Genotyping of *Echinococcus Granulosus* (Hydatid Cyst) Isolated from Domestic Animals in Kurdistan - Iraq

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***Abstract*—Hydatid disease is a silent helminthic disease caused by metacestode of dog tapeworm *Echinococcus granulosus*, with medical and economic impact, the recently DNA markers allowed to identify 10 distinct genotypes of *E. granulosus* (G1-G10). The present study is to genotyping of *E. granulosus* (hydatid cyst) isolated from intermediate host. The partial DNA sequences of *mtCO1* gene followed to detect genetic variation of *E. granulosus*. The result of nucleotide sequence alignment with the recorded nucleotide in the NCBI indicated that all samples belong to the sheep strain, and there is a slight variation among sheep strain. A new variant strain was reported and deposited in the GenBank under variant G1 (Kurdistan strain) with accession number G1 (JX878691), sheep strain G1 (JX878689) and (JX878693). The present study concluded that the sheep strain is predominant genotype of *E. granulosus* in Kurdistan - Iraq, also it can be concluded that sheep strain generally responsible for hydatid disease among animal and human.**

***Index Terms*—CO1, *Echinococcus*, Hydatid cyst, Sheep strain.**

I. Introduction

Hydatid cyst is a potential zoonotic disease of man and animals, caused by the metacestode of the dog tapeworm *Echinococcus granulosus* [1]. The cyst can be grown in all organs, whereas the common sites of infection are the liver and lungs [2]. The hydatid cyst has two main forms which differ in pathology and morphology; cystic hydatid disease belongs to *E. granulosus* and alveolar hydatid disease (AHD) which is caused by *Echinococcus multilocularis* [3]. Based on DNA sequence, analysis of the *mtCO1*, dehydrogenase subunit 1 (*NDI*) gene, and *ITS1* 10 genotypes (G1-G10) have been described in a world isolated from sheep, pigs, cattle, horses, camels, goats, and human [4,5]. The molecular characterization of *E. granulosus* had been studied by many researchers in the different parts of the word [6-14]. The sheep strain is common in human and animal at least seven of these strains have been isolated from humans [15]. The hydatid disease is regarded as a neglected disease, and it remains public health concern because surgical removal of the cyst is the only choice and the recurrence of hydatid cyst still high depending on the operation accuracy and techniques [16,17]. The genotyping of *E. granulosus* has a significant point for control measures and the means of diagnosis [18]. Many studies indicated that Kurdistan Region Government is an endemic area of *E. granulosus* [19-25]. This study carried out to determine the genetic variation of *E. granulosus* and identification of abundance strain of *E. granulosus* in Kurdistan.

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II. Materials and Methods

A. Sampling

The hydatid cyst was isolated from an animal (10 from sheep, 10 from cattle, and 10 from goats) in Sulaimani modern abattoir. The samples stored separately in a sterile capped containers containing about one volume (v/v) of 70% ethanol at 4°C [26].

B. DNA Extraction

The germinal layer washed 3 times with phosphate buffer saline to remove the ethanol [5]. Modified Tissue extraction kit (Geneaid) followed for DNA extraction, the 0.5 g of each sample was diced to a small piece, then grinded with liquid nitrogen instead of mechanical grinding using micropistil, which provided with the kit, then followed the instruction of the company [27]. The extracted DNA dissolved in 50 µl TE buffer and stored at −20°C until use.

C. Polymerase Chain Reaction (PCR) Process

Nejad and his collogues [28] protocol were followed to amplify a fragment of *mtCO1* gene, in the 50 µl volume

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using a master mix (Cinagene, Iran) contains 10–50 ng of DNA and 50 pmol of each primer forward (5' TTT TTT GGG CAT CCT GAG GTT TAT 3') and reverse (5' TAA AGA AAG AAC ATA ATG AAA ATG 3). The PCR condition sets as follows: An initial stepping at 95°C for 4 min followed by 35 cycles at 94°C for 45 s, 55°C for the 30s, 72°C for 45 s, and 72°C for 7 min, as a final extension step. The result of PCR was detected on 1.5% ethidium bromide-stained agarose gel under UV light and digitally photographed.

D. Sequence Analysis

The GFX PCR DNA product and Gel Band Purification Kit used for PCR product purification (Amersham, UK). The purified PCR product of 12 samples which have a good DNA quantity and purity selected for sequencing in the Center for Research and Biotechnology for Agriculture of Malaya University in Malaysia. Multiple sequence alignments were done using the Clustal W method (Bioedit DNA analysis program).

III. Results

In the present study, the partial *mtCO1* gene of all samples was successfully amplified, and the expected band 443 bps product was detected on 1.5% agarose gel. Amplification of mitochondrial cytochrome subunit 1 (*CO1*) gene was detected in all samples, the 369 bp obtained from DNA sequencing for each sample. The multiple sequence alignments carried out with previously published in GenBank of *E. granulosus* under the following accession numbers, Japan sheep strain G1 (U50464), Iran and Jordan sheep strain (JQ250817), G2 (M84662), G3 (M84663), G4 (M84664), G5 (M84665), G6 (M84666), G8 (DQ144021), G10 (AF525457, G1c (AY686565), G1c Argentina (AF458873), and G1 (JN792925), Iran [27,29].

The multiple sequence alignments (Fig. 1) showed that the six isolated samples (sh1, sh4, sh5, sh6, sh7, and go4) were 100% identical to the Japan sheep strain (U504664), whereas five isolates (go1, go3, go5, sh2, and ca2) were 99% identical with sheep strain G1c (AY686565), one sample from goat was differ from the all recorded strains of *E. granulosus* (Table I). The results of the present study deposited in the GenBank as the first record of variant G1 (Kurdistan strain) in Iraq and Kurdistan under the following accession numbers G1 (JX878689), G1 (JX878693), and G1 variant (JX878691).

IV. Discussion

Different molecular markers were described for strain identification and molecular characterization of *E. granulosus* with different efficiency to determine the microvariants and strains [26]. The specific primers for *E. granulosus* strains are reliable for molecular epidemiological study and applicable for the large sample [30]. Many molecular studies support the current study [31] in Iran, [8] in China, [9] in Italy, [10] in India, and [16] in Iraq. They stated that the *CO1* has the ability and good efficacy to determine the strains and genetic variations.

The current study revealed that the sheep strain (G1) is the common strain in Kurdistan - Iraq, which has an ability to infect most intermediate hosts including sheep, goats, cattle, and human [28]. The strain identification of *E. granulosus* has a medical important value for control and prevention, whereas the majority of human infection of *E. granulosus* belonged to sheep strain [32]. The high prevalence of sheep strain (G1) and variant G1 (Kurdistan strain) in Sulaimani province and Kurdistan may be due to the intimate commercial contact with Iran; the most imported animals were from Iran which is a concern as an epidemic region of *E. granulosus* [33]. The occurrence of G1 is of significant value for epidemiological and public health importance due to their abılity and infectivity to humans.

The DNA sequence of *pmtCO1* gene of sheep strain (G1), Tasmanian sheep strain (G2), and Buffalo strain (G3) is very similar [26], these genotypes G1, G2, and G3 are included in a single cluster, named *E. granulosus* sensu stricto, as suggested by Nakao, *et al.* [34] and previously by other authors [35,36].

V. Conclusion

This study concludes that the sheep strain and variant G1 (Kurdistan strain) of *E. granulosus* are mostly responsible for human and animal hydatid cyst in Kurdistan - Iraq, and the Kurdistan - Iraq is the endemic area of *E. granulosus*.

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TABLE I
The Base Pair Change and Mutation of PMT CO1 Sequence Compared With the Common Sheep Strain G1 (U50464)

Sample	Intermediate host	Type of cysts	Nucleotide change	Nucleotide position	Genotype	Genebank accession No.
sh1, sh4, sh5, sh6 and sh7	Sheep	Sterile	100% Identity	-	G1	JX878689
go4	Goats	Sterile	100% Identity	-	G1	
go1, go3 and go5	Goats	Sterile	C→T	32	G1c	JX878693
sh2	Sheep	Fertile	C→T	32	G1c	
Ca1	Cattle	Fertile	C→T	32	G1c	
go2	Goats	Fertile	C→T	32	Variant G1 (Kurdistan strain)	JX878691
			T→C	339		

Sh: Sheep, go: Goat, Ca: Cattle

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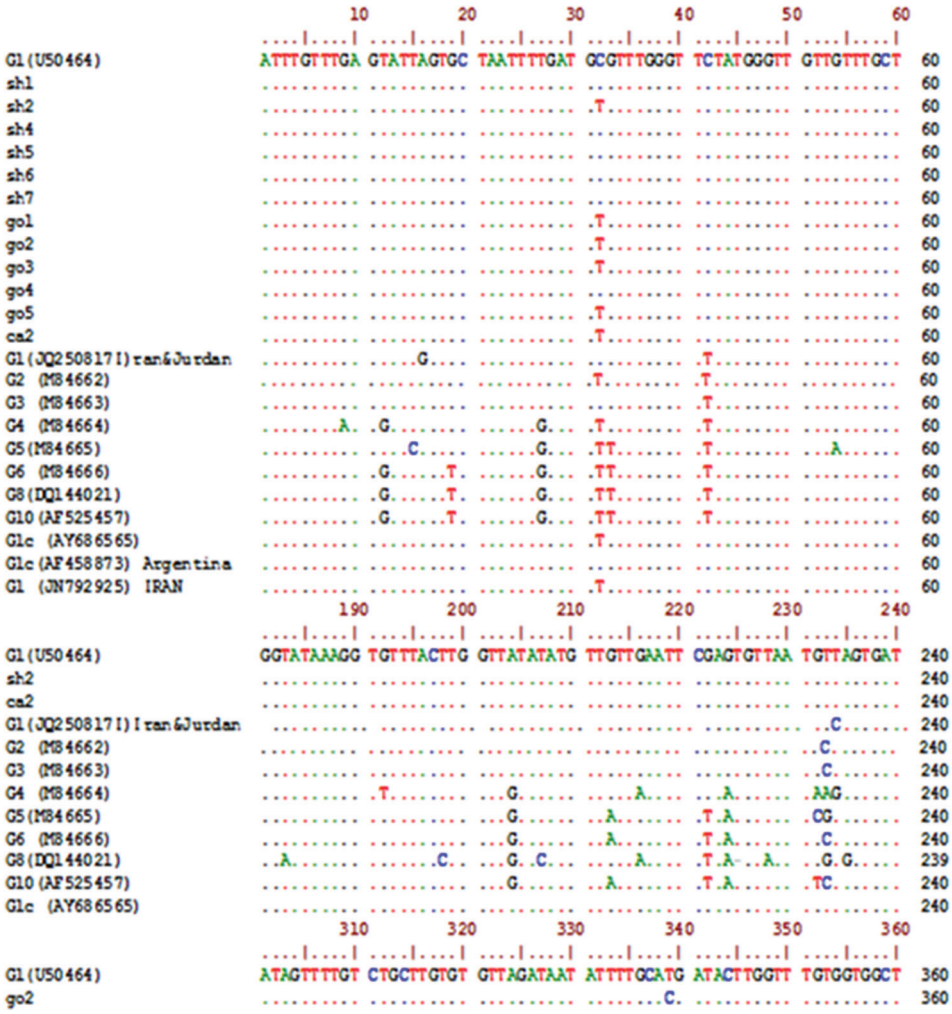

Fig. 1. Representative variable regions of partial nucleotide sequences of 369 bps fragment of the mitochondrial *CO1* of hydatid cysts aligned with previously published results.

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