Original Article

Genetic Characterization of *Echinococcus granulosus* Strain Causing for Cystic Echinococcosis in Human

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Abstract

Background: Cystic echinococcosis is caused by the larval stage of *Echinococcus granulosus*. Humans become infected by ingestion of parasite eggs with contaminated water and food or by direct contact with definitive hosts. Molecular studies have identified 10 genotypes (G1–G10) within *E. granulosus*. **Objectives:** The current study is conducted to determine genotypes (strains) of *E. granulosus* that cause cystic echinococcosis in humans in Thi-Qar province, Iraq, using polymerase chain reaction (PCR) technique and sequence analysis of the mt *12S rRNA* gene. **Materials and Methods:** The molecular study was conducted on 15 hydatid cysts isolated from 15 patients with cystic echinococcosis who underwent surgical operations at the Imam Hussein Teaching Hospital, located in the center of the Al-Nasiriyah city, Thi-Qar province, Iraq, using PCR technique and sequencing of the mt *12S rRNA* gene. **Results:** The PCR technique was utilized for amplifying a single fragment of 254 bp length of the mt *12S rRNA* gene in all hydatid cysts. Sequence analysis of mt *12S rRNA* gene showed the presence of *E. granulosus* genotype G1 (Sheep strain) in human hydatid cysts when compared with selected sequences in the present study with the reference sequences of *E. granulosus* genotype G1 in GenBank database. In addition, five sequences in the present study were registered in GenBank database for the first time under accession numbers (LC712863, LC712864, LC712865, LC712866, and LC712867). Genetic variation within genotype G1 (the G1 variant G163T) was observed in a hydatid cyst sample isolated from the human kidney with accession number LC712863.1. **Conclusion:** The current results concluded that the *E. granulosus* genotype G1 is responsible for human cystic echinococcosis in Thi-Qar province.

Keywords: Cystic echinococcosis, *Echinococcus granulosus*, genetic characterization

Introduction

Cystic echinococcosis is a zoonotic parasitic diseases with a global distribution caused by the larval stage of *Echinococcus granulosus sensu lato*. The life cycle of *E. granulosus* parasite includes dogs and other canids as final hosts of the adult worm, while herbivorous animals as the intermediate hosts in which the larval stage or hydatid cyst develops.[1] Humans (accidental hosts) become infected by accidental ingestion of *E. granulosus* eggs in contaminated water and food or by direct interaction with the final hosts.[2] Hydatid cysts are found in the liver, lungs, and other internal organs of intermediate hosts, which comprise domestic and wild animals, while the human is represented as the end stage, and therefore, the life cycle of *E. granulosus* cannot be completed.[3]

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Ten different genotypes (G1–G10) have been distinguished within *E. granulosus*, relying on the biological and genetic analysis of nuclear and mitochondrial genes.[4] These genotypes include *E. granulosus sensu stricto* (G1, G2, G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* cluster (G6–G8 and G10) and *E. felidis*.[5] The genotype G1 (sheep strain) is the most predominant genotype with a global distribution and is responsible for the majority of cases of human cystic echinococcosis.[6]

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Cystic echinococcosis is a common health concern in Iraq, as many epidemiological studies have shown that the E. granulosus is endemic in different provinces of Iraq [7-11]. The annual surgical incidence of cystic echinococcosis in Basrah governorate is 4.5/100,000[12] and 5.6/100,000 in Slemani city.[13] Hansh[7] registered 58 cases of human cystic echinococcosis in the Thi-Qar province.

The use of molecular techniques for genetic identification of *E. granulosus* is considerable for understanding that the *E. granulosus* genotypes have had an important impact on the phenotypic features, transmission dynamics, pathology, sensitivity to chemotherapeutic agents, and vaccine development strategies.[14] The diagnosis of *E. granulosus* genotypes has a significant index in the application of control programs.[15] Presently, mitochondrial 12S ribosomal RNA (*mt 12S rRNA*) gene is one of the accepted genetic markers to study the molecular characterization of helminthic parasites.[16] The present study aimed to identify the *E. granulosus* strain (genotype) responsible for cystic echinococcosis in humans in the Thi-Qar province based on sequence analysis of mt *12S rRNA* gene.

Materials and Methods

Collection of samples

The present study was conducted on 15 hydatid cysts isolated from humans (one kidney, eight livers, and six lungs) collected directly from 15 patients postoperatively in the Imam Hussein Teaching Hospital in Thi-Qar province during the period from the beginning of October 2020 to January 2021. The patients were primarily from Thi-Qar province and distributed in the following district: Al-Nasiriyah city (seven cases, four females and three males), Al-Shatrah (two females), Al-Rifai (two females), Al-Gharraf (one male), Al-Nasr (two females), and Al-Chibayish (one male). Their ages ranged between 25 and 55 years. Protoscolices were isolated from hydatid cysts and microscopically examined to ensure the viability and fertility of protoscolices by using an eosin stain. The sediment of protoscolices was preserved by freezing until its use in molecular studies.[17]

Molecular study

Molecular study was performed at the Laboratory of Biotechnology at Mazaya university college. DNA was extracted from protoscolices by using the gSYNC DNA Extraction Kit (Geneaid). The genomic DNA was stored at −20 °C.

Polymerase chain reaction

The polymerase chain reaction (PCR) was performed to amplify the *mt 12S rRNA* gene for all samples of genomic DNA prepared from protoscolices. The primers, previously determined by Dinkel *et al*.,[18] targeting the *mt 12S rRNA* gene, consisted of the forward primer, E.g. ss1 for. (5′ GTA TTT TGT AAA GTT GTT CTA 3′) and the reverse primer, E.g. ss1 rev. (5′ CTA AAT CAC ATC ATC TTA CAA T 3′). The PCR reaction was performed in a final 25 µL volume by adding 1 µL of each primer (forward and reverse), 5 µL of DNA sample, and 13 µL of nuclease-free water from Bioneer Accupower PCR PreMix. The reaction was carried out in a thermocycler under the following conditions: one cycle of 94°C for 5 min (initial denaturation), followed by 40 cycles for 30 s denaturation at 94°C, 60 s annealing at 57 °C and extension of 40 s at 72°C, and one cycle of final extension at 72°C for 5 min.[18] The PCR product was loaded on tris-borate ethyle diamine tetraacetic acid agarose gel 1.5%. The gel was stained with 0.1–0.3 µL of ethidium bromide stain. Electrophoresis was carried out at 70 V for 90 min. The bands were examined for visualization of amplified PCR products using a UV transilluminator and digitally photographed.[19]

Sequencing of mt *12S rRNA* gene

The PCR products of the mt *12S rRNA* gene were sent to the biotechnology company (Macrogen) for sequencing. Five sequences, including one from the kidney, three from the liver, and one from the lung, were registered in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) for the first time. BLAST alignment was used for the comparison of sequences in the present study with sequences of *E. granulosus* genotypes recorded in the GenBank database. Phylogenetic analysis was conducted using MEGA7 and the UPGMA method was used to construct the phylogenetic tree.[20] Network V.10 program was used for haplotype network analysis.

Ethical approval

All hydatid cysts were collected and used in this study after obtaining official approval from 15 patients and the medical staff at the Imam Hussein Teaching Hospital, according to document no. 532 dated January 27, 2019, and in agreement with the ethical principles of the Declaration of Helsinki.

Results

Amplification of *mt 12S rRNA* gene

The current study showed that all hydatid cysts isolated from the human kidney, liver, and lungs were fertile [Figure 1]. DNA extracted from protoscolices for 15 hydatid cysts was tested using PCR technique. A single amplicon of 254 bp length was successfully amplified using a specific primer of mt *12S rRNA* gene [Figure 2].

Sequence analysis

The results of sequence analysis demonstrated that all the samples in the present study belong to the genotype G1 (sheep strain) of *E. granulosus*, depending on the sequences analysis by BLAST multiple alignment with

available reference sequences to the *E. granulosus* genotype G1 in GenBank. Five sequences were submitted to the GenBank and recorded for the first time under accession numbers (LC712863, LC712864, LC712865, LC712866, LC712867) [Table 1].

Genetic variation within genotype G1 observed in only one hydatid cyst isolated from the human kidney with accession number LC712863.1, represented by nucleotide mutation (transversion mutation), producing a single nucleotide polymorphism at position 163, where guanine replaced thymine (the G1 variant G163T), while the other sequences in the current study from LC712864.1 to LC712867.1 contain thymine at the 163 position [Figure 3].

Phylogenetic analysis

Phylogenetic analysis exhibited that all five Iraqi samples grouped into genotype G1. In phylogenetic analysis, the sample with acc. no LC712863.1 differed from the other samples in the current study due to genetic variation (the G1 variant G163T) and therefore took a major branch (A); in addition, sample LC712863.1 showed high similarity with genotype G1 sequences in which genetic variation was observed at locus G>T9311 for the mitochondrial genome of *E. granulosus*, registered under accession number KU925365 in which the sample shared with genotype G1 sequences from the following countries: Bangladesh KU695149.1, Turkey MW421619.1, Slovenia MT253550.1, and China KY767925.1. The other samples (LC712864.1 to LC712867.1) showed maximum similarity with genotype G1 sequences recorded in China KY767931.1, Iran MH395774.1, Estonia KY766883.1, and Slovenia MT253556.1 within the second main branch (B) [Figure 4].

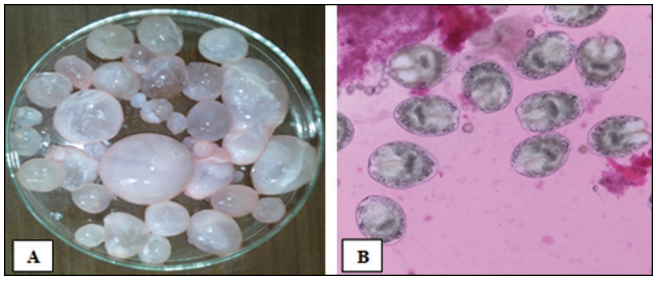

Figure 1: (A) Daughter hydatid cysts isolated from human liver. (B) Protoscolices isolated from the hydatid cyst

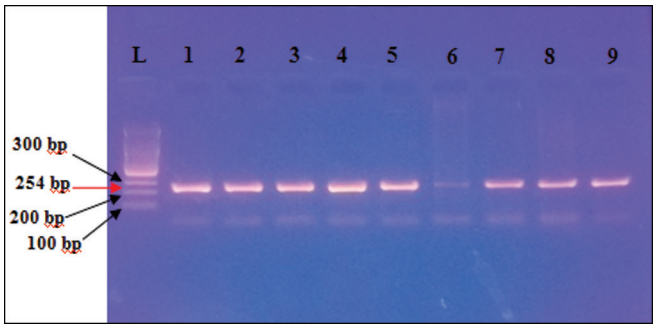

Figure 2: PCR products of *mt 12S rRNA* gene (254bp). L: DNA Ladder (1500 bp). Lane (1 to 9) represents PCR products of hydatid cysts isolated from humans: (1) kidney, (2, 3, 4, 5) liver, and (6, 7, 8, 9) lung

Haplotypes of *E. granulosus* G1

The results of haplotypes network analysis using the network V.10 program exhibited three haplotypes: H1, H2, and H3. Based on the comparison of nucleotide sequences from different countries (China, Iran, Turkey, Bangladesh, Estonia, and Slovenia) with the sequences in the current study registered under accession numbers (LC712863 to LC712867). The current results showed two independent haplotypes, H1 and H2, where the H1 haplotype was shared by Iraq, Iran, Turkey, Estonia, and Bangladesh, with a branch to this haplotype H3 in which China and Slovenia participated due to the mutation occurring at position 9348.A>G of the mitochondrial genome, as registered under accession number KU925365, while the independent H2 haplotype was shared by Iraq, China, Iran, Turkey, Estonia, and Slovenia [Figure 5].

Discussion

Cystic echinococcosis (hydatidosis) has long been regarded as one of the most significant health issues in the Middle East, and it has been reported widely in Iran, Turkey, and Iraq.[6,12,21] The sequence analysis of mt *12S rRNA* gene (254 bp) in the present study showed that the genotype G1 (sheep strain) of *E. granulosus* is a prevalent

Table 1: New submission to GenBank accession numbers and haplotypes of *E. granulosus* genotype G1 in humans identified by mt *12S rRNA* gene sequence. ND: No sequence diversity

Haplotypes	New submission to GenBank acc. No.	Host	Source of hydatid cysts	Nucleotide substitution (12S rRNA gene)
1	LC712863	Human	Kidney	163G>T
2	LC712864	Human	Lung	ND
3	LC712865	Human	Liver	ND
4	LC712866	Human	Liver	ND
5	LC712867	Human	Liver	ND

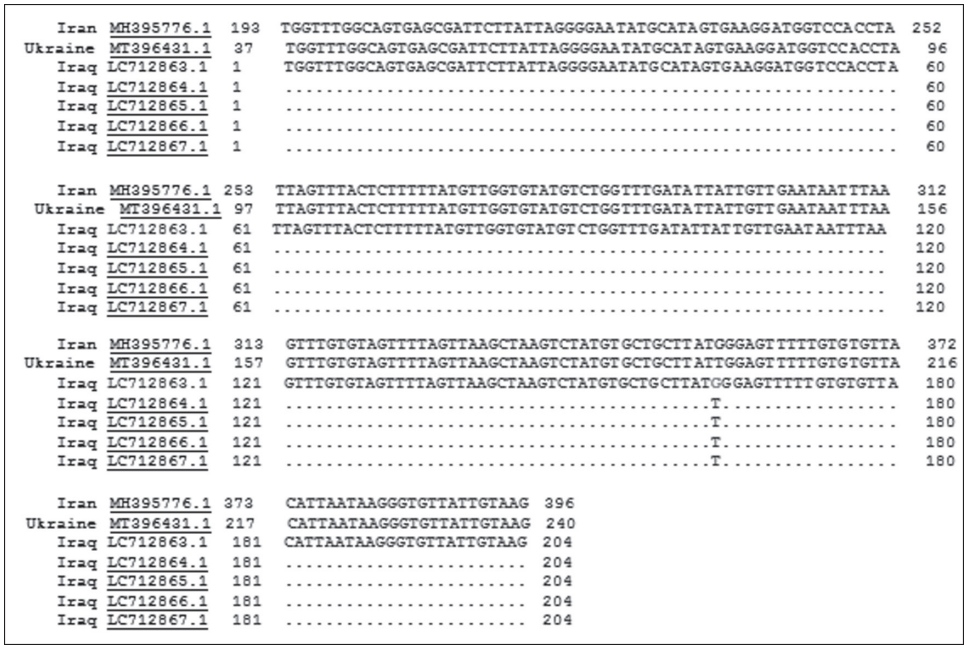

Figure 3: Sequence alignments of *mt 12S rRNA* gene: Reference sequences for *mt 12S rRNA* gene of *E. granulosus* G1 from GenBank through databases from Iran (MH395776.1) and Ukraine (MT396431.1) shown at the top with sample sequences (LC712863.1 to LC712867.1) from humans in Thi-Qar province, Iraq using BLAST pairwise. The dots indicate the similarity of nucleotide sequences to the *mt 12S rRNA* gene sequence from GenBank

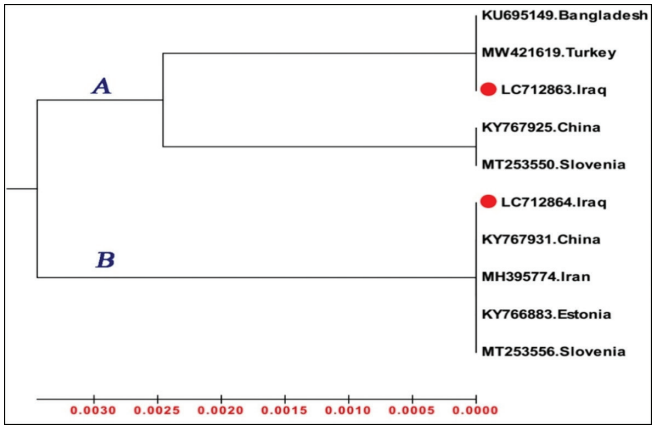

Figure 4: Phylogenetic relationships between mt *12S rRNA* gene sequences in the present study and reference sequences of *E. granulosus* genotype G1

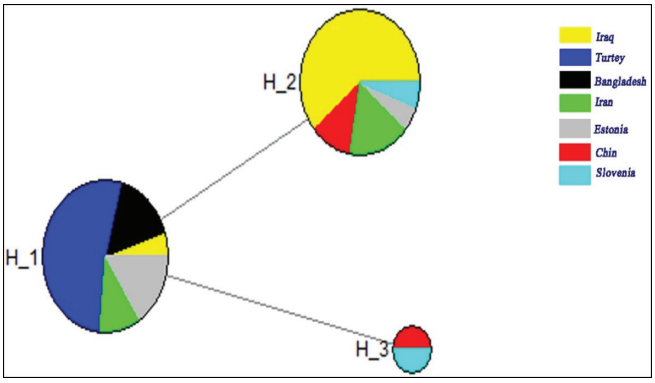

Figure 5: Haplotypes network analysis of *E. granulosus* G1 using the network V.10 program

genotype causing human cystic echinococcosis in Thi-Qar province. The current findings proposed the existence of dominance of the sheep–dog life cycle of *E. granulosus* in Thi-Qar province. In the current study, mitochondrial DNA sequence data represented by the *mt 12S rRNA* gene was used to identify the genotypes of *E. granulosus*. Von Nickisch-Rosenegk *et al.*[22] showed that the *12S rRNA* gene is the most common mtDNA gene for analyzing the phylogeny, inter- and intraspecific divergence, and evolution of parasitic helminths. Laurimae *et al.*[4] reported that the mitochondrial genes are used much better than other genome sources in determining *E. granulosus* genotypes, as well as information gained from mitochondrial DNA can help researchers solve taxonomy problems of *E. granulosus* strains.

The present study agrees with many molecular studies that were carried out in Iraq that relied on nuclear and mitochondrial genes, such as Hansh and Awad,[23] who reported the most prevalent genotype G1 in humans using PCR–restriction fragment length polymorphism and sequencing of *ITS1*. Also, Al-Asadi and Awad[24] (2023) confirmed that genotype G1 is the cause of human hydatid cysts in Basrah and Maysan provinces. The current results were emphasized by Hama *et al.*,[25] who demonstrated that the sheep strain G1 is predominant in humans in the Kurdistan region. Likewise, Barazesh *et al.*[26] exhibited that the genotype G1 and the less influential G3 have main roles in the transmission of hydatidosis in Iran and

Turkey. The consistency with the results of the current study is perhaps due to the closed geographical borders between these two countries and Iraq. Zhong *et al*.[27] reported that the most common genotype G1 in hydatid cysts isolated from humans was in China.

Unlike the present results, Hansh[7] recorded the buffalo strain G3 in hydatid cysts isolated from human lungs in addition to the sheep strain G1 in Thi-Qar province. Rahi and Ali[28] reported two genotypes in Wasit province: sheep strain G1 and buffalo strain G3 in humans. The present study differed from Fadhil and A'aiz,[29] who demonstrated three genotypes, G1, G3, and G6 (camel strain) in human and domestic animals in Al-Qadisiyah province. In Iran, Rostami *et al*.[30] reported four genotypes, G1 (54.4%), G6 (40.8%), G2 (0.8%), and G3 (1%), causing human cystic echinococcosis. The difference in the present results from the previous studies may be due to the difference in geographical areas and methods of diagnosis.

Sequence analysis of a hydatid cyst sample isolated from human kidney showed G163T mutation within *E. granulosus* genotype G1 in the present study; this type of genetic variation represents a genotypic variant. Genetic variation within *E. granulosus* was observed in many studies, such as Hama *et al*.,[31] who observed slight genetic variation within genotype G1 in hydatid cysts of humans. Thompson and McManus[32] exhibited that genetic variability leads to the diagnosis of variants or genotypes within a species, and this diversity may be related to life cycle patterns, host specificity, transmission of parasites, pathogenesis, and rate of development.

The phylogenetic tree revealed a great similarity between sheep strain G1 in Thi-Qar province, Iraq, and genotype G1 in different Asian and European countries. These results indicate close evolutionary relationships depending on the phylogenetic analysis of *12S rRNA* gene sequences; therefore, they were clustered within one clade (genotype G1). 12S ribosomal RNA and Cytochrome c oxidase subunit 1 gene sequences have been extensively applied for the molecular taxonomy of tapeworms.[16] Furthermore, haplotype network analysis relied on 12S rRNA gene sequences exhibited two haplotypes within *E. granulosus* G1 in the present study and was similar to other *12S rRNA* gene sequences from different geographical regions (China, Iran, Turkey, Bangladesh, Estonia, and Slovenia). The haplotype analysis of the mt *12SrRNA* gene demonstrated slight nucleotide variation in the isolate (LC712863), resulting in a new haplotype (H1). A recent study showed 6 and 7 haplotypes within *E. granulosus sensu lato* based on phylogenetic analysis of mt *12S rRNA* gene.[33] The emergence of a new haplotype is perhaps interpreted by the influence of environmental factors that induce genetic mutations, causing the difference in the hereditary structure.

Conclusion

Echinococcus *granulosus* genotype G1 (sheep strain) was specified to be the only genotype in all 15 hydatid cysts isolated from humans; additionally, mt *12 rRNA* gene (254 bp length) scanned in the present study supported the genetic information among the genotypes of *E. granulosus* in Thi-Qar province.

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Conflicts of interest

The author announces there is no conflict of interest.

References

1. Thompson RC. Biology and systematics of echinococcus. Adv Parasitol 2017;95:65-109.
2. Ito A, Budke CM. The echinococcosis in Asia: The present situation. Acta Trop 2016;176:11-21.
3. Zeibig EA. Clinical Parasitology. A Practical Approach. 2nd ed. Saunders, an imprint of Elsevier Inc. 2013; p. 256 – 258.
4. Laurimae T, Kinkar L, Romig T, Omer RA, Casulli A, Umhang G, *et al*. The benefits of analyzing complete mitochondrial genomes: deep insights into the phylogeny and population structure of *Echinococcus granulosus* sensu lato genotypes G6 and G7. Infect Gene Evol 2018;64:85-94.
5. Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, *et al*; World Association of Echinococcosis. The world association for online education. International consensus on terminology to be used in the field of echinococcoses. Parasite 2020;27:41.
6. Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, *et al*. Chapter six global distribution of alveolar and cystic echinococcosis. Advan Parasitol 2017;95:315-493.
7. Hansh WJ. Biological study and molecular identification on Hydatidosis in intermediate hosts depending on sequence analysis for *rDNA – ITS1* and *mtCOX1* genes in Thi-Qar province. Ph.D. Thesis, Coll. Edu. Pure, Sci. Univ. Basrah, 2016; 186 P.
8. Al-Mosawi AMA, Al-Joborae FFM, Al-Joborae HF, Al-Saadi MAK, Al-Charrakh AH. Cytokines profile in patients with hydatidosis in Babylon Province. Iraq. Med J Babylon 2023;20:212-4.
9. Al-Mukhtar A, Qasim IK. Serological survey of hydatid disease in asymptomatic peoples in Mosul City, Iraq. Rafidain J Sci 2017;26:1-8.
10. Al-Marsomy WA. Epidemiology of hydatid disease in Iraq: A study of hydatidosis patients in Baghdad province. India J Forens Med Toxicol 2021;15:3525-30.
11. Hussien FH. Immunological profile of serum antibodies, multiplex cytokines, and complement proteins. Med J Babylon 2023;20:891-6.
12. Abdulhameed MF, Habib I, Al-Azizz SA, Robertson I. A retrospective study of human cystic echinococcosis in Basrah province, Iraq. Acta Trop 2018;178:130-3.
13. Barzanji AA, Saida LA. Echinococcosis in Kurdistan Iraq: Prevalence of cystic hydatidosis in man with a survey of *E. granulosus* eggs in stray dogs in Kalar City, Sulaymania province, Kurdistan—Iraq. J Raparin Univ 2019;6:114-28.
14. Faruk MO, Siddiki AZ, Karim MF, Masuduzzaman M, Chowdhury S, Islam MS, *et al*. Molecular characterization of

Echinococcus granulosus through amplification of *12S rRNA* gene and *Cox1* gene fragments from cattle in Chittagong, Bangladesh. Intern J Anim Vet Sci 2017;11:301-5.
15. Barazesh A, Sarkari B, Sarısu G, Hami M, Mikaeili F, Aydın A, *et al*. Comparative genotyping of *Echinococcus granulosus* infecting livestock in Turkey and Iran. Turkiye Parazitol Derg 2019;43:123-9.
16. Rostami S, Salavati R, Beech RN, Sharbatkhori M, Babaei, Z, Saedi S, *et al*. Cytochrome c oxidase subunit 1 and 12S ribosomal RNA characterization of *Coenurus cerebralis* from sheep in Iran. Vet Parasitol 2013;197:141-51.
17. Nasrieh MA, Abdel-Hafez SK. *Echinococcus granulosus* in Jordan: Assessment of various antigenic preparations for use in the serodiagnosis of surgically confirmed cases using enzyme immuno assays and the indirect haemagglutination test. Diagn Microbiol Infect Dis 2004;48:117-23.
18. Dinkel A, Njoroge EM, Zimmermann A, Wa¨lz M, Zeyhle E, Elmahdi IE, *et al*. A PCR system for detection of species and genotypes of the *Echinococcus granulosus* complex, with reference to the epidemiological situation in eastern Africa. Intern J Parasitolo 2004;34:645-53.
19. Green MR, Sambrook J. Molecular Cloning: A Laboratory Manual. 4th ed. New York: Cold Spring Harbor Laboratory Press; 2012. p. 448.
20. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 2021;37:3022-7.
21. Sarkari B, Hosseini F, Abdolahi Khabisi S, Sedaghat F. Seroprevalence of cystic echinococcosis in blood donors in Fars province, southern Iran. Parasite Epidemiol Control 2017;2:8-12.
22. Von Nickisch-Rosenegk M, Lucius R, Loos-Frank B. Contributions to the phylogeny of the *Cyclophyllidea* (Cestoda) inferred from mitochondrial *12S rDNA*. J Mol Evol 1999;48:586-96.
23. Hansh WJ, Awad AHH. Genotyping Study of Hydatid Cyst by Sequences of *ITS1–rDNA* in Thi-Qar–Southern of Iraq. Int J Curr Microbiol App Sci 2016;5:350-61.
24. AL-Asadi SAW, Awad AHH. Complete characterization of NADH dehydrogenase subunit 1 gene in human hydatid cysts. Baghdad Sci J 2024;21:1457-69.
25. Hama AA, Mero WMS, Jubrael JMS. Molecular identification of *Echinococcus granulosus* (G1) strain in human and animals. J Univ Zakh 2013;1:1-7.
26. Barazesh A, Sarkari B, Shahabi S, Halidi AG, Ekici A, Aydemir S, *et al*. Genetic diversity of *Echinococcus granulosus* isolated from humans: A comparative study in two cystic echinococcosis endemic areas, Turkey and Iran. BioMed Res Intern 2020;1:1-7.
27. Zhong X, Wang N, Hu D, Wang J, Liu T, Gu X, *et al*. Sequence Analysis of cytb Gene in *Echinococcus granulosus* from Western China. Korean J Parasitol 2014;52:205-9.
28. Rahi AA, Ali MA. A first DNA sequencing of hydatid agent isolated from human in Iraq. J Pur Appl Microbiol 2016;10:1015-20.
29. Fadhil SA, A'aiz NN. Genotyping of cystic echinococcosis isolates from clinical samples of human and domestic animals. Iraqi J Vet Sci 2016;30:33-9.
30. Rostami S, Torbaghan SS, Dabiri S, Babaei Z, Mohammadi MA, Sharbatkhori M, *et al*. Genetic characterization of *Echinococcus granulosus* from a large number of formalin-fixed, paraffin-embedded tissue samples of human isolates in Iran. Am J Trop Med Hyg 2015;92:588-94.
31. Hama AA, Mero WMS, Jubrael JMS. 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. 13-14, 2012 Bali (Indonesia). 2012.
32. Thompson RCA, McManus DP. Etiology parasites and lifecycles In: Eckert J, Gemmell MA, Meslin FX, Pawłowski ZS, editors. WHO/OIE. Manual of Echinococcosis in Human and Animals: A Public Health Problem of Global Concern. Paris, France, 2002; 17 – 19.
33. Dehghani M, Mohammadi MA, Hemmati S, Nasibi S, Rostami S, Fasihi Harandi M. Cystic echinococcosis of camels: 12S rRNA gene variation revealed changing pattern of genetic diversity within *Echinococcus granulosus sensu lato* in the Middle East and North/ Sub-Saharan Africa. Front Vet Sci 2020;7:618.