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Molecular Detection of *Echinococcus granulosus* (G1) Strain in human and other intermediate host using molecular marker.

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ABSTRACT

Echinococcus granulosus is a cosmopolitan parasite causing cystic echinococcosis in humans and livestock. Hydatid disease is still a serious public health and economic problem. Is characterized by high intra- specific variability genotypes (G1- G10), the most common strain is sheep strain (G1) which is mostly responsible of human Echinococcosis.

The aim of this study was to prove molecular characterization of *Echinococcus granulosus* (G1) isolates which were obtained from human and livestock (sheep, cow and goat) by using polymerase chain reaction (PCR) in Kirkuk province of Iraq. A total of (40) isolates were obtained from patients after surgical operation and from Kirkuk-slaughter house, DNA samples were extracted from germinal layer of (7) human, (11) sheep, (17) cow and (5) goat. The specific primer for sheep strain G1 was provided for molecular characterization studies.

PCR resulted in a specific product of 400 bp. All isolates genotyped were clearly demonstrated G1 strain using nicotinamide adenine dinucleotide dehydrogenase subunit 1 (nad1) gene. This is the first molecular study report genotyping of Echinococcus isolates in Kirkuk province, Iraq.

INTRODUCTION

The larval stage of *Echinococcus granulosus* (EG) causes cystic echinococcosis (CE) in humans and domestic animals. Cystic echinococcosis considered a critical public health and economic losses problem (Thompsone, 2008). In Iraq, many reports of cystic echinococcosis have been described in humans and animals (Kadir and Saaid, 1997; Al-Nakeeb, 2004; Al-Hadithi and Al- Khamesi, 2010; Hama *et al*., 2012). Transmission of *E. granulosus* occurs predominantly in synanthropic cycles where dogs act as the definitive host. The worm shows high fertility in sheep and is mainly transmitted in a sheep-dog cycle (Prsaei *et al.*, 2012).

Currently, 10 distinct genotypes of EG designated as G1-G10 have been described worldwide on the basis of genetic diversity related to nucleotide sequences of the mitochondrial cytochrome c oxidase subunit1 (cox1) and NADH dehydrogenase ssubunit1 (NADH1) (Eryildize and Sakru, 2012). *Echinococcus granulosus* has been split into *E. granulosus sensu strict* (G1- G3), *E. equines* (G4), *E. orteppi* (G5) and *E. canadensis* (G6-G10) (Nako *et al*., 2007; Maurelli and Rinaldi, 2009].

MATERIAL AND METHODS

Studying Period

This work had been done during the period January 2014- April 2015. Materials used in this study included different types of specimens, equipments and chemical reagents.

Hydatid Cyst/ Germinal Layers

The exposed surface of cyst were cleaned by 95% ethanol, germinal layers isolated from liver hydatid cysts of different intermediate hosts included human (7) cyst (were collected through surgery from two hospital in Kirkuk), sheep (17) cyst, goat (5) and cow (11) which were collected from Kirkuk slaughterhouse in province. The germinal layers were stored in 70% ethanol until used (Kalaf *et al.*, 2014).

Extraction of DNA and Quantification

Genomic DNA was extracted using the genomic DNA extraction kits Miniprep Tissue was provided by Geneaid (Korea) according to the manufacture's protocol, was designed specifically for purifying total DNA, allowing DNA to be easily bound by the glass fiber matrix of the spin column (Step 1). Once any contaminants have been removed, using a wash buffer (containing ethanol), the purified DNA is eluted by a low salt elution buffer or TE.

Quantification of DNA was performed based on the spectrum measurement at the defined pathway. The concentration of extracted DNA was measured by using spectrophotometric method [J. Sambrook *et al.*, 1989]. One µl of the sample can be measured without using a cuvette or capillaries by using nanodrop (ND-1000 UV-Vis Spectrophotometer, Thermo Scientific, USA). The nanodrop ND-1000 is a full-spectrum spectrophotometer (UV and visible spectrum, 220-750 nm) for measuring the absorbance of DNA, RNA, proteins and dyes.

Specific primer

The lyophilized primers (forward and reverse) were, synthesized by (Operon Technologies, USA) were dissolved in sterile deionizer distilled water to give a final concentration of 0.01 µl (10 pmol) as recommended by provider by adding 900µl sterile deionized water to 10 µl of the stock primers according to manufactures instruction. The primers and their sequences are listed in Table (1).

Table 1: Oligonucleotide primer sequences for (gene) (Calderini *et al.*, 2012).

Primer Name		Accession number	Sequence (5́-3′)
nad1	**JB11** **JB12**	**G1: DQ856470**	**AGATTCGTAAGGGGCCTAATA** **ACCACTAACTAATTCACTTTC**

PCR Master Mix

PCR Master Mix (Bioneer, South Korea) used to amplification of genomic DNA was performed with the master amplification reaction. PCR master mix (final reaction volume = 25 µl)

PCR Program

Number of cycles = 35 cycles between initial denaturation and final extension

Initial denaturation	Temp.: 94°C	Time:4min
Denaturation	92°C	0.5 min
Annealing	56°C	0.5 min
Extension	72°C	2 min
Final Extension	72°C	7 min

Approximately 5µl of PCR amplified products were separated by electrophoresis in 1.2 % agarose gels (2 hrs, 5V/cm, 1X Tris-borate buffer). Gels were stained with ethidium bromide, PCR products were visualized by U.V transilluminator then imaged by gel documentation system (Varcasia *et al*., 2007; Busi *et al*., 2007). The amplified products usually estimated by comparing with the marker DNA ladder (100-2,000).

RESULT

DNA Extraction and Quantification

The genomic DNA extraction from 40 fertile liver hydatid cysts isolated from

different intermediate hosts including (7 human, 11 sheep, 17 cow and 5 goat) Figure (1). The results showed the genomic DNA extraction was performed successfully from 3mg tissue of the *Echinococcus* germinal layers and the DNA quantification data obtained by nanodrop method, which showed the concentrations (702-355) ng/µl and the purity (1.8-2.0) A260/A280. According to the results obtained the DNA samples were pure and suitable to do the PCR technique.

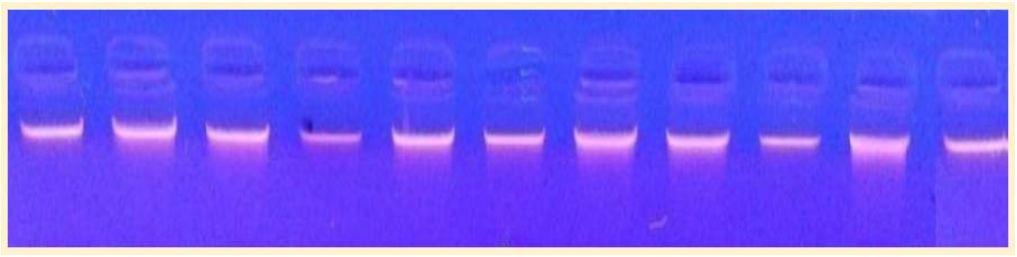

Fig. 1: Agarose gel electrophoresis of the total genomic DNA for some intermediate host tissue sample of *Echinococcus granulosus*, 1% agarose gel, ethidium bromide stained.

Polymerase Chain Reaction (PCR)

PCR program was successfully used in this study, which designed to amplify samples. It was found that 25 µl was the optimum reaction volume for this study for all genes. In this study, the PCR reaction was targeted against the normal chromosomal gene in *Echinococcus granulosus.*

Specific Primer for *nad1* gene

Amplification of nicotinamide adenine dinucleotide dehydrogenase subunit 1(*nad1*) gene isolated from (40) intermediate host of fertile liver hydatid cyst, The results of PCR products of *nad1* primer showed high sensitivity and specificity in detecting *nad1* gene, DNA isolated from tissues were first used as template and PCR carried out with the two primers for portion of *nad1* gene, an amplicon corresponding to 400 bp in size was seen after agarose gel electrophoresis PCR was tested with 40 different samples included the groups distributed to illustrate the effects of infection with *Echinococcus granulosus G1*. Figures (2: A, B, C, D) showed that the genomic DNA of samples were recognized and complementary to *nad1* gene sequence and represented by presence of single band (400 bp) molecular weight.

DISCUSSION

This is the first study to genetically characterize the sheep strain (G1) using nad1 gene. The common sheep strain was founded to cycle among livestock in Kirkuk and appear to be highly host adopted to producing fertile cyst in all domestic livestock. There is a little published information about the genetic characterization of *E. granulosus* in sheep and other intermediate host in Iraq.

Our study demonstrates that hydatid disease is still highly endemic in Kirkuk / Iraq. The presence of the common sheep strain (G1) strain of *E. granulosus* in livestock in Iraq coupled with the challenge of illegal controlling, may explain why the incidence of human echinococcosis is so high in the area.

The nad1 gene with JB11/ JB12 primers was yielded a 400 bp sized product in the all (40) samples. The PCR results these samples were give positive individual amplicon represented single band on agarose gel and there was no detectable size variation on agarose gel among amplicon derived from cysts from same host; for *Echinococcus granulosus* sheep strain (G1).

Moro *et al*., 2009, found that E. granulosus senso strict (sheep strain or G1) was the most prevalent in human patients, sheep, cow. Our result agreed with previous results done; where showed the size of nad1 gene was 400 bp long (Pezeshki *et al*., 2012), and in agreement with (Zhang *et al*., 1998) in

Iran, showed that G1 predominant genotype in livestock and humans. Sheep strain G1 genotype was found to be more infectious genotype in world with a wide range of hosts (Kamenetzky *et al.,* 2002; Criag *et al*., 2003; Sanchez *et al*., 2010).

Our study was in agreement with (Altintas, N., 2013) in which Eighteen hydatid cyst samples obtained, were successfully amplified nad1 gene (4oobp), and the results showed parallelism with other studies had been done (Utuk, *et al*., 2008; Simsek *et al.,* 2010). Also our study was in agreement with (Hama *et al*., 2012) who confirmed that the common strain G1 was isolated from human and animal, that where G1 sheep strain is predominant in Kurdistan/ Iraq because all samples (44) were referred to sheep strain.

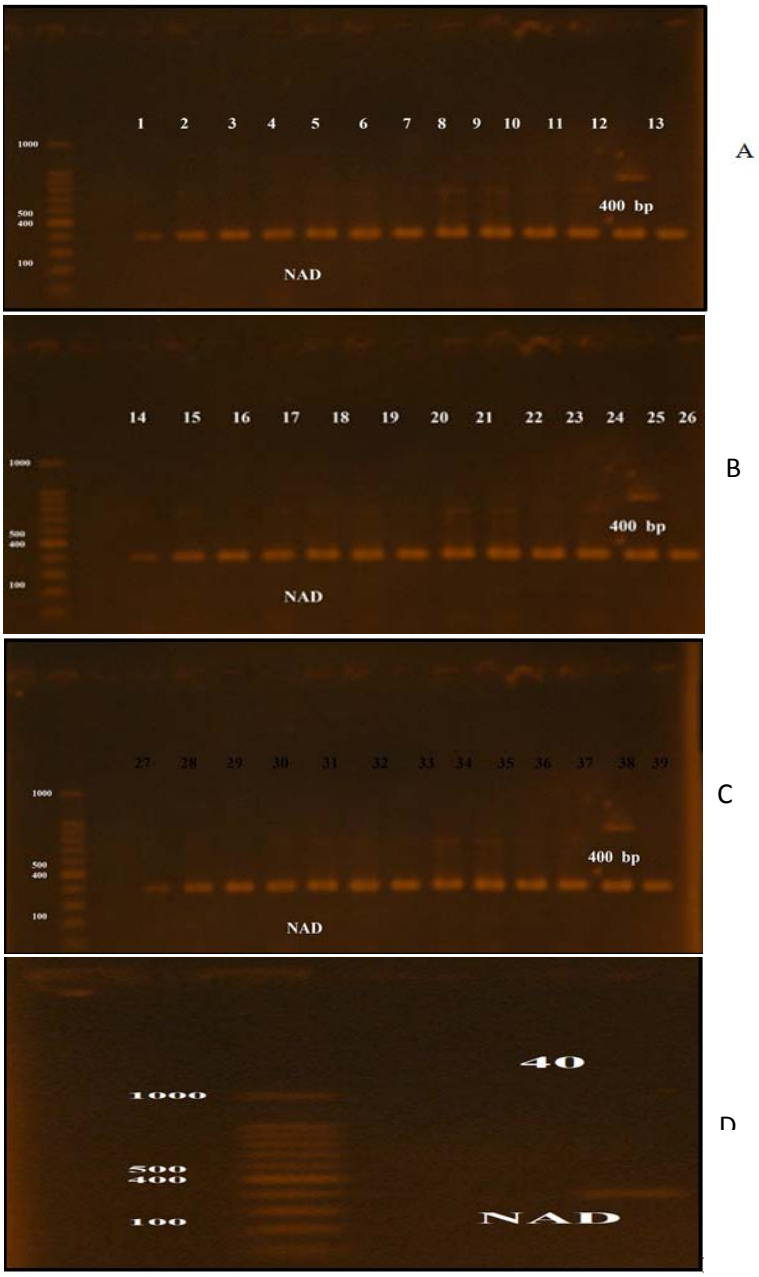

Fig. 2 (A, B, C, D): Gel electrophoresis of the PCR products of *nad* gene, with the products size 400 bp, on 1.2% agarose gel bromide TBE buffer using 5V/cm, for 60 min. M: 1000 bp DNA ladder, NC: negative control, (1.40) samples lane 1-40 Line amplification of sample in the fourty intermediate host (1-7 human, 8-18 sheep, 19-23 goat and 24-40 cow) respectively.

CONCLUSION

The present study is the first report about the genetic characterization of *Echinococcus granulosus* G1 isolates from human and other intermediate host in Kirkuk province of Iraq using nad1gene marker, but further molecular studies are necessary to performed for defining other strains of EG in different intermediate hosts and we propose the nad1 gene as a routine method to distinguish *Echinococcus* species in epidemiological surveys.

REFERENCES

Al-Nakeeb, SAR. (2004). Seroepidemiological and therapeutic study on hydatid cyst infection in Kirkuk and Tikrit province. MSc. Thesis, College of Medicine, Univ. of Tikrit.

Al-Hadithi, I. A. and Al-Khamesi, M. B. (2010). Effect of temperature on viability of human hydatid cyst & study the volume of hydatid cyst. Baghdad Sci. J., 7(4): 1372-1376.

Altintas, N.; Oztatlici, M.; Altintas, N.; Unver, A. and Sakarya, A. (2013). Molecular analysis of cattle isolates of *Echinococcus granulosus* in manias province of Turkey. Kfkas Univ Vet Fak Derg, 19(3): 455- 459.

Busi, M.; Snabel, V.; Varcasia, A.; Garippa, G.; Perrone, V. and De Liberato, C. (2007). Genetic variation within and between G1 and G3 genotypes of *Echinococcus granulosus* in Italy revealed by multilocus DNA sequencing. Veterinary Parasitology, 150(1-2): 75-83.

Calderini, P.; Gabrielli, S.; Cancrini, G. (2012). Is the goat a new host for the G3 Indian buffalo strain of *Echinococcus granulosus*. The Scientific World J., 5. http://dx.doi.org/2012/286357

Criag, P.S. and Rogan, M.T.; (2003). Camppse- Ponc, M. Echinococcosis: disease, detection and transmission. Parasitology, 127: S5- S20.

Eryildize, C. and Sakru, N. (2012). Molecular Characterization of human and animal isolates of *Echinococcus granulosus* in the Thrace Region, Turkey. Balkan Med. J., 29: 261-7.

Hama, Ab. A.; Mero, W.M.S. and Jubrael, J.M.S. (2012). Molecular characterization of *Echinococcus granulosus* (G1) strain in human and animals. 2nd International conference on Ecological, Environmental & Biological sciences (EEBS' 2012) Oct. 13-14. Bali (Indonesia).

J. Sambrook, E.F. Fritsch and T. Maniatis, (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Vols. 1, 2 and 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1: 626.

Kadir, AM and Saaid, L. A. (1997). The effect of albendazole and mebendazole on *Echinococcus granulosus* protoscolices. Med. J. Tikrit Univ., 3: 115-120.

Kalaf, M.S.; Al-Faham, M.A; Al- Taie, L.H.; Alhussian, H.A. (2014). Genotyping of *Echinococcus granulosus* in samples of Iraqi patients. IOSR J. Pharmacy and Sciences (IOSR-JPBS), 9(3): 06-10.

Kamenetzky, L.; Gutierrez, A.M; Canova, S.G.; Haag, K.L.; Guarnera, E.A.; Parra, A. (2002). Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. Infect. Genet. Evol., 2: 129-136.

Maurelli, M.P. and Rinaldi, L. (2009). Development of a real-time PCR for the differentiation of the G1 and G2/G3 genotypes of *Echinococcus granulosus*. Parasitol Res, 105: 255–259.

Moro, P.L.; Nakao, M.; Ito, M.; Schantz, P.M.; Cavero, C. and Cabrera, L. (2009). Molecular identification of Echinococcus isolates from Peru. Parasitol. Int.

Nako, M.; McManus, DP.; Schantz. PM. and Craig, PC. (2007). Ito, A. Amolecular phylogeny of the genus Echinococcus inferred from complrt mitochondrial genomes. Parasitology, 134: 713-22.

Pezeshki, A.; Akhlaghi, L.; Sharbatkhori, M.; Razmjou, E.; Oormazdi, H.; Mohebali, M. and Meamar, A.R. (2012). Genotyping of *Echinococcus granulosus*

from domestic animals and humans from Ardabil province, northwest Iran. J. Helmintholo., 1-5. http//journals. Combridge.org.

Prsaei, P., Mohammad, O.; Anari, M.; Riahi, A. and Rahimi, E. (2012). Prevalence of hydatidosis in slaughtered animals of south West of Iran. Global Veterinaria, 9(40): 430-433.

Sanchez, E.; Caceres, O.; Naquira, C.; Garcia, D.; Silvia, H.; Volotao, A.C. (2010). Molecular characterization of *Echinococcus granulosus* from Peru by sequencing of the mitochondrial cytochrome C oxidase subunit 1 gene. Mem Inst Oswaldo Cruz, Rio De Taneiro, 105(6): 806-810.

Simsek, S.; Balkaya, I. and Koroglu, E. (2010). Epidemiological survey and molecular characterization of *Echinococcus granulosus* in cattle in an endemic area of eastern Turkey. Vet. Parasitol., 172: 347- 349.

Thompsone, RC. (2008). The taxonomy, phylogeny and transmission of Echinococcus. Exp. Parasitol, 119: 439- 446.

Utuk, A.E. (2008). *Echinococcus granulosus'un* Dogu ve Guneydogu Anadolu bolgesi izolatlarinin moleckular ayrimi. Doktora Tezi, Firat Univ. Saglik Bil. Enst.,

Varcasia, A.; Canus, S.; Kogkos, A.; Pipia, A.P.; Scala, A.; Garippa, G. (2007). Molecular characterization of *Echinococcus granulosus* in sheep and goats of Peloponnesus, Greece. Parasitology Research, 101(4): 1135-1139.

Zhang, L., Eslami, A.; Hossein, S.H. and McManus, D.P. (1998). Indication of the presence of two distinct strains of *Echinococcus granulosus* in Iran by mitochondrial DNA marker. American J. Trop. Med. Hygiene., 59: 171- 174.