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# Molecular detection of *Nad5* in cystic echinococcosis in human and livestock animals in Erbil

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## Abstract

**Background and objective:** *Echinococcus granulosus* can cause hydatidosis, or echinococcosis, in both herbivores and omnivores. Hydatid cysts are the larval stage of this tapeworm. As a zoonotic illness, it affects people all over the world. Devastating effects on human and animal health as well as massive economic losses, disproportionately felt in the agriculture sector, characterize this disease. Genomic segments from cox1 and nad1 genes have allowed for the identification of distinct genotypes of *Echinococcus granulosus* sensu stricto (s.s.) in both humans and domesticated animals. The current investigation aimed to distinguish between the G1/G3 genotypes of *Echinococcus granulosus* (s.s.) in cattle via the nad5 gene fragment.

**Methods:** The present study was conducted in Erbil city. Sheep, goat, and cattle hydatid cyst samples (36 isolates) were collected from the Erbil slaughterhouse. In addition, 11 fresh human hydatid cyst samples were taken from patients with hydatid cysts who underwent surgical operations in Rizgary Teaching Hospital. The hydatid cysts were subjected to examination for fertility and viability in addition to DNA extraction for genotyping based on the *Nad5* gene.

**Results:** Out of 42 samples, 15 samples were sequenced, and all samples belonged to the G1 genotype (sheep strain). DNA sequencing of hydatid cysts from both animal and human sources showed mutations in various positions of the Nad5 gene, consistent with the dominant G1 genotype. In human samples, several variations overlap with other hosts, but unique ones include human 11,12 glycine/alanine at 142 arginine/glutamine, glycine/ threonine at 303 valine/phenylalanine, T/A at 334 valine/aspartic acid, and T/C at 361 arginine/histidine, which result in 99% identity to the G1 strain.

**Conclusion:** Humans and animals in Erbil most commonly harbor the G1 genotype of *E. granulosuss*. s.s. according to the study. The findings provided additional evidence that the Nad5 gene accurately distinguished between humans and cattle, sheep, goats, and *E.granulosus* G1/G3 isolates.

**Keywords:** *Echinococcus granulosus*; G1; Genotype; Nad5.

## Introduction

The pathogen that causes cystic echinococcosis (CE) is *Echinococcus granulosus* sensu lato (s.l.), specifically its larval stage. A significant zoonotic disease.(1) This worm's larvae infect a wide variety of intermediate host species, including domestic animals. There are many stages to this parasite's life cycle. There are hydatid cysts (HC) in almost every herbivore organ, including the brain, spleen, liver, and intestines of adult stage in the intestine of canine hosts.(2) Intermediary host organ dysfunction results from HC infection.(3)

Reliable methods for diagnosing infections caused by *E. granulosuss*. l. include imaging techniques and serological

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assays. However, there have been instances where the immunodiagnostic tests have failed to reliably detect antibodies unique to *Echinococcus* due to limits in their sensitivity and specificity;[4] it is not uncommon for antibody titers to be undetectable in individuals with echinococcal cysts in organs other than the liver or in cysts that are in the early, medium, or late stages of development.

The several strains of *E. granulosus*s.l exhibit distinct host specificity and lifecycle characteristics. Sensu stricto (genotypes G1–G3), equinus (G4), ortleppi (G5), and *E. granulosus*s.l. genotypes (G6–G10) are the four major categories into which the ten *E. granulosus* genotypes that have so far been found using various molecular approaches fall.[5] Current molecular phylogenetic investigation using six nuclear genes suggests that genotypes G6/G7 and G8/G10 of *E. granulosus*s.l. should be considered two separate species; hence, the designations of genotypes G6-G10, which include *E. canadensis* and *E. intermedius*, remain debatable.[6]

Two closely related sensu stricto strain genotypes, G1 and G3, account for the vast majority of hydatid cyst genotypes documented globally. [7] Phylogenetic study of *E. granulosus* has made use of a number of mitochondrial genes, including cox1, nad1, and atp6, or a fragment sequence of the 12SrRNA gene and the internal transcribed spacer (ITS1) genomic region.[8] Different studies have extensively utilized sequences of cox1 and nad1 genomic segments to distinguish between G1 and G3 genotypes.[9] But after many decades of studying these genomes in their whole, researchers have shown that these markers cannot differentiate between the two genotypes.[10] One recent piece of work used a 680 bp nad5 genomic fragment of *E. granulosus s.s*. to establish a practical and easy way to distinguish between the G1 and G3 genotypes. Human and animal hydatid cysts are most commonly found in Iran and Turkey.[11] Using cox1 and nad1 genomic segments, various parasite genotypes were specified of *E. granulosus* s.s. isolates from Iranian and bovine samples from Turkey and people living there.[7]

The aim of the present study is to screen and confirm the G1 and G3 genotypes of *E.granulosus* s.s isolated from livestock and human hydatid cyst samples in Erbil Governorate. The current work utilized primers that targeted the NAD5 genomic fragment of hydatid cyst tissue.

## Methods

### Study design and setting

Liver and lung of sheep, goat, cattle hydatid cyst samples (36 isolates) were collected from the Erbil slaughterhouse from September 21, 2024, to January 15, 2025; 11 fresh human liver and lung hydatid cyst samples were taken from patients with hydatid cysts who underwent surgical operations in Rizgary Teaching Hospital and other private hospitals in Erbil city, which was the setting for the current investigation.

### Data collection

The data was designed for human samples that included sociodemographic information like age, gender, occupation, size, number of cysts, and cyst location and was filled out by all patients (Table 1).

The data was designed for animal samples, which included sources of animals, types of animals, cyst locations, and numbers of cysts, as in Table 2.

### Collection of *E. granulosus* hydatid cysts sample

Human hydatid cyst samples collected after surgery from Rizgary Teaching Hospital and other private Hospitals, meanwhile animal samples collected during post-mortem inspection included liver and lung samples of infected sheep, cattle, and goats with several hydatid cysts collected from an Erbil slaughterhouse (one specimen for every animal).

Careful handling of each cyst occurred after samples were moved to the Erbil international university laboratory to reduce the likelihood of contamination, each cyst

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was rinsed many times with a normal saline solution. The next step was to rinse the cysts with 70% ethanol. Transferring the contents of the cysts to the sterile test tubes allowed them to centrifuge at 5000 rpm for 5 minutes and then wash three times with PBS at pH 7.2 at room temperature. Only 1.5 mL of the precipitate at the bottom of the test tube was transferred to 1.5 mL non-pyrogenic microcentrifuge tubes after the supernatant was removed.[12] Preserved with 70% ethanol at -20 in a deep freezer for DNA extraction.

**Microscopic examination of protoscoleces**

The presence of protoscoleces allowed for the determination of fertility by microscopically examining a single drop of cyst fluid (Figure 1.2A).

**Viability test (Eosin exclusion test)**

Each cyst fluid was centrifuged at 5000 × rpm for 5 minutes to estimate the viability rate of viable echinococcal cysts. Afterwards, utilizing a sterile pipette, a single drop of the precipitate was transferred to a pristine mixture with one drop of a 0.1% aqueous eosin solution (v/v) on a glass slide. The mixture was then covered with a cover slip and observed under a 40× × magnification.[13] The dead protoscoleces stained red, living ones did not (Figure 1 A and B).

**DNA extraction**

After removing the 42 hydatid cyst tissue samples from the deep freezer. A 1.5 ml sterile Eppendorf tube was used to transfer about 30-50 mg of GL or 100 µL of PSCs from each sample. The next step was to fill the tube with deionized water. The solution was resuspended using a vortex, and the liquid phase was removed using aspiration. To get rid of the ethanol, this procedure was carried out three times. The commercial AccuPrep® Genomic DNA Extraction Kit (Bioneers kit protocol) protocol) was used to extract DNA from the PSCs and/or GL of the hydatid cyst tissues.

**DNA amplification**

A thermostable DNA polymerase (Taq polymerase), two synthetic oligonucleotide primers, and four deoxyribonucleoside triphosphates interact with the DNA template to amplify a pre-selected section of DNA in multiple copies.

A PCR consists of four major processes that are repeated 25-40 times; this is accomplished using an automated cycler that can quickly heat and cool the tubes containing the reaction mixture.

1. Denaturation: Heat the process at 94°C for 30 seconds to break hydrogen bonds between complementary bases, releasing single-stranded DNA as templates for subsequent DNA synthesis.
2. Annealing step: Lower the reaction temperature to 50°C in 30 seconds to allow primers to adhere to the templates.
3. Taq polymerase performs best at 72°C during the extension/elongation stage. During this stage, the DNA polymerase creates a new DNA strand that is complementary to the DNA template. The extension time is determined by the length of the DNA fragment being amplified.
4. After the last PCR cycle, final elongation is conducted at 72°C for 5 minutes to fully lengthen any remaining single-stranded DNA.

The primers used in the molecular studies of *E. granulosus* were designed by Macrogen (Korea), as shown in (Table 1A). We checked and blasted them in NCBI to make sure they were genuine. After the primer tubes were dried and cleaned with tissue papers, they were centrifuged for a short period of time at 3000 rpm.

To create a stock solution of 100 µM from lyophilized primers, the datasheet advised adding the specified volume of nuclease-free water. Afterwards, a functional solution for the PCR process was made by adding 1 µM of 100 µM to 9 µM of free nuclease water, resulting in 10 µM concentrations. Preserved at -20°C, each well in the PCR run used 1 1M of primer aliquots.

For identifying *E. granulosus*, DNA amplification for NAD5 genes was performed at 95°C for 5 minutes in the thermal cycler to ensure the complete

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denaturation of DNA templates. The PCR was then continued with the program: 95°C for 60 sec, 58°C for 60 sec, and 72°C for 60 sec. Thirty cycles of these segments were repeated with a final extension of 5 min at 72°C as shown in (Table1B).[14, 15]

**Gel electrophoresis**

The gold standard for DNA visualization and purification in the laboratory is gel electrophoresis, which separates DNA by size (bp). The protocols for this technology consist of a three-step process: processing of DNA fragments through electrophoresis and visualization after their production in an agarose gel.[16]

Agarose (1.2%) was prepared by adding 1.2 g into a conical glass flask (250 mL) containing 100 mL of 1× TAE buffer solution and swirled to mix well. The safe red dye was added at 10 µL/100 mL of agarose gel and mixed well to mix the safe red dye. Desired DNA samples were to be loaded, pipetted up 0.2 volumes of loading dye**,** and then the DNA sample and loading dye were mixed well by filling and emptying the pipette a few times.

The gel tank closed, the power supply switched on, and the gel was run at five Volt/cm. Then, the voltage increased to 75-100 volts in 45 min.

**Gene Sequencing**

Because of cost of sequencing the 15 samples of PCR product of (NAD5 gene) 8 human sample and 3 cattle, 3 sheep and 1 goat sample (A 20 µL of PCR product aliquot with 80µl, using only forward primer-NAD5 (5'-GTTGTTGAGTTGATTGTTTTGTTTG -3') have undergone sequencing at Korea's Microgen Company using the ABI Prism Terminator Sequencing Kit (Applied Biosystem). This study used the Finch TV program software to alter incomplete chromatograms and check base calls.

**Data analysis**

Finch TV chromatogram viewer software was used to convert the chromatograms to FASTA format. Manual editing of the DNA sequences in the ABI file was done using BioEdit v.7.0.5. To determine the degree of homology with the nearest species, the results of the sequence editing process were examined using the BLAST (Basic local alignment search tool) NCBI.

**Sequence alignment**

For this study, we used the Basic Local Alignment Search Tool (BLAST), which is accessible at the National Center for Biotechnology Information (NCBI) website, to compare and align the NAD5 partial gene sequences with other biological sequences to discover more similarities with Echinococcus and find out variant nucleotides between Iraqi and other country of *Echinococcus granulosus.*

**Ethical consideration**

The study project was approved by the research ethics committee with number (Sc.EC:1011) approved in 9/9/2024 in the College of Health Science Hawler Medical University and by written informed agreement form participants were obtained with assuring for those who agreed to participate in the study then information will keep private.

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**Table 1 A** Primer sequences were used for PCR

| Primer name | Sequence (5' to 3') | PCR product size bp | Reference |
|---|---|---|---|
| Nad5 forward | 5'-GTTGTTGAAGTTGATTGTTTTGTTTG-3' | 750 | (17) |
| Nad5 reverse | 5'-GGAACACCGGACAAACCAAGAA-3' | 750 | |

**Table 1B** PCR Amplification Reagents

| No. | PCR components | Concentration | Volume (µl) |
|---|---|---|---|
| 1 | Master Mix | 2x | 25 |
| 2 | Forward Primer | 10 Pmol | 2 |
| 3 | Reverse Primer | 10 Pmol | 2 |
| 4 | DNase Free Water | - | 18 |
| 5 | Template DNA | 50 ng/µl | 3 |
| Total | | | 50 |

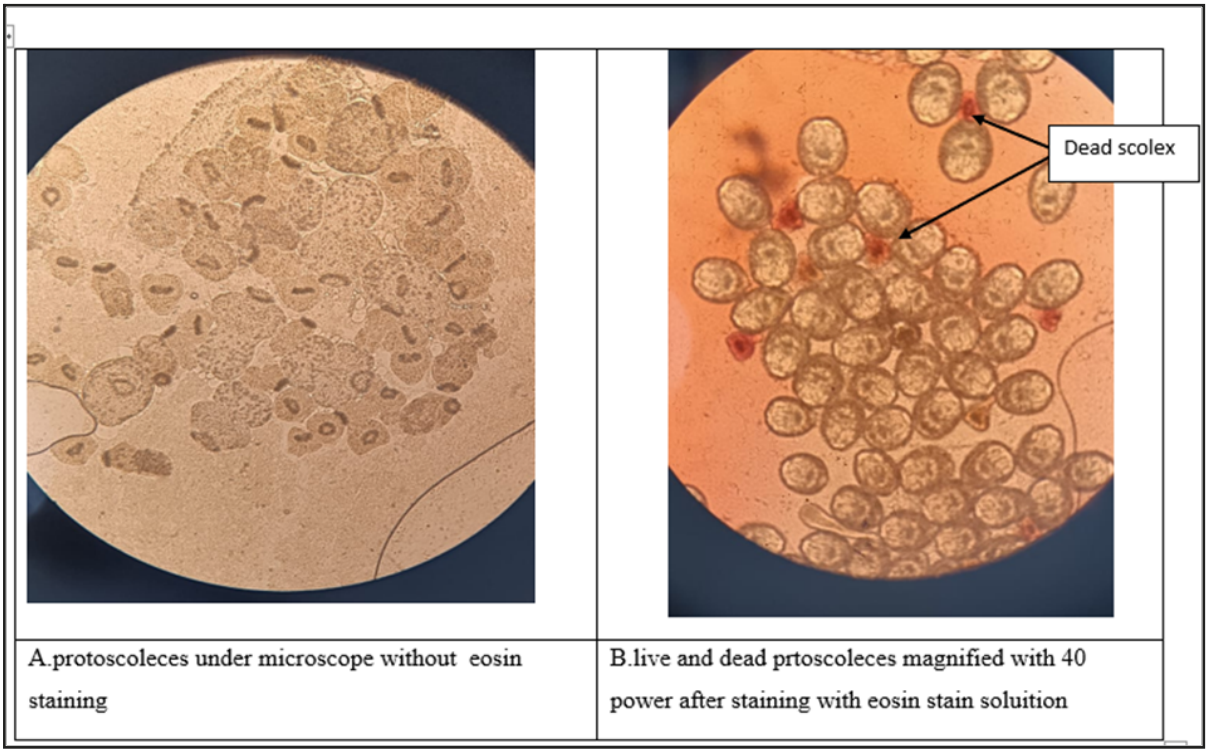


**Figure 1** A light microscopic examination by wet mount drop. B**:** Viability of *E. granulosus* protoscoleces by eosin exclusion test

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## Results

Socio-demographic information was collected and filled out by all patients Table 2.

The data was designed for animal samples which included sources of animals, type of animals, cyst location, and number of cysts Table 3.

**Table 2** Demographic characteristics of hydatid disease of human origin

| Host and number of Isolates | Organ | Fertility | Symptoms | Age | Gender | Location | Occupation |
|---|---|---|---|---|---|---|---|
| (8) Human | Liver | Sterile | Severe abdominal pain | 41 years old | Female | Erbil | Housewife |
| (9) Human | Liver | Fertile | Severe abdominal pain | 13 years old | Female | Makhmur | Student |
| (10) Human | Liver | Fertile | Abdominal pain and nausea | 31years old | Female | Kalak | Housewife |
| (11) Human | Liver | Fertile | No signs | 20 years old | Male | Taq Taq | Farmer |
| (12) Human | Liver | Sterile | Chest pain &coughing | 21yrs old | Female | Sidakan | farmer |
| (13) Human | Liver+ Lung | Fertile | Coughing &chest pain shortness in breath | 18 yrs old | Female | Erbil | Student |
| (14) Human | Liver | Fertile | Abdominal pain | 35 yrs old | Male | Erbil | Teacher |
| (15) Human | Liver | Fertile | No sign | 44 yrs old | Female | Erbil | Housewife |
| (16) Human | Liver | Fertile | No sign | 25 yrs old | Male | Erbil | Food handler |
| (17) Human | Lung +liver | Sterile | Chest pain &abdominal pain | 37 yrs old | Female | Shaqlawa | Housewife |
| (18) Human | Liver | Sterile | No sign | 42yrs old | Male | TaqTaq | Teacher |

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**Table 3** Features of hydatid cyst of animal origin

| Host | Organ | Fertility | Source |
|---|---|---|---|
| 1.Sheep | Liver & lung | Fertile | Local |
| 2.Sheep | Liver | Fertile | Syria |
| 3.Sheep | Lung | Fertile | Syria |
| 4. cattle | Liver | Sterile | Local |
| 5.Cattle | Liver | Sterile | Local |
| 6.Cattle | Liver | Fertile | Local |
| 7.goat | Liver | Sterile | Local |
| 19.Sheep | Liver | Fertile | Local |
| 20. Sheep | Liver | Fertile | Local |
| 21.sheep | Lung | Fertile | Local |
| 22.Cattle | Liver | Fertile | Local |
| 23.Sheep | Liver | Fertile | Local |
| 24. Cattle | Liver | Fertile | Local |
| 25. Sheep | Liver | Fertile | Local |
| 26. Sheep | Liver+Lung | Fertile | Local |
| 27. Sheep | Liver | Fertile | Local |
| 28.Cattle | Liver | Fertile | Local |
| 29. Sheep | Liver+Lung | Fertile | Local |
| 30. Sheep | Liver | Fertile | Local |
| 31. Sheep | Liver+Lung | Fertile | Local |
| 32. Cattle | Liver | Sterile | Local |
| 33. Cattle | Liver | Fertile | Local |
| 34. Sheep | Liver+Lung | Fertile | Local |
| 35.Sheep | Liver | Fertile | Local |
| 36.Sheep | Liver | Fertile | Local |
| 37.Cattle | Lung | Fertile | Local |
| 38.Sheep | Lung | Fertile | Local |
| 39.Sheep | Liver | Fertile | Local |
| 40.Sheep | Liver+Lung | Fertile | Local |
| 41.Sheep | Liver | Fertile | Local |
| 42.Sheep | Liver | Fertile | Local |
| **Negative** | **Samples** | | |
| 43.Cattle | Lung+Liver | Sterile | Local |
| 44.Cattle | Liver | Sterile | Local |
| 45.Sheep | Liver | Sterile | Local |
| 46.Sheep | Lung | Sterile | Syria |
| 47.Sheep | Liver | Sterile | Local |

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The genomic DNA was extracted from 25 mg of GL and 100 µL of *E. granulosus* PSC of each of 42 cysts isolated from intermediated hosts (11 humans and 36 animals).

The isolated DNA was electrophorized in 1.5% Agarose gel Figure 2. The mitochondrial Nad5 gene was amplified for *E. granulosus* strain identification. After amplification, the PCR products were electrophoresed and visualized under a UV transilluminator, which showed clear and visible bands. The size of the bands was the same in cysts isolated from animals and humans, as shown in Figure 3.

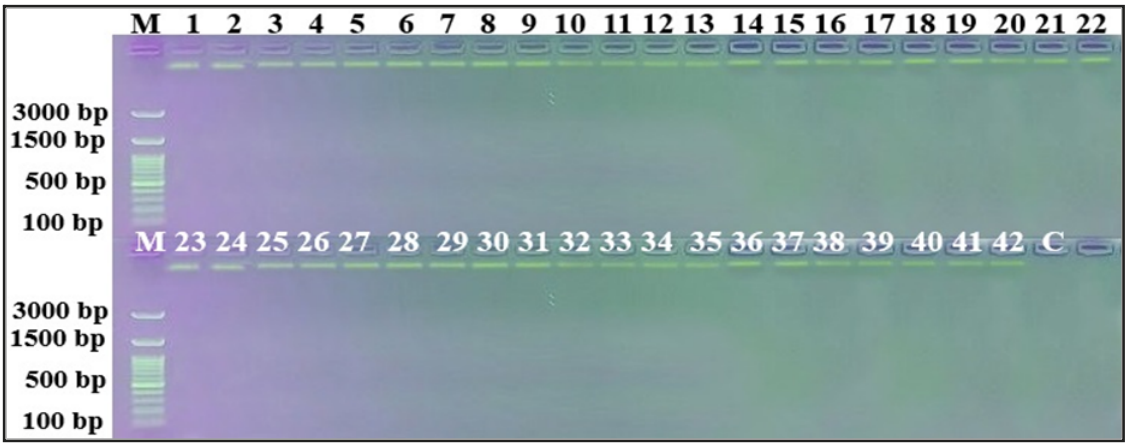


**Figure 2** Bands of extracted DNA from GL and PSCs of hydatid cyst tissue in *Echinococcus granulosus* tapeworm this is confirmed that DNA extracted successfully. The safe red dye was added at 10 µL/100 mL of agarose gel.

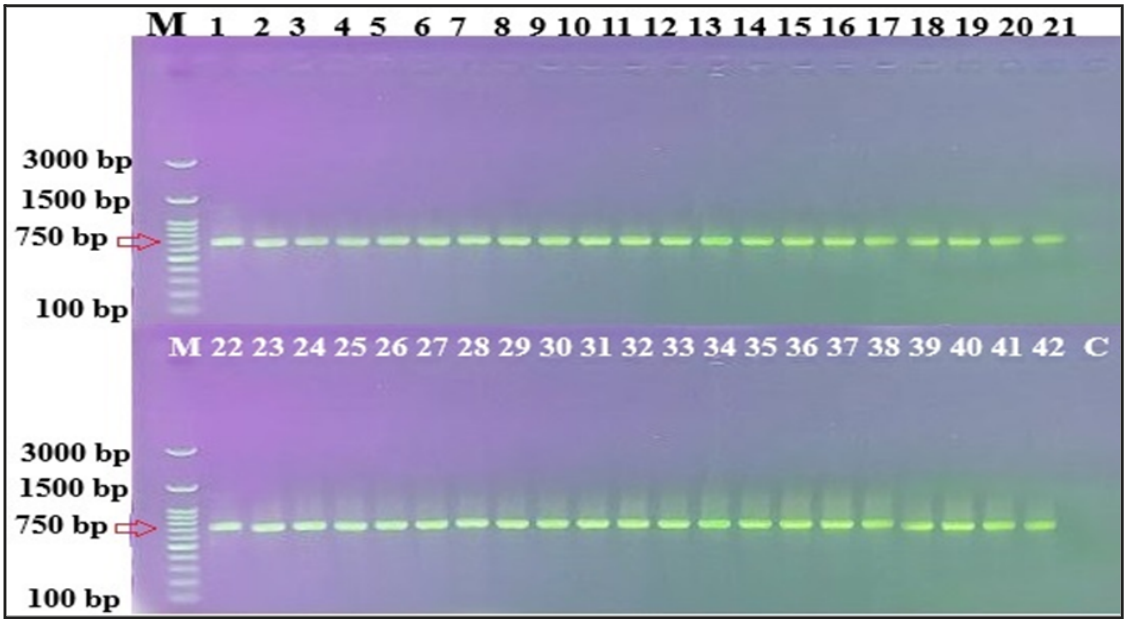


**Figure 3** PCR amplification of partial *NAD5* gene bands with 750 bp from 42 *Echinococcus granulosus* hydatid cyst sample from human, sheep, cattle and goat, M: DNA marker size (3000-100bp Ladder), lane (8 to 18) indicates Template DNA isolated from GL of human hydatid cysts, and lane (1to 7 and 19 to 42) represent Amplicon isolated from germinal layer &protoscoleces of animal hydatid cysts, lane (C) denotes negative control PCR, The safe red dye was added at 10 µL/100 mL of agarose gel.

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The data obtained from sequencing of 15 samples showed that the entire single and multiple cysts (GL and PSCs) isolated from humans and livestock animals were *E. granulosus*, with accession number (PV023162, PV023163, PV023164 for sheep) (PV023165 for goat) (human from PV023166 to PV023176) as shown in Table 4.

**Table 4** BLAST analysis to verify human and animal hydatid cyst samples

| GenBank Accession no. | Species | Host | Country | Genotype |
|---|---|---|---|---|
| PV023162 | *Echinococcus granulosus* | Sheep | Iraq | G1 |
| PV023163 | *Echinococcus granulosus* | Sheep | Iraq | G1 |
| PV023164 | *Echinococcus granulosus* | Sheep | Iraq | G1 |
| PV023165 | *Echinococcus granulosus* | Goat | Australia | G1 |
| PV023166 | *Echinococcus granulosus* | Cattle | Iraq | G1 |
| PV023167 | *Echinococcus granulosus* | Cattle | Iraq | G1 |
| PV023168 | *Echinococcus granulosus* | Cattle | Iraq | G1 |
| PV023169 | *Echinococcus granulosus* | Human | China | G1 |
| PV023170 | *Echinococcus granulosus* | Human | China | G1 |
| PV023171 | *Echinococcus granulosus* | Human | China | G1 |
| PV023172 | *Echinococcus granulosus* | Human | Iraq | G1 |
| PV023173 | *Echinococcus granulosus* | Human | Iraq | G1 |
| PV023174 | *Echinococcus granulosus* | Human | Iraq | G1 |
| PV023175 | *Echinococcus granulosus* | Human | Iraq | G1 |
| PV023176 | *Echinococcus granulosus* | Human | Iraq | G1 |

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The BLAST tool was used to evaluate and compare the results with similar *E. granulosus* sequences that were deposited in GenBank. Based on GenBank reference sequences, related *E. granulosus* sequences that are in GenBank. According to the sequences in GenBank. All samples all belong to genotype G1 (sheep strain) in different hosts and countries.

Sequencing outcomes of nad5 gene of various host species sheep, goat, cattle, and human revealed considerable similarity (98.6%–99%) with sequences of *Echinococcus granulosus* that have been previously reported in the NCBI GenBank database. All the samples showed high homology with accession numbers that were previously reported, confirming their identity as *Echinococcus granulosus* Table 5.

**Table 5** Sequences in NCBI-BLAST homology results among human and animal hydatid source

| Samples | Accession Numbers | NCBI-BLAST Homology Sequence | | |
|---|---|---|---|---|
| | | Identic Number % | GenBank Accession Number | GenBank Parasite Species Identification |
| | PV023162 | 98.6 | LC843908 | Echinococcus granulosus |
| Sheep | PV023163 | 98.6 | LC843895 | Echinococcus granulosus |
| Sheep | PV023164 | 98.6 | PQ843381 | Echinococcus granulosus |
| Sheep | PV023165 | 98.6 | LC84389 | Echinococcus granulosus |
| Goat | PV023166 | | | Echinococcus granulosus |
| Cattle | PV023167 | 98.6 | KJ559023 | |
| Cattle | PV023168 | | | Echinococcus granulosus |
| Cattle | PV023169 | 99 | KC756248 | |
| Human | PV023170 | | | Echinococcus granulosus |
| Human | PV023171 | 99 | OR269146 | |
| Human | PV023172 | | | Echinococcus granulosus |
| Human | PV023173 | 99 | KC756247 | |
| Human | PV023174 | | | Echinococcus granulosus |
| Human | PV023175 | 99 | AF297617 | |
| Human | PV023176 | | | Echinococcus granulosus |
| Human | | 99 | NC_044548 | |
| | | 99 | OR269149 | Echinococcus granulosus |

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The nad5 gene sequence mutations observed among the different isolates from sheep, goat, cattle, and human indicate several nucleotide substitutions, which include some at the amino acid level and some silent mutations (Figure 4).

The comparison of different amino acid codon variants of the NAD5 gene among human and animal isolates of E. granulosus reveals that genotype G1 is predominant in all host species. mutations—particularly at positions 640, 697, and 704–706—are uniformly prevalent, confirming the widespread circulation of the sheep strain (G1). While a few of the human and cattle isolates have additional nucleotide and amino acid changes, these are likely sub-variants or intra-strain polymorphisms and not indicators of another genotype Table 6 and Table 7.

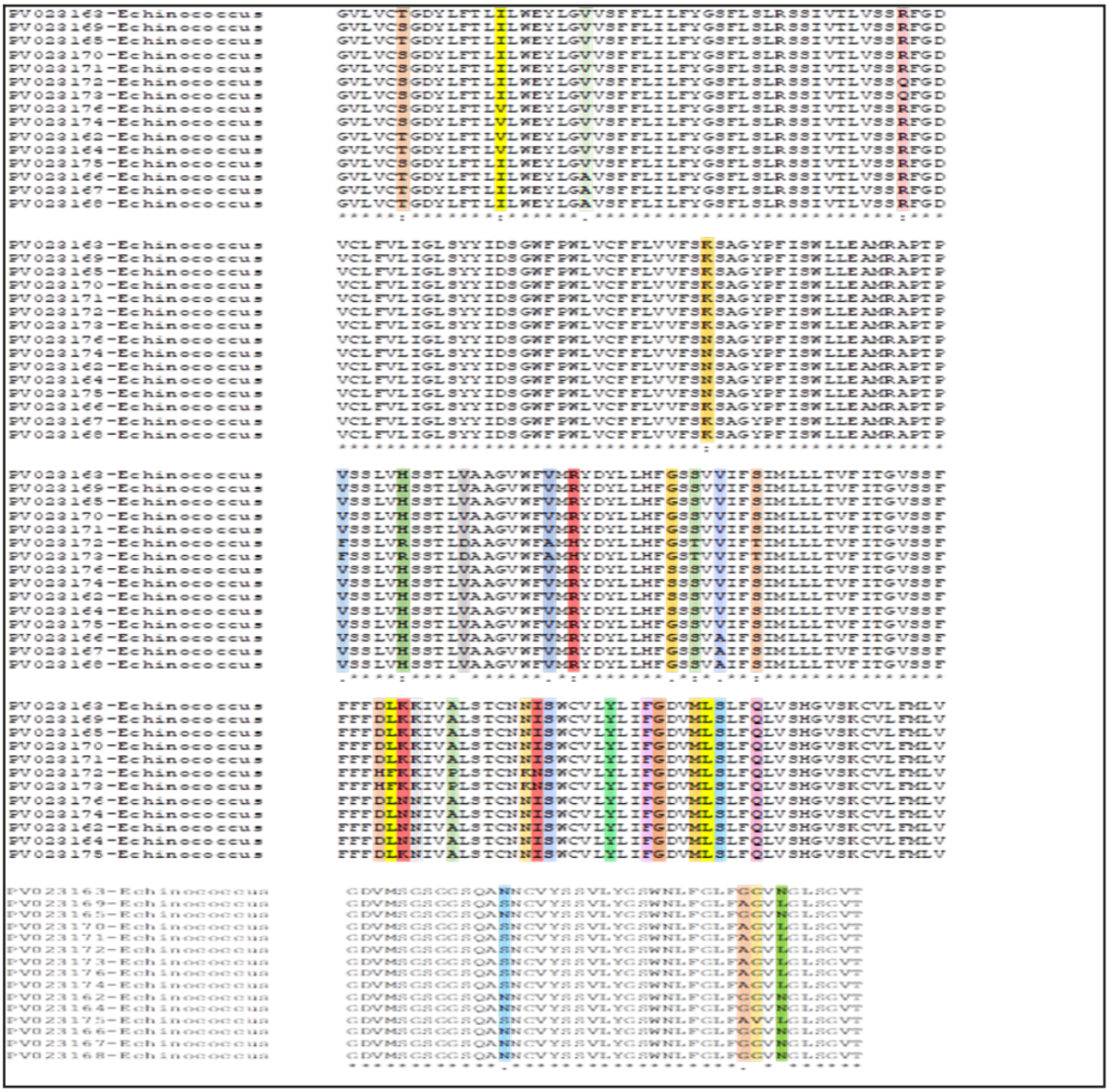

**Figure 4** Multiple protein sequence alignment analysis of *NAD5* gene among 15 submitted sequences *Echinococcus granulosus*

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**Table 6** Multiple variant amino acid codons of *NAD5* among human and animal *Echinococcus granulosus*

| Isolate No. | ACC. No. | Nucleotide change | Nucleotide position | Amino acid change | Amino acid position |
|---|---|---|---|---|---|
| Sheep | PV023162 | G/C | 19 | S/T | 6 |
| | | T/C | 362 | No change | 121 |
| | | A/G | 488 | No change | 164 |
| | | G/C | 632 | No change | 212 |
| | | G/A | 640 | S/N | 214 |
| | | C/G | 697 | A/G | 232 |
| | | T/A,C/A,T/A | 704,705,706 | L/N | 235 |
| Sheep | PV023163 | G/C | 19 | S/T | 6 |
| | | A/C | 632 | No change | 212 |
| | | G/A | 640 | N/S | 214 |
| | | G/A | 641 | No change | 215 |
| | | C/G | 697 | A/G | 233 |
| | | T/G,T/G | 698,700 | V/G | 234 |
| | | T/A,C/A,T/A | 704,705,706 | L/N | 235 |
| | | C/A | 723 | P/T | 242 |
| Sheep | PV023164 | G/C | 632 | No change | 212 |
| | | G/A | 640 | S/N | 214 |
| | | C/G | 697 | A/G | 233 |
| | | T/G,T/G | 698,700 | V/G | 234 |
| | | T/A,C/A,T/A | 704,705,706 | L/N | 235 |
| | | C/A | 723 | P/T | 242 |
| Goat | PV023165 | G/C | 19 | S/T | 6 |
| | | C/T | 443 | No change | 149 |
| | | G/C | 632 | No change | 212 |
| | | G/A | 640 | S/N | 214 |
| | | C/G | 697 | A/G | 233 |
| | | T/A,C/A,T/A | 704,705,706 | L/N | 235 |
| Cattle | PV023166 | G/C | 19 | S/T | 6 |
| | PV023167 | T/C | 64 | V/A | 22 |
| | PV023168 | T/C | 397 | V/A | 132 |
| | | G/T | 461 | D/Y | 154 |
| | | G/A | 471 | K/N | 157 |
| | | G/C | 480 | A/P | 160 |
| | | A/T, A/T | 499 ,498 | N/L | 166 |
| | | T/C | 504 | S/P | 168 |
| | | T/A | 519 | Y/N | 174 |
| | | T/C | 529 | F/S | 176 |
| | | T/C, G/A | 530,532 | G/D | 177 |
| | | T/A | 439 | M/L | 180 |
| | | G/A | 542 | L/F | 181 |
| | | G/C, T/C | 546 ,545 | S/P | 182 |
| | | G/A | 640 | S/N | 214 |
| | | C/G | 697 | A/G | 233 |
| | | T/A,C/A,T/A | 704,705,706 | L/N | 235 |

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| | | | | | |
|---|---|---|---|---|---|
| Human | PV023169 | T/C | 243 | No change | 148 |
| | | A/G | 632 | No change | 211 |
| | | G/A | 641 | No change | 214 |
| Human | PV023170 PV023171 | T/C | 243 | No change | 148 |
| | | A/G | 632 | No change | 211 |
| | | G/A | 641 | No change | 214 |
| | | T/G, T/G | 700 ,698 | V/G | 233 |
| | | T/A | 707 | No change | 236 |
| | | C/A | 723 | P/T | 241 |
| Human | PV023172 PV023173 | G/A | 142 | R/Q | 48 |
| | | G/T | 303 | V/F | 102 |
| | | A/G | 319 | H/R | 107 |
| | | T/A | 334 | V/D | 112 |
| | | T/C | 355 | V/A | 118 |
| | | G/A | 361 | R/H | 120 |
| | | G/C | 406 | S/T | 125 |
| | | G/C | 464 | D/H | 154 |
| | | G/C | 480 | A/P | 160 |
| | | T/G, T/A | 502 ,500 | I/N | 167 |
| | | G/A | 641 | No change | 214 |
| | | T/G, T/G | 700 ,698 | V/G | 233 |
| | | T/A | 707 | No change | 236 |
| | | C/A | 723 | P/T | 241 |
| Human | PV023174 | A/G | 42 | I/V | 14 |
| | | G/A | 245 | No change | 82 |
| | | G/A | 384 | G/S | 128 |
| | | G/A | 470 | K/N | 157 |
| | | T/G, T/G | 700 ,698 | V/G | 233 |
| | | T/A | 707 | No change | 236 |
| | | C/A | 723 | P/T | 241 |
| Human | PV023175 | G/A | 470 | K/N | 157 |
| | | T/G | 719 | No change | 721 |
| Human | PV023176 | A/G | 42 | I/V | 14 |
| | | G/A | 245 | No change | 82 |
| | | G/A | 384 | G/S | 128 |
| | | G/A | 470 | K/N | 157 |
| | | T/G, T/G | 700 ,698 | V/G | 233 |
| | | T/A | 707 | No change | 236 |
| | | C/A | 723 | P/T | 241 |

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**Table 7** Summarizing of mutations and changes among nucleotide and amino acids with common amino acids changing position based on nad5 gene

| Isolate number | Number of mutations | Number of Amino acid changes | Common amino acid changes position |
|---|---|---|---|
| PV023162 Sheep | 7 | (4) S/T (6), S/N (214), A/G (232), L/N (235) | 6(S/T) 214(N/S) |
| PV023163 Sheep | 8 | (6) S/T (6), N/S (214), A/G (233), V/G (234), L/N (235), P/T (242) | 6(S/T) 214(N/S ) 233(A/G ) |
| PV023164 Sheep | 6 | (5) N/S (214), A/G (233), V/G (234), L/N (235), P/T (242 | 214(N/S) |
| PV023165 Goat | 6 | (4) S/T (6), N/S (214), A/G (233), L/N (235) | 6(S/T) 214(N/S ) 233(A/G ) |
| PV023166 Cattle | 1 | (1) S/T(6) | ------ |
| PV023167 Cattle | 1 | (1) V/A(22) | ------ |
| PV023168 Cattle | 15 | 15 V/A (132), D/Y (154), K/N (157), A/P (160), N/L (166), S/P (168), Y/N (174), F/S (176), G/D (177), M/L (180), L/F (181), S/P (182), N/S (214), A/G (233), L/N (235) | 6(S/T) 214(N/S ) 233(A/G ) |
| PV023169 Human | 4 | 0 No change | ---- |
| PV023170 Human | 6 | 0 No change | ----- |
| PV023171 Human | 6 | 2 V/G (233), P/T (241) | 233(A/G ) |
| PV023172 Human | 1 | 1 R/Q (48) | ----- |
| PV023173 Human | 13 | V/F (102), H/R (107), V/D (112), V/A (118), R/H (120), S/T (125), D/H (154), A/P (160), I/N (167), V/G (233), P/T (241) | 233(A/G ) |
| PV023174 Human | 7 | 5 I/V (14), G/S (128), K/N (157), V/G (233), P/T (241) | 233(A/G ) |
| PV023175 Human | 1 | 1 K/N (157) | ---- |
| PV023176 Human | 7 | 5 I/V (14), G/S (128), K/N (157), V/G (233), P/T (241) | 233(A/G ) |
| Total | 89 | 49 | 8 |

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Table 8 shows the prevalence of mutations in 15 samples taken from various host species. Nearly one-quarter of the samples had four mutations, while fewer samples had three or one mutation. The statistics presented here pertain to the distribution of mutations in all of the examined sequences for each host species.

Table 9 displays the average amount of nucleotide mutations and amino acid changes. In terms of nucleotide mutations, sheep had the highest mean value (7.00), while humans had the lowest mean value (2.38), indicating a lower number of mutations that could be functional. The data demonstrates that different hosts exhibit different patterns of mutation.

**Table 8** Mutation frequency distribution among different species

| Frequency | Percent | Valid Percent |
|---|---|---|
| 4 | 26.7 | 26.7 |
| 1 | 6.7 | 6.7 |
| 4 | 26.7 | 26.7 |
| 3 | 20.0 | 20.0 |
| 1 | 6.7 | 6.7 |
| 1 | 6.7 | 6.7 |
| 1 | 6.7 | 6.7 |
| 15 | 100.0 | 100.0 |

**Table 9** The Average frequency of nucleotide mutations and amino acid changes in various host species

| Host | | Num Mutations | Num AA Changes |
|---|---|---|---|
| **Cattle** | Mean | 5.67 | 5.67 |
| | N | 3 | 3 |
| **Goat** | Mean | 6 | 4 |
| | N | 1 | 1 |
| **Human** | Mean | 5.63 | 2.38 |
| | N | 8 | 8 |
| **Sheep** | Mean | 7 | 5 |
| | N | 3 | 3 |
| **Total** | Mean | 5.93 | 3.67 |
| | N | 15 | 15 |

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## Discussion

Parasitic infestations and other infectious illnesses are major concerns for animal and human health because they lead to serious illness and financial losses.[18] Due to diminished production and illness, parasite-borne diseases generate substantial economic losses.[19]

Molecular investigations aid in the better characterization of the several Echinococcus genotypes (G1, G3) that cause CE. Molecular and phylogenetic analyses of *E. granulosus* in closely related species have made use of various nuclear and mitochondrial genomes. The current investigation divided hydatid cysts from cattle, sheep, goat and humans into G1 and G3 genotypes using the nad5 genomic segment in Erbil city**.**

Socio-demographic characteristics, hydatidosis affects wide age groups and both sexes with a significant number of female patients who are housewives and students. They were predominantly hepatic although pulmonary and combined localizations were also identified. Symptoms varied from intense abdominal or chest pain to completely asymptomatic, demonstrating the variability of the disease. Consistent with previous research in the Kurdistan area and elsewhere in Iraq, including Theqar, this study found that the CE rate was greater in females than in males .[20]

Fertile cysts prevailed in animal sample examination, and such is particularly the case with sheep and cattle. Sheep were common intermediate hosts with predominant hepatic and pulmonary localizations. It is also important to mention that animals derived from the vicinity of each locality and the animals imported from the Syrian Arab Republic had fertile cysts, highlighting the potential cross border spread of infected animals and the relatedness of livestock trade to the distribution pattern of *Echinococcus granulosus* infections.

Positive molecular analysis of germinal layers and protoscoleces, molecular analysis supports significant quality extraction of genomic DNA from germinal layers as well as protoscoleces, while nad5 mitochondrial gene PCR amplifications resulted topronounced and consistent bands for all samples. This indicates a good performance of nad5 as a genetic marker for *E. granulosus* characterization and typing.

Distinguishing between genotypes G1 and G3 remains contentious, despite the numerous mitochondrial DNA markers developed for molecular identification of *E. granulosus.* Although the mitochondrial DNA segment is relatively tiny at 680 bp, the nad5 gene occupies six crucial locations, making it more reliable in distinguishing G1 and G3 genotypes.[21] Mitochondrial genes NAD1 and NAD5 have recently been the focus of attention for their roles in identifying strains G1 and G3, which are extremely close to one another in terms of strain stricto.[22]

Based on nad5 gene sequencing of 750 bp, all samples detected in this investigation belong to *Echinococcus granulosus*, specifically the G1 genotype when compared to *E. granulosus* sequences found in GenBank, This genotype, often called the "sheep strain," is the most prevalent and contagious one that impacts both humans and animals. According to the research, humans are accidental hosts, whereas sheep, goats, and cattle are intermediate host.

11 samples screened belong to Iraq with additional samples related to China and Australia It appears that *E. granulosus* is quite common in Iraq, maybe because of the country's large livestock population, the proximity of humans to animals, and the traditional slaughtering practices. While other research in several Iraqi provinces has found the same thing. Investigations like the one by Hammad et al. have used either all of cox1, nad1, or atp6, or just one of them.[23] and found in the governorates of Kirkuk and Sulaimania, as well as Thi-Qar and Misan, and G3 strains exclusively.[24] Aqrah and Koya cities and

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northern Iraq, confirm and record the G1 strain, and these results contrasted from those of Hamoo et al,[25] Abdulla et al,[26] Alignment of NAD5 sequences revealed mutations at locations 19, 640, 697, 704-706 in three of the sheep samples (2, 3, and 4). Most of the alterations occur at positions 640 (G/A), which impact amino acid Serine /Threonine and 697 (C/G), which alter Alanine /Glycine. A mutation at positions 704–706, which causes an amino acid change from Lucine / Asparagine. is seen in several samples. There is a greater variety of mutations in the cattle samples 6, 7, and 8. Mutations are also present at positions 19, 640, 697, 704-706. Mutations at T/C 64Valine /Asparagine, G/T 461 Aspartic acid /Tyrosine, and A/T 498 Alanine /Proline may suggest that the 439 Methionine /Leucine position is unique to cattle. Whereas the goat sample 5 has fewer variations G/C at 19 Serine/ Threonine and/A at 640 Serine/Threonine, are also observed in sheep. C/T at 443 shows "No change," meaning it's a silent mutation. So, they were considered the G1 strain.

In human samples Several variations overlap with other hosts, but unique ones include: human 11,12 Glycine/Alanine at 142 Arginine/Glutamine, Glycine/Threonine at 303 Valine/phenylalanine ,T/A at 334 Valine/Aspartic acid ,T/C at 361 Arginine/ Histidine, that result in 99% identity to G1 strain as From human in turkey.[27]

Strong statistical evidence is provided for the varied distributions of mutation frequencies and amino acid alterations among host species. Because of increased genomic diversity or host-due mutation pressure, isolates obtained from sheep had the highest nucleotide mutation numbers. Protein evolution in humans was influenced by natural selection, as evidenced by the reduced frequency of amino acid-changing mutations in isolates originated from humans.

Our result demonstrated that G1 genotypes identical 99% to reported G1 genomes from previous papers as shown that these results show that the G1 strain of Echinococcus is the most infectious strain of this species globally. Because it is capable of infecting specific types of hosts (Alvarez Rojas et al., 2014; Thompson, 2017). This made the genetic variety within the research area's single genotype obvious; the large variation in individual patterns may be due to the interplay between domestic and animal hosts. (Haag et al., 2008). Also from cattle in Iraq,[28] from humans in Iran,[29] Mutations in NAD5 could affect parasite survival, making these sites potential drug targets. If certain mutations are linked to more virulent strains, they could help in tracking infections and developing treatments. Human samples show the highest variation, possibly due to stronger immune pressures or longer adaptation. These insights can aid in disease management, vaccine development, and understanding parasite evolution.

## Conclusion

This research found that the G1 genotype of *E. granulosus s.s.* is the most common in both humans and cattle, sheep, goat in Erbil. The findings provided additional evidence that the nad5 gene accurately distinguished between human and cattle, sheep, goat *E. granulosus* G1/G3 isolates. The prevalence of the G1 genotype of *Echinococcus granulosus* among human and animal hosts in Erbil indicates the imperative need for targeted public health interventions. Future studies must aim towards intensification of genotypic monitoring all over Iraq to trace out strain pattern distribution and variation more widely. Also, the identified genetic differences, especially in human samples.

## Competing interests

The authors declare that they have no competing interests.

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