Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: January 2021 **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2021.18.2.0238

Employing NADH Dehydrogenase Subunit 1 in the Determination of *Echinococcus granulosus* Strain in Sheep, Cattle and Human in Thi-Qar Province, Iraq

***Sarmad Awad Mozan AL-Asadi*[1*]** ***Wesam Jasim Hansh*[2]**
***Abdul-Hussien Habash Awad*[1]**

[1] Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq
[2] General Directorate of Education in Thi-Qar, Iraq
[*]Corresponding author: sarmad.mozan@uobasrah.edu.iq[*],wjhansh81@gmail.com, abdulhussein.awad@uobasrah.edu.iq
[*]ORCID ID: https://orcid.org/0000-0001-9161-753X[*], https://orcid.org/0000-0002-1891-4279, https://orcid.org/0000-0001-7618-7762

Received 26/11/2019, Accepted 15/3/2020, Published Online First 11/1/2021, Published 1/6/2021

Abstract:

Echinococcosis is a zoonotic disease caused by the larval stage of the tapeworm *Echinococcus granulosus*. This disease is an important public health and a significant economic issue in Iraq, where the lungs and livers are the popular places of infection. The aim of the current study focused on using the molecular techniques in the detection of an *E. granulosus* strain that causes cystic echinococcosis to human, sheep and cattle in Thi-Qar province, Iraq. In the current study, thirty isolates of *E. granulosus* were collected from 10 human hydatid cysts through surgery done at Al-Hussein Imam Teaching Hospital in Thi-Qar province and 10 sheep with 10 cattle hydatid cysts were obtained from the slaughterhouse in Thi-Qar province, Southern of Iraq to identify strains of *E. granulosus* which infect human and other intermediate hosts (sheep and cattle). The molecular study was carried out on the isolates and a specific primer set for the mitochondrial dehydrogenase NADH subunit 1 (NAD1) gene was used. This primer set was amplified 400 bp of the NAD1 gene in all selected isolates. The PCR products for the twelve selected isolates of *E. granulosus* (4 isolates per intermediate host) were sequenced and the results for these twelve isolates showed that all sequenced isolates, except one isolate Eg_5, belonged to the sheep strain G1 and a slight genetic diversity was observed with the reference sequences of the strain G1. The exception was in the isolate Eg_5 isolated from a cattle liver, which was similar to the buffalo strain G3. This study concludes that the common *E. granulosus* strain in Thi-Qar province is G1.

Key words: *Echinococcus granulosus*, NADH dehydrogenase subunit I, Thi-Qar.

Introduction:

Echinococcus stays considerable public health problem cosmopolitan and, in some areas, the causing agents of hydatidosis or echinococcosis are extending their range (1-3). Through the previous 40 years, laboratory and field studies have shown there is a considerable variation in phenotypic and genetic patterns between samples of *E. granulosus* isolated from different intermediate hosts (4, 5).

In *E. granuslosus*, many different phenotypic features like host specificity, antigenicity, development rate and geographical distribution may be affected by genetic variabilities. Monitoring schemes such as vaccines and the discovery and development of diagnostic reagents were confirmed through the genetic variation between *E. granulosus* populations in addition to the genetic variation within these populations (1, 6, 7). The description of *E. granulosus* strains recently depends on some morphological, molecular and biochemical techniques. To date, 9 separate strains (G1 – G9) of *E. granulosus* which were characterized (8). These strains include G1 (sheep strain), G3 and G5 (bovine strain), G4 (horse strain), G6 (camel strain), G7 (pig strain) and G8 (cervid strain). In addition to this, in Poland, G9 has been identified in human and pigs (9). However, partial sequences of NADH dehydrogenase subunit 1 (NAD1) and mitochondrial cytochrome *c* oxidase subunit 1 (CO1) genes show 10 *E. granulosus* strains (1, 5, 10). Thus, the 4 species of *E. granulosus* including *E. granulosus sensu stricto*

(G1 - G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6 – G10) are considered as the valid species, nowadays (11). The aim of the current study focused on the detection of an *E. granulosus* strain that causes cystic echinococcosis to humans, sheep and cattle in Thi-Qar province, Iraq.

Materials and Methods:

Ethics Approval

Ethic statements for collection hydatid cysts from animal and human samples were accepted by the Ethics Committee at the University of Basrah.

Collection of Hydatid Cysts

The present study was continued from the beginning of January 2019 to the end of April 2019. Hydatid cyst samples were collected from the slaughterhouse of Nassiriyah municipality in Thi-Qar province. These samples included 10 hydatid cysts for sheep (5 livers and 5 lungs), 10 hydatid cysts for cattle (6 livers and 4 lungs). In addition to this, 10 hydatid cysts from human (5 livers and 5 lungs) were collected from Al-Hussein Imam Teaching Hospital in Thi-Qar province. According to Hanifian *et al.* (12), the hydatid cyst contents (fluid and protoscolices) were aspirated aseptically then the sediment of protoscolices was stored at -20^{o}C until it was needed.

DNA Extraction

Total *E. granulosus* DNA was extracted from protoscolices stored as described above using DNA Extraction Kit from tissues (Geneaid, catalogue number GS100) according to the manufacturer's instructions. The concentration of the eluted DNA was determined using a Thermo Scientific NanoDrop® 1000 spectrophotometer. The genomic DNA was stored at -20^{o}C until it was needed.

Polymerase Chain Reaction (PCR)

The PCR was performed for amplifying the mitochondrial NAD1 gene for all samples of genomic DNA extracted from protoscolices. The primers used were forward: 5'-CGTAGGTATGTTGGTTTGTTTGGT-3' and reverse: 5'-CCATAATCAAAT GGCGTACGA T-3' that were previously designed by Sharbatkhori *et al.* (13). Each PCR reaction was carried out in a final volume of 25 µl by adding 0.5 µl of each primer, 5 µl of the genomic DNA and 14 µl of nuclease free water from Bioneer Accupower® PCR PreMix. The reaction was carried out in thermocycler under the following conditions: a pre-denaturation step at 94^{o}C for 5 min followed by 30 cycles of denaturation at 94^{o}C for 30 sec, annealing at 50^{o}C for 45 sec and extension at 72^{o}C for 30 sec plus a final extension step at 72^{o}C for 5 min. DNA samples were loaded into the appropriate wells of the TAE agarose gel (1.5% (w/v)) stained with 1 – 3 µl ethidium bromide dye. The agarose gel was run at 70V for 90 minutes. The DNA within agarose gel was visualized using UV transilluminator.

DNA Sequencing and Phylogenetic Analysis

The PCR products obtained as described above were purified utilizing the Geneaid DNA Cleanup kit (catalogue number DP100). Nucleotide sequencing of the purified PCR products was done in both directions using the forward or reverse primer for NAD1 gene. The sequencing was done by a South Korea public biotechnology company (Macrogen). The sequences of samples of the hydatid cysts were obtained from 2 hydatid cysts of liver sheep, 2 hydatid cysts of lung sheep, 2 hydatid cysts of liver cattle, 2 hydatid cysts of lung cattle, 2 hydatid cysts of liver human and 2 hydatid cysts of lung human and were dispatched to The National Centre for Biotechnology Information Service (NCBI, https://www.ncbi.nlm.nih.gov/) and the accession numbers were assigned for these sequences. The sequences of the current study were aligned and compared with each other and with those of *E. granulosus* sequences obtained from the GenBank database accessed through the "Nucleotide database" on the NCBI website. The phylogenetic tree was generated using the Neighbor-joining method accessed through Molecular Evolutionary Genetic Analysis (MEGA) version 7.0. The compute pairwise distances were also performed using MEGA version 7.0.

Results:

Mitochondrial NAD1 Gene

DNA extracted from protoscolices of 30 samples of *E. granulosus* derived from sheep (10 samples), cattle (10 samples) and human (10 samples) were examined for the molecular analysis using mitochondrial NAD1 primers. For all these samples, a fragment of ~ 400 bp was successfully PCR-amplified using the gene-specific primers (Fig. 1).

The partial sequencing of NAD1 produced a sequence of $\leq$ 400 bp for each of the chosen 12 isolates obtained from selected hosts (Table 1). The sequences obtained in the current study were deposited in the GenBank database under the accession numbers MK894989.1, MK894990.1, MK894991.1, MK904486.1, MK904489.1, MK904490.1, MK894994.1, MK894995.1, MK894992.1, MK904487.1, MK904488.1 and MK894993.1 (Table 1).

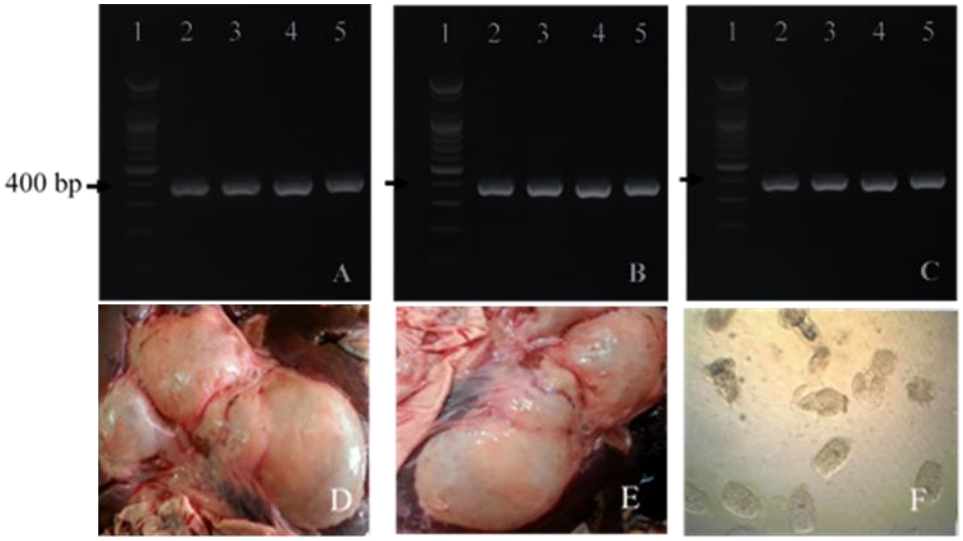

Figure 1. PCR products of NAD1 gene of *E. granulosus*. Lane 1 contains a DNA ladder and lanes 2 and 3 contain PCR products of Hydatid cysts derived from liver of sheep (A), cattle (B) or human (C). Lane 4 and 5 contain PCR products of Hydatid cysts derived from lung of sheep (A), cattle (B) or human (C). Panels D and E represent hydatid cysts and the panel F represents protoscolices of *E. granulosus*.

Table 1. *E. garnulosus* genotypes in different hosts (sheep, cattle and human) identified by NAD1. Hosts were from Thi-Qar province. – indicates a hydated cyst isolated either from liver or lung.

Haplotype	Accession No.	Host	Liver	L
1	MK894989.1	Sheep	-	
2	MK894990.1	Sheep	-	
3	MK894991.1	Sheep		
4	MK904486.1	Sheep		
5	MK904489.1	Cattle	-	
6	MK904490.1	Cattle	-	
7	MK894994.1	Cattle		
8	MK894995.1	Cattle		
9	MK894992.1	Human	-	
10	MK904487.1	Human	-	
11	MK904488.1	Human		
12	MK894993.1	Human		

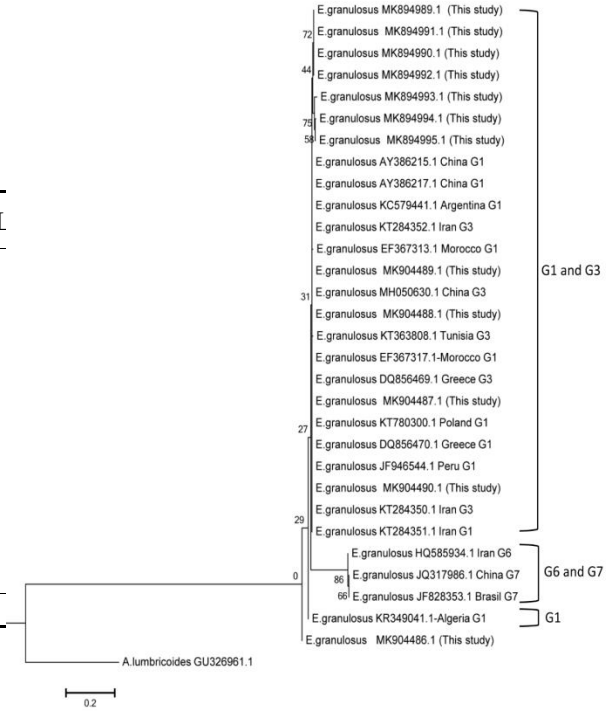

Figure 2. A Phylogenetic tree showing the relationships between reference sequences of the NAD1 genes from *E. granulosus* strains G1, G3, G6 and G7 with NAD1 sequences of the present study. An *Ascaris lumbricoides* NAD1 gene was used as the out-group to root the tree.

Phylogenetic Analysis

Figure 2 shows the phylogenetic relationships between NAD1 sequences obtained in the present study and those of reference sequences of *E. granulosus* strains (G1, G3, G6 and G7). All our isolates, except the isolate with an accession number MK904486.1, clustered together with *E. granulosus* NAD1 sequences from strains G1 and G3. In addition to this, all these isolates, including the isolate with an accession number MK904486.1, were clearly separated from the other *E. granulosus* NAD1 sequences that belong to strains G6 and G7. Thus, the majority of the isolates obtained in the current study were more similar to the common strains G1 and G3 that are responsible for the human hydatid disease in the Southern part of Iraq.

The Evolutionary Divergence between *E. granulosus* NAD1 Sequences

NAD1 nucleotide sequences of the present isolates had an identity value of 97.3 – 100% with reference sequences of *E. granulosus* strains G1 and G3 displayed in Table 2. Thus, estimates of evolutionary divergence between NAD1 nucleotide

sequences of the current study and with reference sequences of *E. granulosus* strains G1 and G3 were performed (Table 2). The sequences of isolates Eg_1 (MK894989.1) and Eg_3 (MK894991.1) had a genetic distance value of 0. 5 – 0. 8% with sequences of *E. granulosus* strain G1 whereas they possessed a genetic distance value of 0.8 –1.4% with sequences of *E. granulosus* strain G3. In addition to this, the sequences of isolates Eg_4 (MK904486.1) and Eg_8 (MK894995.1) owned genetic distance values of 0.8 – 1.1% and 1.4 – 1.6% with sequences of *E. granulosus* strain G1 while they owned genetic distance values of 1.1 – 1.6% and 1.6 – 2.2% with sequences of *E. granulosus* strain G3, respectively. Like isolates Eg_1, Eg_3, Eg_4 and Eg_8, the sequences of isolates Eg_2 (MK894990.1), Eg_11 (MK904488.1) and Eg_12 (MK894993.1) possessed a genetic distance value of 1.1 – 1.4% with sequences of *E. granulosus* strain G1 while they possessed a genetic distance value of 1.4 – 1.9% with sequences of *E. granulosus* strain G3. Similarly, sequences of isolates Eg_6 (MK904490.1), Eg_7 (MK894994.1), Eg_9 (MK894992.1) and Eg_10 (MK904487.1) possessed a genetic distance value of 0.0 – 0.3% with sequences of *E. granulosus* strain G1 while they possessed a genetic distance value of 0.3 – 0.8% with sequences of *E. granulosus* strain G3. Unlike other isolates, the isolate Eg_5 (MK904489.1) had a genetic distance value of 0.8% with *E. granulosus* strain G3 from Iran, China and Greece whereas it had a genetic distance value of 1.1 – 1.4% with sequences of *E. granulosus* strain G1. These results clearly indicate that all isolates, except the isolate Eg_5, were closely related to the strain G1. However, the isolate Eg_5 was closely related to the strain G3.

Table 2. The percentage of genetic distances among NAD1 sequences of 12 present isolates of *E. granulosus* and 12 selected sequences (strains G1 and G3) from GenBank

No.	Species	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	Eg_1_This study		0.5	0.0	1.4	0.5	0.5	0.5	1.4	0.5	0.5	1.4	1.1	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.4	0.8	0.8	0.8
2	Eg_2_This study			0.5	1.9	1.1	1.1	1.1	1.9	1.1	1.1	1.9	1.6	1.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.9	1.4	1.4	1.4
3	Eg_3_This study				1.4	0.5	0.5	0.5	1.4	0.5	0.5	1.4	1.1	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.4	0.8	0.8	0.8
4	Eg_4_This study					1.9	0.8	0.8	2.2	0.8	0.8	1.9	1.9	1.1	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.6	1.1	1.1	1.1
5	Eg_5_This study						1.1	1.1	1.9	1.1	1.1	1.9	1.6	1.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.4	0.8	0.8	0.8
6	Eg_6_This study							0.0	1.4	0.0	0.0	1.1	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
7	Eg_7_This study								1.4	0.0	0.0	1.1	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
8	Eg_8_This study									1.4	1.4	1.1	0.8	1.6	1.4	1.4	1.4	1.4	1.4	1.4	1.4	2.2	1.6	1.6	1.6
9	Eg_9_This study										0.0	1.1	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
10	Eg_10_This study											1.1	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
11	Eg_11_This study												0.3	1.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.9	1.4	1.4	1.4
12	Eg_12_This study													1.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.9	1.4	1.4	1.4
13	Eg_Algeria_G1														0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.1	0.5	0.5	0.5
14	Eg_Morocco_G1															0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
15	Eg_Iran_G1																0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
16	Eg_China_G1																	0.0	0.0	0.0	0.0	0.8	0.3	0.3	0.3
17	Eg_Greece_G1																		0.0	0.0	0.0	0.8	0.3	0.3	0.3
18	Eg_Poland_G1																			0.0	0.0	0.8	0.3	0.3	0.3
19	Eg_Peru_G1																				0.0	0.8	0.3	0.3	0.3
20	Eg_Argentina_G1																					0.8	0.3	0.3	0.3
21	Eg_Tunisia_G3																						0.5	0.5	0.5
22	Eg_Iran_G3																							0.0	0.0
23	Eg_China_G3																								0.0
24	Eg_Greece_G3																								

Nucleotide Sequence Comparison of *E. granulosus* NAD1 Genes

Figure 3 shows an alignment of NAD1 nucleotide sequences from *E. granulosus* strains G1 and G3 and *E. granulosus* isolates obtained in the current study. The reference sequences of *E. granulosus* strain G1 from Iran and China shared the same nucleotide sequences, except nucleotide adenine at the position 357 was replaced by a nucleotide guanine, with *E. granulosus* strain G3 from Iran and China. The present *E. granulosus* NAD1 sequences were highly similar to those reference sequences of strain G1 and G3 from Iran and China. However, there were 14 polymorphism sites among these NAD1 sequences obtained in the current study (Figure 3). At the position 8, the nucleotide Adenine found in strains G1 and G3 is conserved in most present isolates, except for 3 isolates (Eg_8, Eg_11 and Eg_12) it was replaced by nucleotide thymine. At the site 9, the nucleotide

thymine was replaced by nucleotide adenine in 2 isolates (Eg_11 and Eg_12) but it was conserved in the isolates Eg_1 to Eg_10. At the position 10, the nucleotide guanine was only replaced by nucleotide thymine in 5 isolates (Eg_1, Eg_2, Eg_5, Eg_8 and Eg_12) and was replaced by nucleotide cytosine in the isolate Eg_11. At the position 11, the nucleotide thymine found in strain G1 and G3 was only substituted with nucleotide guanine in 4 isolates (Eg_1, Eg_2, Eg_3 and Eg_5) whereas at the position 12, the nucleotide thymine was only substituted with nucleotide guanine in 3 isolates (Eg_8, Eg_11 and Eg_12). The nucleotide guanine was conserved in the position 28 in all isolates, except that for the isolate Eg_4 it was replaced by nucleotide cytosine. At positions 33 and 41, the nucleotides adenine and cytosine were substituted with nucleotide thymine in the isolate Eg_4, respectively. At positions 79, and 210, the nucleotides thymine was only substituted with nucleotide cytosine in the isolate Eg_8 whereas at positions 264 and 353, it was only substituted with nucleotide cytosine in isolates Eg_5 and Eg_2, respectively. In addition to this, the nucleotide thymine was only substituted with nucleotide adenine in the isolate Eg_2 at the position 358. At the position 357, the nucleotide adenine found only in strain G1 was conserved in most isolates of the current study (Eg_1, Eg_2, Eg_3, Eg_4, Eg_6, Eg_7, Eg_8, Eg_9, Eg_10, Eg_11 and Eg_12) while the nucleotide guanine found only in strain G3 was conserved in the isolate Eg_5. These results indicate the occurrence of a single nucleotide polymorphism in the isolates of the current study. The sequence of isolates Eg-7, Eg_9 and Eg_10 was identical to the sequence of the *E. granulosus* strain G1 but the sequence of other isolates was slightly different. The sequence of the isolates Eg_5 was similar to the sequence of the *E. granulosus* strain G3.

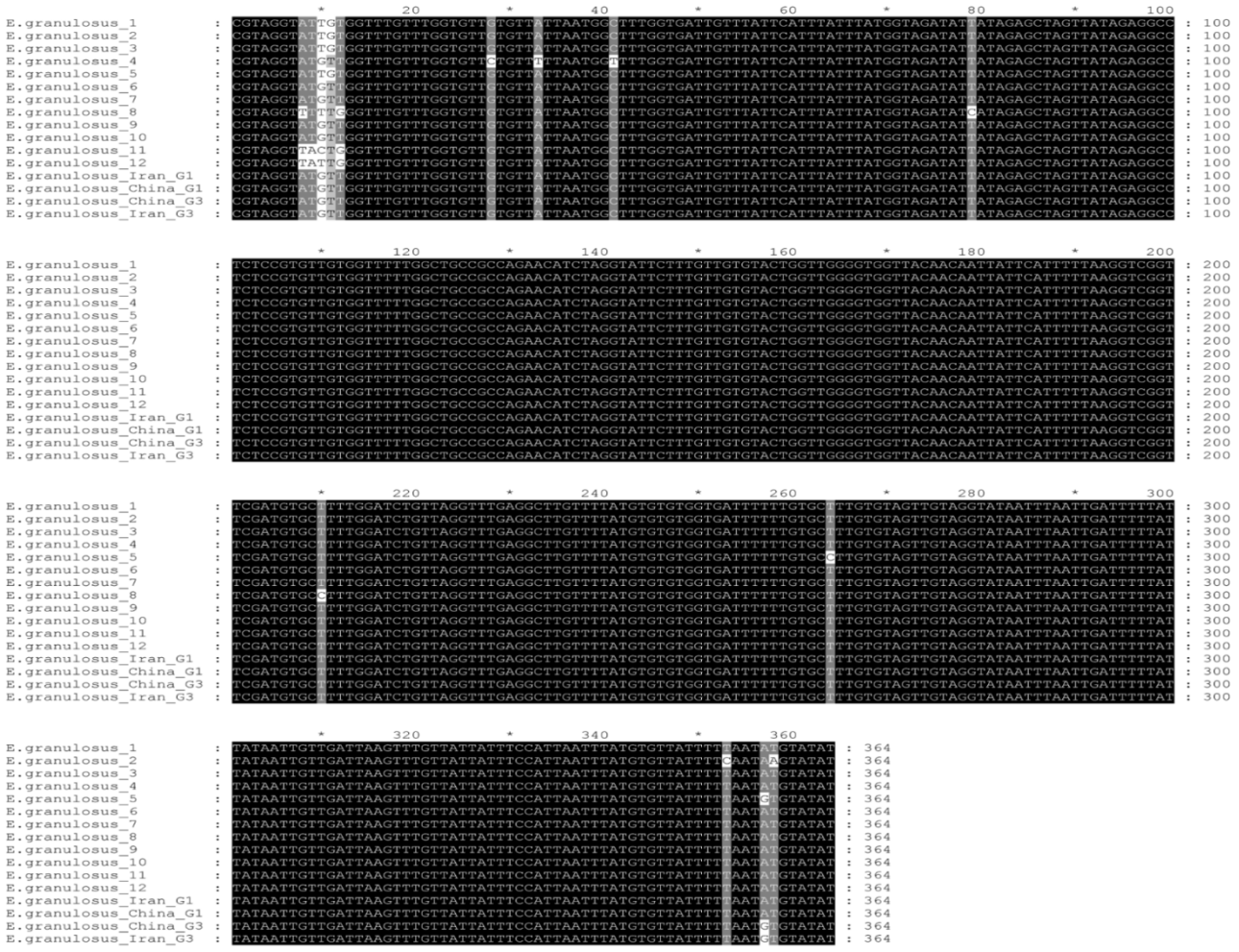

Figure 3. A nucleotide sequence alignment of NAD1 genes from *E. granulosus* strains G1 and G3 and NAD1 sequences from the present study.

Amino Acid Sequence Comparison of *E. granulosus* NAD1 Proteins

Figure 4 shows an alignment of NAD1 amino acid sequences from *E. granulosus* strains G1 and G3 and *E. granulosus* isolates obtained in the current study. The reference sequences of *E. granulosus* strain G1 from Iran and China shared the same amino acid sequences, except the isoleucine (I) residue at the position 119 was replaced by a methionine (M) residue, with *E. granulosus* strain G3 from Iran and China. The majority of the *E. granulosus* NAD1 proteins obtained in this study were highly similar to the *E. granulosus* NAD1 proteins of strain G1, especially in the isolates Eg_6, Eg_7, Eg_9 and Eg_10 in which the amino acids remained the same. On the other hand, the amino acid sequences of the isolate Eg_5 was highly similar to the *E. granulosus* NAD1 proteins of strain G3. In total, there were 7 non-synonymous substitutions, causing in the amino acid change in the isolates Eg_1, Eg_2, Eg_3, Eg_4, Eg_5, Eg_8, Eg_11 and Eg_12. The tyrosine (Y) residue at the position 3 was replaced by a leucine (L) residue in the isolates Eg_11 and Eg_12 whereas the valine (V) residue at the position 4 was replaced by either a cysteine (C) residue in the isolates Eg_1, Eg_2, Eg_3 and Eg_5 or a leucine residue in the isolates Eg_8, Eg_11 and Eg_12. In the isolate Eg_4, at the position 11, the leucine residue was substituted with a phenylalanine (F) residue while at the position 14, the A residue was substituted with a valine residue. The tyrosine residue was only replaced by a histidine (H) residue in the isolate Eg_8 at the position 27. In the isolate Eg_2, at the position 118, the leucine residue was substituted with a serine (S) residue whereas at the position 120, the cysteine residue was substituted with a serine residue.

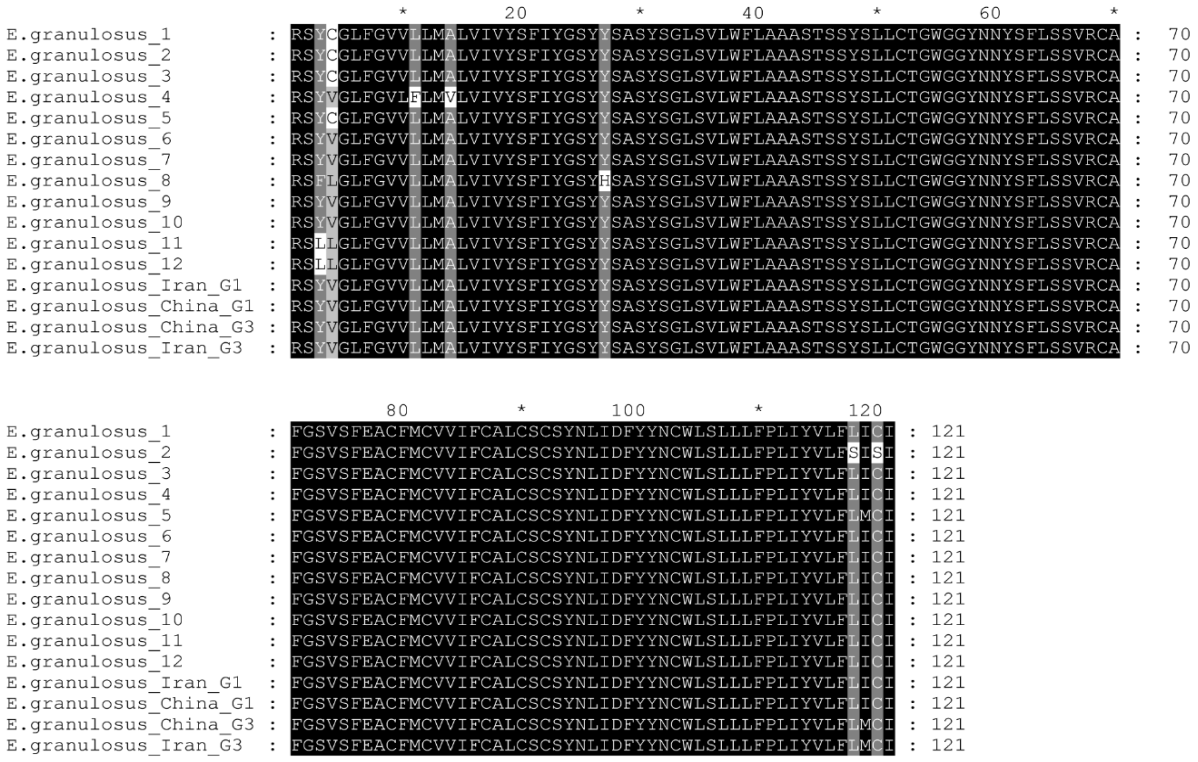

Figure 4. An amino acid sequence alignment of NAD1 proteins from *E. granulosus* strains G1 and G3 and NAD1 proteins from the present study.

Discussion

Phylogenetic analysis of the NAD1 sequences (≤ 400 bp) obtained in the current study showed the presence of the G1 strain (G1 - G3 complex) in *E. granulosus* samples collected from Thi-Qar province. Eleven isolates out of twelve confirmed the *E. granulosus* sheep strain G1 whereas 1 isolate confirmed the *E. granulosus* buffalo strain G3. These results suggest that the predominate strain of *E. granulosus* in Thi-Qar province is the sheep strain.

Globally, ten different strains of *E. granulosus* (G1 – G10) have been pronounced according to the sequence analyses of the mitochondrial genes such as cytochrome *c* subunit 1 (CO1), NAD1 (5, 10, 13, 14). *E. granulosus* isolates have been characterized in some Arabic countries such as Sudan, Lebanon and Jordan using molecular techniques (14, 15). In Iraq, few studies have

employed molecular techniques as a tool in the characterization of *E. granulosus* strains (16). One of these studies conducted in Thi-Qar province by Hansh and Awad (17). Like the results of the current study, Hansh and Awad (17) reported in their study on 75 hydatid cysts collected from human, sheep, cattle, buffaloes and camels that the predominant strain of *E. granulosus* is the sheep strain G1 depending on the rDNA-internal transcribed spacer 1 (ITS1) gene followed by PCR-restriction fragment length polymorphism (RFLP) analysis. Similarly, it was observed that the *E. granulosus* sheep strain is responsible for the hydatid disease in Kurdistan, Iraq (18). In Iran, it was reported that every sheep, cattle and goat is infected by the sheep strain of *E. granulosus* (12). Moreover, based on rDNA-ITS1 sequences, Vahedi, Mahdavi (19) demonstrate in Iran that all samples of hydatid cysts isolated from different organs of human are similar to the sheep strain. In Turkey, it was observed that the predominant strain of *E. granulosus* is the sheep strain (20). Shahzad, Abbas (21) confirmed the presence of the *E. granulosus* sheep strain in sheep, cattle, goats and buffaloes in Pakistan as well. Thus, our results are in accordance with other results from Iraq and other countries that the strain G1 is the dominate strain of *E. granulosus*.

Unlike the results of the current study, in Mosul province, two strains of *E. granulosus* which are dog/sheep and dog/cattle were demonstrated depending on morphological characters (22). Again, in contrast to our results, a study in Baghdad province amplifying the rDNA-ITS1gene demonstrated that most of patients with hydatidosis is related to the sheep strain G1, the horse G4, the buffalo strain G3, the cattle strain G5, the camel strain G6 and the pig strain G7 of *E. granulosus* (23). Therefore, the variety in the results of the previous studies, conducted in Mosul and Baghdad provinces, from our results could be explained by the difference in geographic areas, the type of samples and the employed methods as well. A definitive host for *E. granulosus*, the domestic dog, plays a significant role in the spread of infection with hydatid cysts in Middle East countries by contamination of the environment, sheep, goats, cattle, camels, buffaloes, pigs and donkeys (8, 16, 24).

Although the nucleotide NAD1 sequences of the present isolates were highly similar to reference sequences of *E. granulosus*, there were 14 polymorphism sites among these NAD1 sequences observed in the present isolates. It seems to be the most important variation that occurred in the isolate Eg_5 at the position 357 via replacement of the nucleotide A found in the sheep strain G1 with the nucleotide G found in the buffalo strain G3 (Fig. 3). This was reflected on the amino acid sequence of the isolate Eg_5 which shared the same M residue found only in the strain G3 at the position 119. Thus, the amino acid sequence of the isolate Eg_5 was highly similar to the *E. granulosus* NAD1 proteins of strain G3. This result indicates that the isolate Eg_5 isolated from the liver of cattle could belong to the buffalo strain G3.

Conclusion:

The results of the current study showed the presence of the popular strains G1 and G3 of *E. granulosus* in the samples collected from Thi-Qar province. This study also reported, based on the nucleotide sequences of NAD1 gene, that the predominate strain of *E. granulosus* in Thi-Qar is the strain G1. Further studies on *E. granulosus* in different geographic areas and different intermediate hosts with a large sample size need to be performed using molecular techniques in Iraq.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

References:

1. Bhutani N, Kajal P. Hepatic echinococcosis: A review. Ann Med Surg. 2018 Dec 1;36:99-105..
2. Ma X, Zhang L, Wang J, Luo Y. Knowledge Domain and Emerging Trends on Echinococcosis Research: A Scientometric Analysis. Int J Environ Res Public Health. 2019;16(5):842.
3.Velasco-Tirado V, Alonso-Sardón M, Lopez-Bernus A, Romero-Alegría Á, Burguillo FJ, Muro A, et al. Medical treatment of cystic echinococcosis: systematic review and meta-analysis. BMC Infect Dis. 2018;18(1):306.
4. Thompson R, McManus D, Eckert J, Gemmell M, Meslin F, Pawlowski Z. Manual on *Echinococcus* in humans and animals a public health problem of global concern. 2001.
5. Dybicz M, Borkowski PK, Jonas M, Wasiak D, Małkowski P. First Report of *Echinococcus ortleppi* in Human Cases of Cystic Echinococcosis in Poland. BioMed Res Int. 2019; Apr 8 2019.
6. Kinkar L, Laurimäe T, Acosta-Jamett G, Andresiuk V, Balkaya I, Casulli A, et al. Distinguishing

Echinococcus granulosus sensu stricto genotypes G1 and G3 with confidence: A practical guide. Infection, Genet Evol. 2018 Oct 1;64:178-84.
7. Wang N, Wang J, Hu D, Zhong X, Jiang Z, Yang A, et al. Genetic variability of *Echinococcus granulosus* based on the mitochondrial 16S ribosomal RNA gene. Mito DNA. 2015;26(3):396-401.
8. Craig P, Mastin A, van Kesteren F, Boufana B. *Echinococcus granulosus*: epidemiology and state-of-the-art of diagnostics in animals. Vet Parasitol. 2015;213(3-4):132-48.
9. Pearson M, Le TH, HuaZHANG L, Blair D, Dai TH. Molecular Taxonomy and Strain Analysis in. Cestode Zoonoses: Echinococcosis and Cysticercosis: Emerg Glob Prob. 2002;341:205.
10. Khademvatan S, Majidiani H, Foroutan M, Tappeh KH, Aryamand S, Khalkhali H. *Echinococcus granulosus* genotypes in Iran: a systematic review. J Helminthol. 2019;93(2):131-8.
11. Nakao M, Yanagida T, Okamoto M, Knapp J, Nkouawa A, Sako Y, et al. State-of-the-art *Echinococcus* and *Taenia*: phylogenetic taxonomy of human-pathogenic tapeworms and its application to molecular diagnosis. Infect Genet Evol. 2010;10(4):444-52.
12. Hanifian H, Kambiz D, Tappeh KH, Mohammadzadeh H, Mahmoudlou R. Identification of *Echinococcus granulosus* strains in isolated hydatid cyst specimens from animals by PCR-RFLP method in West Azerbaijan–Iran. IJP. 2013;8(3):376.
13. Sharbatkhori M, Mirhendi H, Jex AR, Pangasa A, Campbell BE, Kia EB, et al. Genetic categorization of *Echinococcus granulosus* from humans and herbivorous hosts in Iran using an integrated mutation scanning-phylogenetic approach. Electrophoresis. 2009;30(15):2648-55.
14. Ahmed ME, Salim B, Grobusch MP, Aradaib IE. First molecular characterization of *Echinococcus granulosus* (sensu stricto) genotype 1 among cattle in Sudan. BMC Vet Res. 2018;14(1):36.
15. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 1992;54(2):165-73.
16. Galeh TM, Spotin A, Mahami-Oskouei M, Carmena D, Rahimi MT, Barac A, et al. The seroprevalence rate and population genetic structure of human cystic echinococcosis in the Middle East: a systematic review and meta-analysis. Int J Surg. 2018;51:39-48.
17. Hansh WJ, Awad A-HH. Genotyping Study of Hydatid Cyst by Sequences of ITS1–rDNA in Thi-Qar–Southern of Iraq. Int J Curr Microbiol App Sci. 2016;5(8):350-61.
18. Hama AA, Mero WM, Jubrael JM, editors. Molecular characterization of *E. granulosus*, first report of sheep strain in Kurdistan-Iraq. 2nd International Conference on Ecological, Environmental and Biological Sciences (EEBS™ 2012) Oct; 2012.
19. Vahedi A, Mahdavi M, Ghazanchaei A, Shokouhi B. Genotypic characteristics of hydatid cysts isolated from humans in East Azerbaijan Province (2011-2013). J Anal Res Clin Med. 2014;2(3):152-7.
20. Utuk AE, Simsek S, Koroglu E, McManus DP. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. Acta Tropica. 2008;107(2):192-4.
21. Shahzad W, Abbas A, Munir R, Khan MS, Avais M, Ahmad J, et al. A PCR analysis of prevalence of *Echinococcus granulosus* genotype G1 in small and large ruminants in three districts of Punjab, Pakistan. Pak J Zool. 2014;46(6).
22. Aljawady M. Sheep can be infected with more than one strain of *Echinococcus granulosus*. Asian J Anim Vet Adv. 2009;8(11):2177-80.
23. Khalf MS, Al–Faham MA, Al-Taie LH, Alhussian HA. Genotyping of *Echinococcus granulose* in Samples of Iraqi Patients. IOSR J Pharm Biol Sci. 2014;9(3):06-10.
24. Thys S, Sahibi H, Gabriël S, Rahali T, Lefèvre P, Rhalem A, et al. Community perception and knowledge of cystic echinococcosis in the High Atlas Mountains, Morocco. BMC public health. 2019;19(1):118.

Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: January 2021 **E-ISSN: 2411-7986**

استخدام الجين NADH dehydrogenase 1 في تحديد سلالة الاكياس العدرية في الاغنام والماشية والانسان في محافظة ذي قار، العراق

سرمد عواد موزان الاسدي[1*] **وسام جاسم حنش[2]** **عبد الحسين حبش عواد[1]**

[1] قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، بصرة، العراق
[2] المديرية العامة لتربية ذي قار، العراق

الخلاصة:
يعد داء المشوكات الحبيبية من الامراض المشتركة يسببه دور يرقي لطفيلي الاكياس العدرية *Echinococcus granulosus* والذي يعود لمجموعة الديدان الشريطية. يسبب هذا المرض خسائر كبيرة من ناحية صحية واقتصادية في العراق ويعد الكبد والرئتين من الاماكن العامة المفضلة للاصابة بهذا الطفيلي. هدفت الدراسة الحالية الى استخدام التقنيات الجزيئية في تشخيص سلالة الاكياس العدرية *E. granulosus* التي تسبب داء المشوكات الحبيبية للانسان والاغنام والماشية في محافظة ذي قار جنوب العراق. تم الحصول على 30 عزلة من هذا الطفيلي 10 منها جمعت من مرضى اجريت لهم عملية جراحية في مستشفى الامام الحسين التعليمي في محافظة ذي قار و 10 عزلات من الاغنام و 10 عزلات من الماشية جمعت من مجازر في محافظة ذي قار جنوب العراق. شخصت هذه العزلات من خلال الدراسة الجزيئية الحالية باستعمال بادئ مايتوكوندیري متخصص للجين mitochondrial dehydrogenase NADH subunit 1 (NAD1) والذي يضم 400 زوج قاعدي في جميع العزلات المختارة. اظهر تتابع sequencing لنواتج PCR لاثني عشر عزلة (4 عزلات لكل مضيف متوسط) بانها تعود الى سلالة الاغنام G1 عدا عزلة واحدة Eg_5، لوحظ اختلافات وراثية طفيفة بين سلالة G1 للعزلات الحالية وسلالة G1 للعزلات المرجعية المتوفرة في بنك الجينات العالمي. كما لوحظ اختلاف في العزلة Eg_5 المعزولة من كبد الاغنام وهي مشابه لسلالة G3 الخاصة بالجاموس. استنتج من الدراسة الحالية بان سلالة الاغنام G1 هي السلالة الشائعة لطفيلي *E. granulosus* في محافظة ذي قار.

الكلمات المفتاحية: طفيلي الاكياس العدرية، NADH dehydrogenase subunit I، ذي قار