# Genetic Polymorphism in *ND1* Gene of Hydatid Cyst in Iraqi goats

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***Abstract***
*Hydatid cysts were taken from 18 goats living in different places in Iraq after diagnosing and specifying the place of the injury for the period from July 2016 till February 2018. DNA was extracted from (protoscoleces) by using the mechanical crashing method to gain an enough quantity of the DNA to perform the partial diagnostic. NADH dehydrogenase subunit 1 Gene (ND1) was used as a goal after designing the specific primers for study.*

*Results showed that a DNA fragment was obtained from all the samples that have been studied where the primary detection of the gene depends on the molecular size of about 800 bp after electrophoresis on Agaros gel. After that, the results were refined by using Geneaid Kit, Kuria and the purity of the DNA was measured by using Spectrophotometer by measuring the wave lengths 260 and 280 and then specifying the value of (OD). After receiving the gene ND1 series, they were matched with International Gene Bank NCBI where all the symbols gave matching results with G1 sheep strain.*

**Keywords**: Hydatid cyst in goat, G1 strain, *ND1* gene, Goat, Sequences.

## Introduction

The injury with the Hydatid Cysts by the larval stage of the *Echimococcus granulosus* is considered of global spread and it is considered as one of the most contagious diseases that is transferred between human and animal worldwide[3,24]. Animals get infected when contacting infected dogs with adult worms *Echimococcus granulosus* that live in the intestines of the dogs which will lay their eggs with the stool and eventually human and other animals might get infected by swallowing contaminated food and water with these eggs.[9]

In contrast, *Echimococcus granulosus* will be influenced by the life cycle specially the host and the rate of development and the pathogenicity and at the end it determines the types of the chemotherapy and the enhancements of the vaccination against *Echimococcus granulosus*. In Iraq, Cystic Echinococcosis is considered one of the most epidemic disease which imposes danger of both human and animal at the same time, thus, it is considered of a great effect on the health of human and animal[2,11,13,23]. Recent studies proved that there are 10 distinct genotypes (G1- G10) and they all have been studied and specified for their characteristics all over the world depending on nucleotide sequences analysis of ND1, Co1 genes, these genotype might be limited to infect intermediate hosts like pegs, sheep, goats, camels, cattle, cervides and horses[20,21].

The process of using the DNA to diagnose the infection of *E. granulosus*, is considered extremely important and very delicate in pinpointing the genetic type of the worms that infect a specific geographic area and not others[8,22]. Hydatidosis is considered one of the important disease in the developing countries. Here the contact between human and animal is tight[18]. Goats get infected by close contacting with dogs that are used as Sheppard to control the cattle during herding[25].

This study was aimed to diagnose the genotype causing the *E. granulosus* disease that infects Iraqi goats and estimates the genetic contrast of the species of the disease depending on amplification and sequencing of *NADH dehydrogenase subunit 1 gene*.

## Material and Methods

Eighteen infected samples were collected from different governorates of Iraq from July 2016 to February 2018. At the beginning, the cysts were washed several times by using normal saline to reduce contamination that might occur by the host tissue, after that they were washed by using ethanol 70%. According to McManus et al[12], each cyst was divided to two parts: membrane and intra Cystic fluid with protoscoleces. The cyst content (fluid and protoscoleces) was separated by using sterile syringes and emptied in a flask. Then the cyst was opened longitudinally and what is left of the fluid and protoscoleces was separated and was added to the content of the flask.

After that, the content of the flask was transferred carefully to a sterile test tube and was centrifuged at 3000 rpm for 10 minutes at room temperature to get residue of protoscoleces whereas the remaining of the bag which was Germinal membrane, was separated from the rest of the cyst content, then washed several times using Hanks Saline ($_P$H=2), containing 2% Pepsin to get rid of the rest of the protoscoleces attached to the membrane. After that centrifugation was done to the suspended matter at a rate of 3000 rpm for 10 minutes. The last step was to wash the protoscoleces 3 or 4 times using sterile normal saline. Centrifugation was repeated several times, then washed by

using 70% ethanol and stored in 70% ethanol at 4ºC to be used in the following steps.

Sediment of protoscoleces was washed more than one times with sterile distilled water and phosphate buffer saline (PBS) to take off ethanol prior to DNA extraction[1]. Extraction of DNA was done by utilizing Wizard ®Genomic DNA Purification Kit. (USA) and followed the instruction of manufacture[16]. 20 ng of DNA from sediment of protoscoleces was used in all samples.

**PCR analysis:** Adequate samples of DNA (20 ng) was dissected according to Miller et al[14] by using the dissected methods with some modifications. The mitochondrial *ND1* fragment was enlarged by PCR using *ND1* R. and *ND1* F. primers as in table 1[22]. The thermal conditions of the PCR *ND1* reaction was done by following steps: denaturation for 4 mins at 94ºC, followed by 35 cycles of 45 sec at 94ºC 45 sec at 58ºC and 45 sec at 72ºC and a final extension at 72ºC for 7 min.

Phylogenetic analysis and sequencing of $_{mt}$DNA for eighteen amplicons were chosen and fragments of *ND1* genes were amplified with specific primers manufactured earlier[4,22]. Sequences of DNA were compared with partial *ND1* sequences from prior publications and National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

## Results and Discussion

**PCR amplification:** All the 18 DNA samples extracted from protoscoleces study, were amplified, using specific primers designed to perform this experiment. The processing of the PCR is to amplify the DNA and to get the gene to perform the study where all the eighteen samples gathered from DNA (figure 1) reveal the electrophoresis of PCR products of *ND1* gene. A partial fragment of the *ND1* gene was amplified by using a previously described protocol.

**Sequence analysis:** To specify the genetic types to all the specimen taken from the eighteen sample bags, the ND1 gene was amplified and then sequenced and analyzed, then matching process was carried out with the Gene Bank depending on the references registered in NCBI (figure 2). The process of matching the series and analyzing the results was done using (DNA analysis program) Bioedit and the results achieved through this study were matched with the references that were registered in NCBI to the *E. granulosus*. Results of the study showed the rate of matching was 100% with sheep strain G1 genotype comparing with (MG682543.1)[10].

This study diagnosed the genotype to the parasite causing the disease Hydatid Cyst in Iraqi goats where the gene ND1 was amplified using special primer designed especially for this study.

Result of amplification of ND1 gene gave positive results in all the specimens that had been studied with PCR product of about 800 bp. These results match the previous studies that were executed by earlier studies mentioning[4,7,22] that the size of *ND1* gene was around 800 bp. The genotype G1 is the most common and is considered as the species that infects sheep where this genotype of the *E. granulosus* is most spread all over the world and infects large hosts[5]. The infecting occurs in the developing countries that use dogs for herding and the infecting takes place by close contact between goats and dogs that are used to control the cattle during herding[21,25].

Hydatid Cyst is considered one of the epidemiological diseases and the way it infects, makes it one of the most spreading epidemiological diseases in Iraq and thus sheep genotype (G1) is considered one of the most epidemiological in the country and the results match with what was mentioned earlier[4,5,15,16,21]. The reason for that is the presence of types intermediate hosts, has the ability and the readiness in infecting in *E. graulusus* belongs to sheep strain[19,22].

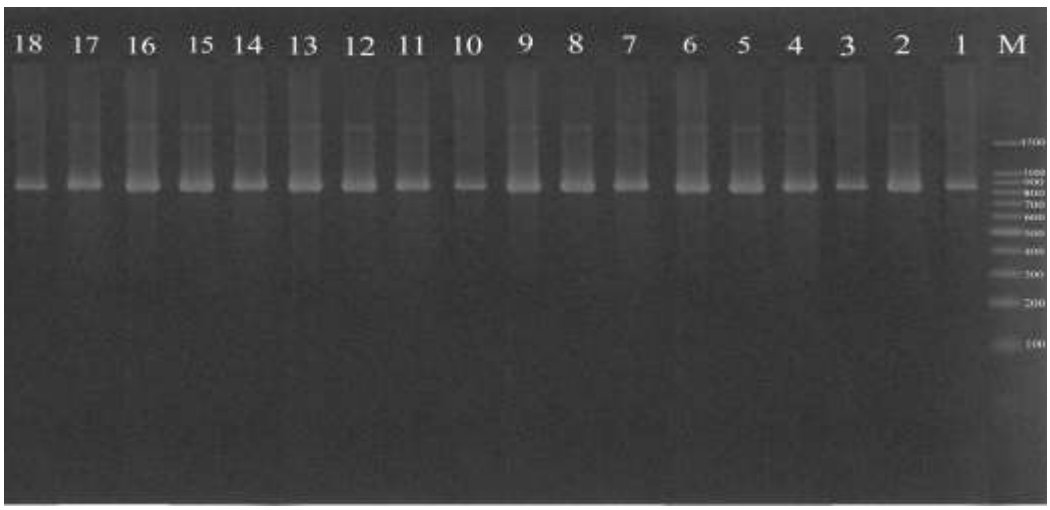


**Fig. 1: Electrophoresis of the amplified *ND1* gene on agarose gel (2%) at 80 volt for 2 hrs, (lanes 1-18: *Echinococcus granulosus* isolates; M: 100 bp DNA ladder).**

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 1201 bits(650) | 0.0 | 650/650(100%) | 0/650(0%) | Plus/Plus |

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Query  1     TTTTTTGTTTTAGGGGAGCGTAAGGTTTTGGGCTATTCTCAGTCTCGTAAGGGCCCTAAC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7341  TTTTTTGTTTTAGGGGAGCGTAAGGTTTTGGGCTATTCTCAGTCTCGTAAGGGCCCTAAC  7400

Query  61    AAGGTTGGTGTGATTGGTTTGTTGCAGAGGTTTGCTGATCTATTGAAGTTGGTAGTTAAG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7401  AAGGTTGGTGTGATTGGTTTGTTGCAGAGGTTTGCTGATCTATTGAAGTTGGTAGTTAAG  7460

Query  121   TTTAAGTGTTTTTACTTCCAAAGTCGTAGGTATGTTGGTTTGTTTGGTGTTGTGTTATTA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7461  TTTAAGTGTTTTTACTTCCAAAGTCGTAGGTATGTTGGTTTGTTTGGTGTTGTGTTATTA  7520

Query  181   ATGGCTTTGGTGATTGTTTATTCATTTATTTATGGTAGATATTATAGAGCTAGTTATAGA  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7521  ATGGCTTTGGTGATTGTTTATTCATTTATTTATGGTAGATATTATAGAGCTAGTTATAGA  7580

Query  241   GGCCTCTCCGTGTTGTGGTTTTTGGCTGCCGCCAGAACATCTAGGTATTCTTTGTTGTGT  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7581  GGCCTCTCCGTGTTGTGGTTTTTGGCTGCCGCCAGAACATCTAGGTATTCTTTGTTGTGT  7640

Query  301   ACTGGTTGGGGTGGTTACAACAATTATTCATTTTTAAGGTCGGTTCGATGTGCTTTTGGA  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7641  ACTGGTTGGGGTGGTTACAACAATTATTCATTTTTAAGGTCGGTTCGATGTGCTTTTGGA  7700

Query  361   TCTGTTAGGTTTGAGGCTTGTTTTATGTGTGTGGTGATTTTTTGTGCTTTGTGTAGTTGT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7701  TCTGTTAGGTTTGAGGCTTGTTTTATGTGTGTGGTGATTTTTTGTGCTTTGTGTAGTTGT  7760

Query  421   AGGtataatttaattgatttttattataattgttgattaagtttgttattatttccatta  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7761  AGGTATAATTTAATTGATTTTTATTATAATTGTTGATTAAGTTTGTTATTATTTCCATTA  7820

Query  481   atttatgtgttatttttaatgtgtATATTGTGTGAAACTAATCGTACGCCATTTGATTAT  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7821  ATTTATGTGTTATTTTTAATGTGTATATTGTGTGAAACTAATCGTACGCCATTTGATTAT  7880

Query  541   GGAGAGGCTGAAAGAGAGTTGGTCAGTGGGTTTAAAGTTGAGTATAGTGGTATTTATTTT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7881  GGAGAGGCTGAAAGAGAGTTGGTCAGTGGGTTTAAAGTTGAGTATAGTGGTATTTATTTT  7940

Query  601   ACGTGTTTATTTGCTTGTGAGTATATTATTATATATGTGTTTTCATGGTT  650
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7941  ACGTGTTTATTTGCTTGTGAGTATATTATTATATATGTGTTTTCATGGTT  7990
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**Fig. 2: Alignment of NADH dehydrogenase subunit 1 (ND1) gene with reported reference sequences of G1 genotype of *Echinococcus granulosus* by using Gene bank with (MG682543.1)**

**Table 1**
**NADH dehydrogenase subunit 1 *ND1* primer**

| Marker | Size | Code | Sequence |
|---|---|---|---|
| *ND1* | 800 bp | *ND1*.F<br>*ND1*.R | 5'-GTT TTT GGG TTA GTC TCT GG-3'<br>5'-ATC ATA ACG AAC ACG TGG -3' |

The process of diagnostic and specifying the identifying of the genotype *E. gramulosus* that infects goat are considered important from the epidemiological point of view within the areas that are considered epidemic and the genotype G1 that infects intermediate host are more inclusive and versatile.[6,17]

## Conclusion

The species causing Hydatid Cyst disease in Iraqi goats are G1 genotype where all the eighteen infected samples that were studied which were gathered from different parts of the country gave positive results depending on diagnosing Partial Sequences of ND1 and this study is considered good in diagnosing the species causing the disease in Iraqi goats.

## References

1. Al-Azawiy A.K., Immunization of experimental mice by DNA of protoscoleces of hydatid cyst: Immunological and histopathological studies, Ph.D. thesis, College of Veterinary Medicine, University of Baghdad, Iraq **(2003)**

2. Al-Jeboori T.I., Hydatid disease, A study of the records of the medical city hospital, *J. Fact. Med. Baghdad*, **18**, 65-75 **(1976)**

3. Altintas N., Oztatlici M., Altintas N., Unver A. and Sakarya A., Molecular Analysis of Cattle Isolates of *Echinococcus granulosus* in Manisa Province of Turkey, *Kafkas Univ Vet Fak Derg.*, **19(3)**, 455-459 **(2013)**

4. Baraak M.J., Molecular Study on Cystic Echinococcosis in Some Iraqi Patients, Ph.D. Thesis, College of Science, University of Baghdad, Iraq **(2014)**

5. Craig P.S., Rogan M.T. and Campos-Ponce M., Echinococcosis: disease, detection and transmission, *Parasitol*, **127**, 5-20 **(2003)**

6. Eckert J. and Thompson R.C., Intraspesific variation of *Echinococcus granulosus* and related species with emphasis on their infectivity to humans, *Acta Trop*., **64**, 19-34 **(1997)**

7. Ergin S., Saribas S. and Yuksel P., Genotypic characterisation of *Echinococcus granulosus* isolated from human in Turkey, *Afr. J. Microbiol. Res*., **4(7)**, 551-555 **(2010)**

8. Eryıldız C. and Şakru N., Molecular Characterization of Human and Animal Isolates of *Echinococcus granulosus* in the Thrace Region, Turkey, *Balkan Med J*., **29**, 261-7 **(2012)**

9. Fasihi H.M., Hobbs R.P., Adams P.J., Mobedi I., Morgan-Ryan U.M. and Thompson R.C.A., Molecular and morphological characterization of *Echinococcus granulosus* of human and animal origin in Iran, *Parasitol*., **125**, 367-373 **(2002)**

10. Kinkar L., Laurimae T., Balkaya I., Casulli A. and Zait H., Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3, *Parasitology*, In press, 1-10 **(2018)**

11. Mahmoud S.S., Studies on hydatid disease in Mosul, M.Sc. Thesis, Collage of Medicine, University of Mosul **(1980)**

12. McManus D.P. and Smyth S.D., Differences in the chemical composition and carbondrate metabolisim of *Echinococcus granulosus* (horse and sheep strains) and E. multilocularis, *Parasitol*., **77**, 103-109 **(1978)**

13. McManus D.P. and Thompson R.C.A., Molecular epidemiology of cystic echinococcosis, *Parasitol*., **127**, S37-S51 **(2003)**

14. Miller S.A., Dykes D.D. and Polesky H.F., A simple salting out procedure for extracting DNA from human nucleated cells, *Nucl. Acids Res.*, **16**, 1215 **(1988)**

15. Muhaidi M.J., Determination of the Infective Strain of Hydatid Cyst in Iraqi Cattle By using Co1 Gene, *Iraqi J. Agric. Sci*., **48(2)**, 644-649 **(2017)**

16. Muhaidi M.J., Ahmed M.N. and Aziz L.M., Identification of *Echinococcus granulosus* Genotype in Iraqi's Sheep by Using ND1 Gene, *J. of Global Pharma Tech*., **10(07)**, 261-26 **(2018)**

17. Mwambete K.D., Ponce-Gordo F. and Cuesta-Bandera C., Genetic identification and host range of the Spanish strains of *Echinococcus granulosus*, *Acta Trop*., **91**, 87-93 **(2004)**

18. Nyero D., Zirintunda G., Omadang L. and Eko J., Prevalence of hydatid cysts in goats and sheep slaughtered in Soroti Municipal Abattoir, Eastern Ugand, *African Journal of Parasitol*., **2(9)**, 148-151 **(2015)**

19. Rinaldi L., Maurelli M.P., Capuano F., Perugini A.G., Veneziano V. and Cringoli S., Molecular update and cystic echinococcosis in cattle and water buffaloes of southern Italy, *J. Blackwell verlag. Zoonosis, Pub. Heal*., **55**, 119-123 **(2008)**

20. Romig T., Dinkel A. and Mackenstedt U., The present situation of echinococcosis in Europe, *Parasitol. Int*., **55**, 187-191 **(2006)**

21. Sanchez E., Caceres O., Naguira C., Garcia D. and Patifio G., Molecular characterization of *Echinococcus granulosus* from Peru by sequencing of the mitochondrial cytochrome C oxidase subunit 1 gene, *Mem Inst Oswaldo Cruz, Rio de Janeiro*, **105(6)**, 221-232 **(2010)**

22. Sánchez E., Cáceres O., Náquir A.C., Miranda E., Samudio F. and Fernandes O., *Echinococcus granulosus* genotypes circulating in alpacas (*Lama pacos*) and pigs (*Sus scrofa*) from an endemic region in Peru, *Mem Inst Oswaldo Cruz, Rio de Janeiro*, **107(2)**, 275-278 **(2012)**

23. Sarıözkan S. and Yalçın C., Estimating the production losses due to cystic echinococcosis in ruminants in Turkey, *Vet. Parasitol*., **163**, 330-334 **(2009)**

24. Thompson R.C., The taxonomy, phylogeny and transmission of *Echinococcus*, *Exp Parasitol*., **119**, 439-446 **(2008)**

25. UBOS, The national livestock census report, UBOS, Kampala, www.ubos.org **(2008)**.