ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Identification of Echinococcus Granulosus Genotype in Iraqi's Sheep by Using Nd1 Gene

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Abstract

Hydatid Cysts were obtained from 15 sheep from hepatic, pulmonary, spleen, heart, and peritoneal cavity, between December 2014 and October 2015. Hydatid cysts (protoscoleces) were used for DNA extraction by using mechanical grinder. The purification of mt DNA was done by (promega kit, USA). The mitochondrial *NADH* dehydrogenase subunit 1 (*ND1*) genes was applied as aim for amplification by using polymerase chain reaction (PCR), all of 15 hydatid cysts yielded amplification products.PCR product for NADH1 800 bp. The PCR products had been purified and fractional sequences were produced. The sequences obtained were found to align with corresponding region for *ND1* gene in the Gene Bank nucleotide database confirming to genotype of sheep strain (G1) in Iraq, Phylogenetic dissection of fractional sequence datum from *ND1* gene for obtained Phylogenetic tree. G1 genotype was the majority widespread taxon and the genuine provenance of infection for Iraqi's sheep. All of 15 specimens were G1 strain (sheep strain) according to the fractional sequences of NADH dehydrogenase 1 (*ND1*).

Keywords: *G1 strain, Hydatid cyst, ND1 gene, sheep, Sequences.*

Introduction

Cystic echinococcosis has a cosmopolitan distribution is partly due to the capacity of *E. granulosus* to adjust to an enormous species of wild and domestic definitive and intermediate hosts [1] .The disease is usually asymptomatic. However, it can clinically manifest as a complicated cyst. The most frequent complication is compression or rupture of pericystic structures [2].

The prevalence of infection was mostly occurred in regions of the temperate area, such as South America, the whole Mediterranean area, China, central Asia, Australia, Russia, and parts of Africa[3]. The extensive difference in *E. granulosus* may influence lifecycle, host specificity, pathology and consequently, rate of development, the design plus sensitivity to development of vaccines and chemotherapeutic agents against *E. granulosus* [4]. In Iraq, CE constitutes one of the major endemic diseases and has serious influence on animal health or human [5-7]. To date ten notable genotypes (G1-G10 strains) were qualified in world according to nucleotide sequences dissection of the (*CO1*), NADH dehydrogenase 1 (*ND1*) genes and intra transcribed spacer 1 (ITS1), these strain were correlating with prominent, intermediate hosts including: sheep, goats, horses, pigs, cervides, cattle and camels [8]. The G1 genotype was likewise specified as the only strain secluded from sheep, cattle and humans [9].

A figure of (DNA) correspondence techniques were used to distinguish *Echinococcus* species and genotypes by used various intermediate and definitive hosts and in several geographical settings [10].The aim of this research to recognize the *E. granulosus* strain actually infecting sheep in Iraq. Polymerase chain reaction was used to evaluation the genetic contraindication within the strains by sequencing the NADH dehydrogenase 1 genes *ND1*gene.

Materials and Methods

The samples of 15 hydatid cysts were collected from different region in Iraq during 2014 to 2015. The cysts were washed more than one times with normal saline to decrease contamination with host tissue, and then they were extensively washed with 70% ethanol. According to McManus [11]. Each cyst was

separated into membrane and intra cystic fluid with protoscoleces. The cyst contents (fluid and protoscoleces) were aspirated aseptically by sterile syringes (10 ml) into a flask. Cysts were opened with longitudinal incision and all the remaining protoscoleces and fluid were aspirated and added to the flask content.

The fluid was carefully and gradually decanted into a sterile test tube with spinning at 3000 rpm for l0 minutes at room temperature collect the protoscoleces' pellet. The germinal membrane was peeled away and washed several times with Hanks saline (pH 2.0) containing 0.2% (w/v) pepsin to release the remaining attached scoleces [12]. The suspension was centrifugated at 3000 rpm for 10minutes, and the pellet of scoleces was collected. Protoscoleces were finally rinsed 3-4 times with sterile normal saline by repeating centrifugation followed by 70% ethanol, and stored in 70% ethanol at 4°C for further analysis. Pellet of protoscoleces were rinsed more than one times with sterile distilled water and Phosphate buffer saline (PBS) to remove ethanol prior to DNA extraction [13]. DNA extraction was done by using Wizard ®Genomic DNA Purification Kit.(USA) and following the instructions of the manufacture [14]. Twenty nanograms of DNA from protoscoleces pellet were used for all samples.

PCR Analysis

20 ng of DNA samples was analyzed by utilizing the described methods with subaltern modifications [14] Table (1). The mitochondrial *ND1* fragment was amplified by PCR using *ND1* F. and *ND1* R. primers Table(2)[9]. The thermal conditions of the PCR *ND1* reaction was as follows, denaturation for four minutes at 94ºC, and then were tuned for 35 cycles of 45 second at 94ºC, 45 second at 58ºC and 45 second at 72ºC and a ultimate extension at 72ºC for 7 minutes.

Table1: PCR condition for *ND1* gene

Criteria of cycle	Conditions (temp / time)
Initialdenaturation	94°C for 5minutes
Amplification	94°C for 45sec;58°C for 45sec;72°C for 45sec
Numberofcycles	35
Finalextension	72°C/7mins

Table 2: NADH dehydrogenate subunit 1*ND1* primer

marker	size	code	Sequence
ND1	800 bp	*ND1*.F	5'-GTT TTT GGG TTA GTC TCT GG-3'
		ND1.R	5'-ATC ATA ACG AAC ACG TGG -3'

mt DNA sequencing and phylogenetic analysis fifteen amplicons, were chosen, and fractions of amplicons*ND1* genes were amplified by using two primers published in [9]. DNA sequences were contrasted with fragment *ND1* sequences from previous publications and NCBI website (http://www.ncbi.nlm.nih.gov).Phylogeneticanal ysis of fractions sequence datum of *ND1* genes.

Results and Discussion

PCR Amplification

The objective DNA was prosperous amplified from 15hydatid cyst isolates were prepared for PCR processby using specific primer. A PCR procedure to amplify DNA as the target gene was set up and completed on all 15 DNA specimens. (Figure 1) display the agarose gel electrophoresis of PCR amplicon of *ND1* gene. A fractional part of the *ND1* gene was amplified following a formerly characterized protocol.

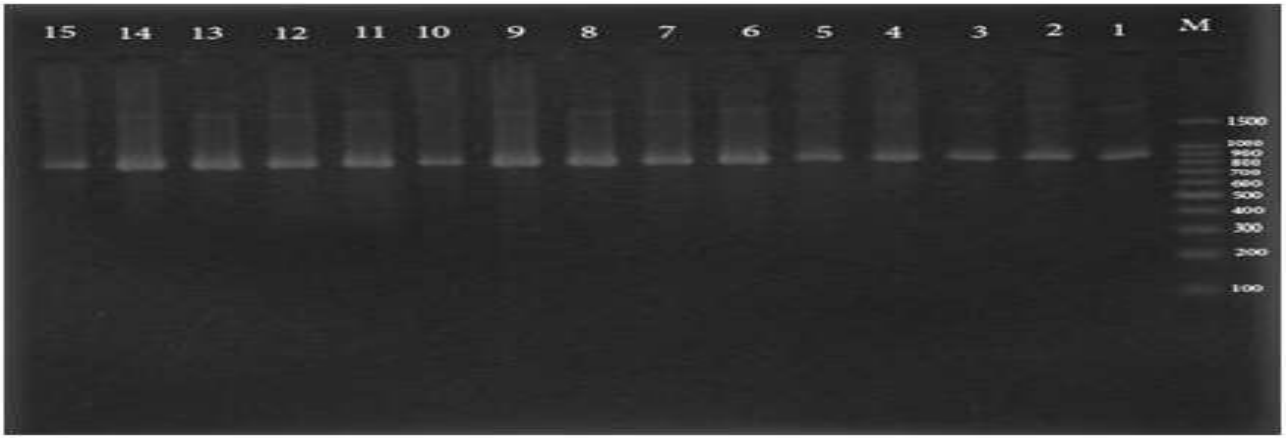

Fig.1: AgarosegelelectrophoresisofPCRampliconof*ND1*gene. Using2 % agarose, 80 V, 70 Am for 1 hrs, (lanes 1-15: *E. granulosus* isolates; M: 100 bp DNA ladder)

Sequence Analysis

For determine the genotypes of 15 isolates of cysts, NADH dehydrogenase subunit 1 gene was amplified by PCR, then sequenced and resolved by alignments with communicated reference sequences of G1 strain of *E*chinococcus *granulosus* using Gene bank (Figure 2). The sequence alignment was done by using Bio edit (DNA analysis program) and compared with previously reported references of *E. granulosus* genotypes. The results revealed 100% were identical with common sheep strain G1 genotype comparing with (ACCESSION KU925430) [15].

Score Expect Identities Gaps Strand
1410 bits (763) 0.0 763/763(100%) 0/763(0%) Plus/Plus

Query 20
GTTTGTTAATAATTGCCTTTTTTGTTTTAGGGGAGCGTAAGGTTTTGGGCTATTCTCAGT 79
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 4480
GTTTGTTAATAATTGCCTTTTTTGTTTTAGGGGAGCGTAAGGTTTTGGGCTATTCTCAGT 4539

Query 80
CTCGTAAGGGCCCTAACAAGGTTGGTGTAATTGGTTTGTTGCAGAGGTTTGCTGATCTAT 139
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 4540
CTCGTAAGGGCCCTAACAAGGTTGGTGTAATTGGTTTGTTGCAGAGGTTTGCTGATCTAT 4599

Query 140
TGAAGTTGGTAATTAAGTTTAAGTGTTTTTACTTCCAAAGTCGTAGGTATGTTGGTTTGT 199
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 4600
TGAAGTTGGTAATTAAGTTTAAGTGTTTTTACTTCCAAAGTCGTAGGTATGTTGGTTTGT 4659

Query 200
TTGGTGTTGTGTTATTAATGGCTTTGGTGATTGTTTATTCATTTATTTATGGTAGATATT 259
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 4660
TTGGTGTTGTGTTATTAATGGCTTTGGTGATTGTTTATTCATTTATTTATGGTAGATATT 4719

Query 260
ATAGAGCTAGTTATAGAGGCCTCTCCGTGTTGTGGTTTTTGGCTGCCGCCAGAACATCTA 319
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 4720
ATAGAGCTAGTTATAGAGGCCTCTCCGTGTTGTGGTTTTTGGCTGCCGCCAGAACATCTA 4779

Query 320
GGTATTCTTTGTTGTGTACTGGTTGGGGTGGTTACAACAATTATTCATTTTTAAGGTCGG 379
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||


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Sbjct                                                                    4780
GGTATTCTTTGTTGTGTACTGGTTGGGGTGGTTACAACAATTATTCATTTTTAAGGTCGG
4839

Query                                                                    380
TTCGATGTGCTTTTGGATCTGTTAGGTTTGAGGCTTGTTTTATGTGTGTGGTGATTTTTT
439
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    4840
TTCGATGTGCTTTTGGATCTGTTAGGTTTGAGGCTTGTTTTATGTGTGTGGTGATTTTTT
4899

Query  440GTGCTTTGTGTAGTTGTAGGtataatttaattgatttttattataattgttgattaagtt  499
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    4900
GTGCTTTGTGTAGTTGTAGGTATAATTTAATTGATTTTTATTATAATTGTTGATTAAGTT
4959

Query  500tgttattatttccattaatttatgtgttatttttaatatgtATATTGTGTGAAACTAATC  559
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    4960
TGTTATTATTTCCATTAATTTATGTGTTATTTTTAATATGTATATTGTGTGAAACTAATC
5019

Query                                                                    560
GTACGCCATTTGATTATGGAGAGGCTGAAAGAGAGTTGGTCAGTGGGTTTAAAGTTGAGT
619
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    5020
GTACGCCATTTGATTATGGAGAGGCTGAAAGAGAGTTGGTCAGTGGGTTTAAAGTTGAGT
5079

Query                                                                    620
ATAGTGGTATTTATTTTACGTGTTTATTTGCTTGTGAGTATATTATTATATATGTGTTTT  679
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    5080
ATAGTGGTATTTATTTTACGTGTTTATTTGCTTGTGAGTATATTATTATATATGTGTTTT
5139
Query                                                                    680
CATGGTTGGGAGTTGTGTTGATGTTTGGTGGCGGTTTTATCGGTATGTTGGTGTTAGTGT
739
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                    5140
CATGGTTGGGAGTTGTGTTGATGTTTGGTGGCGGTTTTATCGGTATGTTGGTGTTAGTGT
5199
Query  740  TTAATTTATTATTTTTTATGTGGGCTCGGGCGACATTACCACG  782
            ||||||||||||||||||||||||||||||||||||||||
Sbjct  5200 TTAATTTATTATTTTTTATGTGGGCTCGGGCGACATTACCACG  5242
```

Fig.2: Alignment of *NAD1* gene of *E. granulosus* by using Gene bank

A Phylogenetic Tree

The phylogenetic of *ND1*genehas been organized utilizing Maximum Likelihood calculate with Tamura-Neirange, as the pattern for development[16] (Fig. 3). The progressive date was conclude by using the ceilingLikelihood style according to the Tamura-Nei technique[16]. The tree with the elevated log likelihood (-980.0307) is reveal. Elementary tree for the investigative study was acquired willingly by employing Neighbor-Join and Bio NJ method for a template of pairwise stretch evaluated utilizing the ultimate installed probability, thereafter choosing the topology with officer

log probability rate. The tree was depicted to gauge, with bough longitude calibrated in the number of replacement per position .The test participatory 15 nucleotide longitude .Co don location inclusive were1st +2nd+ 3rd Non-coding. Whole sites including cavities and absent datum were forsaken. There were overall of 783 sites in ultimate dataset. Development dissection has been performed by using MEGA7 [17].

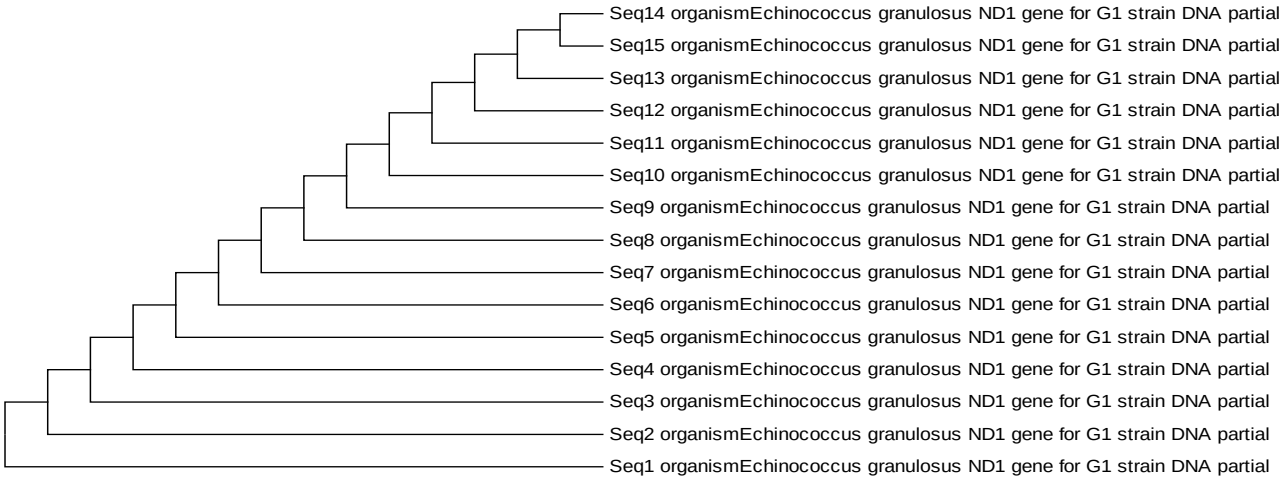

Fig.3: Molecular Phylogenetic analysis by Maximum Likelihood method

The *ND1* fraction was amplified by employing a primer set prepared for the current study,the polymerase chain reaction of *ND1* gene was felicitous in all specimens and created products of nearly 800 bp, this result agreed with previous results done by [18],[9],[19] where showed the size of NADH dehydrogenase subunit 1 (*ND1*) was 800 bp. Genotype G1 was the most common sheep contagious *Echinococcusgranulosus* strain all over the world with a wide variety of hosts [20]. In these districts, dogs are usually feed with livestock bowels that probably lead to infected with *E.granulosus* (8). This efficiency could be adequate to proliferate the present endemic case. From the results above, it can be indicated that the distinguished pervasion of the prevalent sheep strain (G1) was prevalent in hyper-endemic regions of Iraq. This result agreed with[20],[8],[19],[21]possibly because it is the most common one also it is widespread among intermediate hosts [9],[22]. The identification of the strain of the parasite existing in domestic and wild animals within areas endemic in *Echinococcus granulosus* are epidemiologically remarkable, G1 genotype has the ability to infect other domestic animal such as goats [23],[24].

Conclusion

The G1 genotype was the generality popular taxon and the effective provenance of infection of Iraqi's sheep. All of 15 specimens were G1 strain (sheep strain) according to NADH dehydrogenase 1 (*ND1*).

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