 2.The five haplotypes of *T. saginata* COX1 sequences from Iraqi cattle were put to comparison with reference COX1 sequence from Korea (accession number AY684274) for the substitutions of the nucleotide and corresponding amino acid variations.

Haplotypes of the current study	Position of nucleic acid substitution	Amino acid substitution
IQTS-H1	–*	–*
IQTS-H2	197 (A→G)	Glu→Gly
	332 (C→T)	Ala→Val
IQTS-H3	70 (T→A)	Leu→Met
	356 (T→C)	Ile→Thr
IQTS-H4	10 (G→C)	Ala→Leu
	371 (T→C)	Leu→Ser
IQTS-H5	39 (C→T)	Val→Val
	146 (G→A)	Arg→Lys
	195 (G→C)	Gln→His

*No substitution.

Discussion

Phylogenetic studies of distinct *Taenia* species have applied various genomic areas, such as 18-S and 28-S ribosomal RNA, in addition to the mitochondrial genes (Hoberg, 2006; Yan *et al*., 2013). In addition, sequence fragment analysis depending on PCR synthesis of such taeniids' DNA is one of the molecular methods frequently employed for phylogenetic investigations (Nickish-Rosenegk *et al.,* 1999). Gonzalez *et al.* (2011) sequenced the appropriate sequences from all taeniid isolates after PCR-amplifying them using particular primers.

This work studied the mitochondrial COX1 gene diversity of 37 *T. saginata* cattle specimens from Sulaymaniyah, Iraq. COX1 sequence analysis revealed nine nucleotide substitutions, three of which have been nucleotide transversions, in five *T. saginata* haplotypes. The haplotypes detected eight amino acid alterations attributable to eight nucleotide substitutions (Table 2). There is no indication that cytochrome c oxidase amino acid composition changes impact the parasite adaptability or enzyme's function. Yet, it was demonstrated in other parasite species that a single amino acid change could influence the biological fitness of an organism (Tachibana *et al*., 2004; Otsuki *et al*., 2009). COX1 nucleotide variation was determined to be 0.027 – 0.134 across *T. saginata* isolates from this investigation and six other *Taenia* species. Bowles and McManus (1994) and Rostami *et al.* (2015) calculated the predicted nucleotide variations in COX1 in genus *Taenia* to be 0.025 – 0.158 and 0.026 – 0.141, respectively. Compared to other haplotypes of such taeniid, COX1 of *T. saginata* showed a relatively low degree of variation. Similar results were observed by Abuseir *et al.* (2018) in Germany. In contrast, other studies on the genetic divergence of *T. saginata* in Asia have revealed considerably higher haplotype diversity in the COX1 gene (Anantaphruti *et al.,*2013; Sanpool *et al.,* 2017). Furthermore, pairwise comparisons of *T. saginata* from the experiment with existing mitochondrial sequences from Iranian (Rostami *et al*., 2015) and Korean (Jeon *et al*., 2007) cattle revealed 0.000 – 0.018 and 0.000 – 0.008 nucleotide differences in COX1 gene, respectively (Table 1). As a result, the phylogram revealed that the Iraqi *T. saginata* in this study was equivalent to other *T. saginata*, sharing 98.18 – 99.74 % identity with the ones from Iran and Korea. The phylogenetic analysis produced a dendrogram clustered all COX1 haplotypes uniformly to a single clade with a *T. saginata* reference sequence (AY684274 accession number). As distinct sub-clade, other types of the taeniids, including *Echinococcus granulosus, T. solium, T. multiceps, T. asiatica,* and *T. hydatigena,* were grouped together.

Understanding the control and epidemiology of parasitic infections requires molecular characterization of veterinary and medical significance parasites. *T. saginata* can be defined as one of the human and cattle zoonotic parasites with a global range (Rostmai *et al*., 2015). In the molecular epidemiological surveys of the echinococcosis/taeniasis in various host assemblages and geographical settings, DNA methods are often utilized to identify *Echinococcus* and *Taenia* species, strains, and subspecies (McManus, 2006).

Conclusion

The current study added new data about *T. saginata* mt-DNA cattle haplotypes in Iraq. *T. saginata* was made up of five haplotypes clustered in a single clade, according to phylogenetic analysis of the COX1 gene calculated using maximum likelihood. Four new strains with new mutations have been discovered in the research area. More thorough research on nuclear genes is needed to fully understand the amount and relevance of genetic variations within populations of the *T. saginata*.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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