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Direct Detection of *Echinococcus Granulosus* in Human by Using Polymerase Chain Reaction (PCR) Technique

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Abstract: *A total of the 18 hydatid cyst fluid samples were collected from human in different age, sex and counties in Wasit province, Iraq. All human samples were detected for the parasite Echinococcus granulosus by using the polymerase chain reaction (PCR) technique based on the genetic characterization of protoscolices and germinal layer. The incidence rate with E.granulosus was 100% by using PCR obtained on one mitochondrial gene Cytochrome C oxidase subunit 1(COX1). The present investigation revealed that the highest number of patients was in ages (18-45) year and in female more than in male. Also high infection appeared in urban than rural areas.*

Keyword: *PCR, COX1gene, E. granulosus.*

1. INTRODUCTION

Echinococcus granulosus has a cosmopolitan distribution [(1)]. In Iraq, is still a major economic and public health problem, and also is not yet an organized national control program. Disease is endemic and zoonotic[(2)]. Cystic Echinococcosis has a global distribution partly due to the ability of the parasitic adapted a wide variety of domestic and wild intermediate and definitive hosts [(3, 4)]. It's recognized as being one of the world major zoonotic parasites [(5)]. The cystic echinococcosis has a public health importance not only in areas of endemicity but also in countries or regions without endemicity due to migration of infected people and livestock [(6,7)].

The larval or metacestode stage can be pathogenic causing economic losses to livestock and various forms of echinococcosis in human, some of which have a high fatality rate [(8)]. This parasite requires to mammalian hosts to complete life cycle, intermediate host (sheep, cattle, etc.), definitive host (dog or foxes) and the human is not part of life cycle but he accidently host [(9)]. This parasite has a worldwide prevalence but it has a particularly high prevalence in southern Europe, East Africa, Australia, NE Zealand and Latin America [(10)]. Outbreak of this disease have occurred in Asian countries such as Lebanon, Jordan, Iraq, Saudi Arabia and Iran leading to substantial health problems and economic losses [(11)].

The polymerase chain reaction took the world biological laboratories by storm and become the key technology in the field of molecular biology. The generation of un limited amounts of specific PCR products made many new methods of DNA analysis possible , such as downstream modifications (e.g. cloning of the PCR product establish gene libraries) or comprehensive product analysis techniques (e.g. gene expression or genotyping analysis) [(12)].

The PCR purification soluble protein of whole parasite body that's give (100%) protection and determine the strains of *E. granulosus* (G1-G10) and sub strains to facilitate controlling [(13)]. The advantages of this technique is required only DNA from viable or non- viable organisms for positive results and for transmission of CE by examination of soil samples [(14)]. This is the first study in the province of Wasit for parasite hydatid cysts to diagnosis by molecular technique's. The aim of study is diagnosis of parasite *Echinococcus granulosus* by using PCR technique.

2. MATERIALS AND METHODS

2.1. Samples Collection

A total of 18 human hydatid cyst samples were collected from AL-Zahra'a and Al- Karamah teaching hospital in Wasit province and placed in sterile container, then transported to laboratory and stored in freeze (-20C) until genomic DNA extraction step.

2.2. Genomic DNA Extraction

Genomic DNA was extracted from frozen hydatid cyst fluid samples by using (Genomic DNA extraction Kit, Geneaid. USA). The extraction was done according to company instructions by using Proteinase K. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C at freeze until used in PCR amplification.

2.3. PCR Amplification

PCR assay was performed for detection Echinococcus granulosus based on amplification of cox1 gene. The PCR assay was carried out according to (Nikmanesh *et al*., 2014). The primers were provided by (Bioneer Company, Korea) as following table:

Primer	**Sequence**		**Amplicon**
Cox1	F	**TTTTTTGGGCATCCTGAGGTTTAT**	450 bp
	R	**TAAAGAAAGAACATAATGAAAATG**	

The PCR master mix was prepared by using (AccuPower® PCR PreMix kit, Bioneer- Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM,$MgCl_2$ 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10p mole of forward primer and 1.5µl of 10p mole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer, Korea). The reaction was performed in a thermocycler (Techne TC-3000, USA) by set up the following thermocycler conditions; initial denaturation temperature of 95°C for 5 min; followed by 30 cycles at denaturation 95°C for 30 s, annealing 50°C for 30 s, and extension 72°C for 1 minute and then final extension at 72°C for 7 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

3. RESULTS

3.1. Epidemiological Results

Table (1) showed the prevalence of hydatid cyst cases according to age groups, gender and habitat. The results showed higher infection in female than male; the urban than rural areas according ages (18-60) year.

Table1. *Prevalence of hydatid infection in Wasit province*

Age (year)	**Gender**	**District**	**Organ**	**No. of cyst**	**Body condition**	**Presence of dogs**	**Vegetables consumption**
18	F	R	Liver	Uni	Thin	No	Yes
35	F	R	Liver	Uni	Fat	No	Yes
23	F	R	Liver	Uni	Fat	Yes	No
40	F	U	Liver	Uni	Fat	Yes	No
45	F	U	Liver	Uni	Thin	No	Yes
40	F	U	Liver	Uni	Fat	No	Yes
41	F	U	Liver	Uni	Fat	Yes	Yes
32	F	R	Liver	Uni	Thin	No	Yes
30	M	U	Liver	Uni	Fat	Yes	Yes
35	M	U	Liver	Uni	Thin	Yes	Yes
23	M	R	Liver	Uni	Thin	No	Yes
45	M	R	Liver	Uni	Fat	No	Yes
50	F	R	Liver	Uni	Fat	No	Yes
60	F	R	Liver	Uni	Fat	Yes	No
50	F	U	Liver	Uni	Fat	Yes	No
50	F	U	Liver	Uni	Fat	Yes	Yes
60	M	R	Liver	Uni	Fat	No	Yes
50	F	R	Liver	Uni	Fat	No	Yes

**Uni= Unilocular cyst, F= Female, M= Male, R=Rural, U=Urban*

3.2. Molecular Results

A total of the 18 hydatid cyst fluid samples were collected from human in different age, sex and areas in Wasit province, Iraq. The incidence rate with *E.granulosus* was 100% by using PCR obtained on

one mitochondrial gene Cytochrome C oxidase subunit 1(COX1). The COX1 gene of *E. granulosus* has been routinely utilized for variant designation with specific primers identificated for PCR amplification [(15)].

These primers generated the expected 450bp product; individual PCR products were further purified after agarose gel electrophoresis and visualized under UV trans illuminator (Figure1).

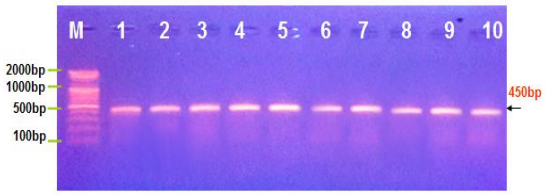

Fig1. *Agarose gel electrophoresis image that show the PCR product analysis of cox1 gene in E. granulosus positive samples. Where M: marker (2000-100bp), lane (1-10) positive cox1 gene at 450 bp PCR product.*

4. DISCUSSION

The identification of the strains in charge of human echinococcosis is nowadays a significant point which has to be taken into consideration in order to concentrate to adapt the control measure and the means of diagnosis [(16)]. The use of specific primer in PCR technique for strain identification is the most reliable method for epidemiological studies: this technique has the advantage that there is no need for DNA sequencing or other DNA markers [(17)]. The purpose of this technique is the amplification of a portion from parasite DNA contains diagnostically useful information, PCR have been developed to detect *E. granulosus* nucleic acids in biological samples [(18)]. The present study appeared that the incidence rate with *E.granulosus* was 100% in Wasit province by taking fluid from unilocular cyst in human patients. In Iraq, there are several studies about hydatid cystic disease (HCD); in Erbil province, the study recorded 149 cases(0.84%) due to cystic echinococcosis in human and slaughtered animals [(19],in Kufa, the study included (74) patients of hydatid cysts were diagnosed by using imaging techniques like (x-ray, ultrasound, MRI) as well as surgical intervention this study showed that (91.9%) of these cases were diagnosed firstly as hydatid

disease by ELISA (IgG), (94.6%) were positive by IFAT (total immunoglobulin) which considered as a confirmed echinococcosis cases and (52.8 %) were positive by using immune chromatographic tests [(20)], in Baghdad province, the study showed that DNA extract from protoscolices were more visible and more concentrated than the germinal layer DNA and appeared at 448bp on electrophoresis, the study revealed that protoscoleces DNA was differed and better than germinal layer DNA [(21)]. Infectious *E.granulosus* genotype in the world with a wide range of hosts [(22)]. This result was considered a normal result especially when Cystic echinococcosis (CE) occurs mostly in poor communities of raising sheep and other livestock, and involving dogs in guarding as well as herding animals. *E. granulosus* is mainly transmitted in a cycle between dog definitive hosts and livestock (mainly sheep), the human behavior also helps to perpetuate the domestic cycle of E. *granulosus* [(23)]. This fact obviously seen in Iraqi urban communities, the practice of animal slaughtering was usually performed in open spaces. Under these conditions, dogs would have free access to feed on livestock viscera, which may harbor hydatid cysts; the infective stage. Dogs play a critical role in the transition of echinococcosis [(24)]. Urban abattoirs are insufficiently equipped and lack efficient veterinary control, efficient waste disposal facilities, and water. Abattoirs are also frequently accessibility to dogs. Lack of adequate health education and ignorance of transmission route. All cysts caused by hydatid parasite were collected in this study were fertile containing a large number of protoscolices. The mean viability of the protoscolices exceeded (90%) when assessed by microscopic examination of evagination and invagination activity and eosin stain. The fertility of the cysts analyzed and viability of extracted protoscolices demonstrate the perfect adaptation of the strain to man [(25)]. The present study revealed that the infection of female more than male because female are usually warm responsible for home chores including feeding dogs, collecting yak dung for fuel and milking livestock, and opportunity to be exposed to Echinococcu-infection dogs and contaminated environmemt[(26)]. It was observed that the most common affected organ was the liver (unilocular cyst). These findings are in accordance with other studies published which reported that the most common ultrasonography features of hydatid cysts are their spherical shape and the fact that they are unilocular

[27]. The higher rate of hepatic infection may be attributed to the fact that the liver acts as the primary filter in the human body and the lung is often thought to be the second filter [28]. A similar picture of organ affection to this has been reported by other studies. It was reported that the most frequent site of hydatid cysts was the liver (50-70%) followed by the lung (20-30%) and less frequently, kidney, heart, bones [29]. Also the infection in rural areas caused by contaminated vegetables by eggs from final hosts (dogs) that caused infection of hydatid cysts in the patients.

5. CONCLUSIONS

The genetic properties of a species can play an important role in the clinical manifestations, pathogenesis, epidemiology, and classification of the parasites.

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