

# Epidemiological study and Molecular characterization of cystic Echinococcosis in Man in Al-Diwanyah Province, Iraq .

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## Abstract

The present study included the percent of infection with hydatid cystic disease (HCD) in human in Al-Diwanyah province, Iraq. from period January 2021 to August 2022 a statistical analysis of documents, 42 cases have been recorded due to cystic Echinococcosis, and treated surgically in governorate hospital ,Al- Diwanyah teaching hospital . The results of this study showed that the number and rate of infection in rural was 22 (52.38%) higher than in urban 20 (47.61%). According to the patients, females were highly infected than males , and among different organs involved liver was higher infected 19 (45.23%) , and then Ovaries 16 (38.09%). In comparison to age groups, the highest rate of infection was found among the age group 21-30 years 12(28.57

%). 42 samples from humans collected from fluids and the germinal layer ,The isolation and identification of *E. granulosus* were done using microscopic visualization followed by confirmation using a polymerase chain reaction (PCR) technique targeting the antigen B (AgB2) gene ,where Antigen B (AgB2) is frequently used for immuno-diagnosis of human cystic Echinococcosis (CE).The microscopic results revealed the presence of characteristic cysts, All *E. granulosus* isolates in human appeared to be contained this gene show one distinct band (MW400 bp) when electrophoresed on agarose gel. The results of this study indicated that the PCR technique had a high specify in the detection of *E. granulosus* especially this species that encoded to AgB2 gene isolated from human .

**Keyword:** *Echinococcus granulosus* , AgB2 gene ,Human, PCR.

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## I. Introduction

The zoonotic disease, CE, is induced by the cestode *Echinococcus granulosus* which considered as an important infection affecting different parts of the world especially countries with poor veterinary and human health systems (1). The disease is initially induced by the larval stage of the cestode, metacestodes, affecting different parts and organs of the human body such as liver and lungs leading to, sometimes, a fatal Echinococcosis (2). For the lifecycle, 2 mammalian hosts are involved in maintaining this cestode. One host, a carnivore, plays as the host for the adult tapeworm inhabiting the small intestines. The adult cestode produces the eggs leading to the generation of the infective oncospheres. However, these eggs are released from the intestines of this host either as free eggs or within the proglottids. After the ingestion of these eggs, the metacestode develops itself in the tissues of animal or human organs. Interestingly, those metacestode produces large numbers of protoscoleces, each can grow up into an adult cestode following its ingestion by a carnivore host (3,4). The form of the CE is widely distributed in the world having important health and economic effects (5). The ingestion of the eggs in humans may lead to developing the metacestode, a small cyst containing clear hydatid fluid, into a 60-to- 70- $\mu$ m-diameter vesicle formed out of the internal layer (germinal layer), cellular and an outer layer, acellular, and the laminated layer. The cyst gradually gets bigger and initiates a granulomatous-host reaction and a subsequent fibrous-tissue reaction, a connective tissue layer (pericyst) (6). The treatment for the CE involves surgery which is recommended in cases with few cysts. The puncture aspiration injection respiration (PAIR) and chemotherapy are recommended in cases with multiple cysts in different body organs (7-9). It remains public health threatened in endemic areas such as Mediterranean countries, North and East Africa, Western and Central Asia, China, South America and Australia.

**Materials and methods**  
**Samples**

This study was conducted in Al-Diwaniyah province From January 2021 to August 2022, 42 CE from 42 patients (6 male, 36 female ) were collected from Al- Diwaniyah teaching hospital where patients were referred for surgery .

**Microscopic visualization**

Microscopic examination was made for cysts fluid to determine the cyst fertility through Investigation of live protoscoleces, the isolation and identification of *E. granulosus* were done using microscopic visualization in the Laboratory of the Parasitology section, School of Veterinary Medicine, University of Al-Qadisiyah.

**PCR**

The DNA was extracted using Genomic DNA extraction kit (Geneaid, china) and following the kit instructions. The DNA was estimated for quantity and quality using a Nanodrop. A characterizing and confirming step was done using a PCR technique targeting the antigen B (*AgB2*) gene. The piece targeted was at 400bp of length. The primers used were from (10), and they are

F:GGATCCTTCGTGGCCGTCGTTCAAGC and R:TCGACAAATCATGTGTCCCGACGCA

The kit employed for the PCR mastermix Bioneer (South Korea ) and following the instructions accompanied with the kit using 10pmol from each primer. For the PCR conditions, the denaturation was at 95°C for 3min, 35 cycles were for the (main denaturing at 95°C for 1min, annealing at 55°C for 1min, and extension at 72°C for 1min), and the ending extension at 72°C for 10min. PCR products were run on 1.5% agarose gel pre- treated with ethidium bromide. The product separation was visualized using a UV imager.

**II. Results**

**Microscopically**

The microscopic results revealed the presence of characteristic cysts, figure 1.



Figure 1: The *E. granulosus* cysts showing the hydatid sand and protoscoleces. X10

Table (1) shows the percentage of infection in males and females of 149 patients according to their age groups. It was found that the age group 21-30 years were 12 (28.57%) being the highest rate compared to other age groups, while the lowest percentage infect 2.38% in the age groups ( $\leq 10$ ) and ( $\geq 61$ ) of patients, and the rate of infection in females 36(85.71%) was more than males 6(14.28 %) as shown in the table 1 .

**Table: 1 Prevalence of infection with hydatidosis according to age and sex in Al-Diwanyah Hospitals.**

Age group	No. of infected person (%)		
	Male	Female	Total
$\leq 10$	1(100)	-	1(2.38)
11-20	-	9(100)	9(21.42)
21-30	2(16.66)	10(83.33)	12(28.57)

31-40	3(27.27)	8(72.72)	11(26.19)
41-50	-	6(100)	6(14.28)
51-60	-	2(100)	2(4.76)
≥ 61	-	1(100)	1(2.38)
<b>Total</b>	<b>6(14.28)</b>	<b>36(85.71)</b>	<b>42(100)</b>

Table(2) shows the percentage of cystic hydatidosis among 42 patients according to their residence, the result showed that, 20 (47.61%) were lived in areas inside the city (urban), while the others 22 (52.38%) were in areas around or outside the city rural.

**Table2: percentage of cystic hydatidosis in 42 patients according to their residence.**

No. of i nfection	Urban (%)	Rural (%)
<b>Males</b>	<b>1(4.54)</b>	<b>5(25)</b>
<b>Females</b>	<b>21(50)</b>	<b>15((75)</b>
<b>Total</b>	<b>20(47.61)</b>	<b>22(52.38)</b>

Among 42 patients infected and treated surgically, liver had the highest rate of infection, 19(45.23%) followed by 16 (38.09%) in Ovaries (Table 3) .

**Table: 3 Predilection sites of hydatid cysts in males and females patients admitted private and governorate hospitals in Al-Diwanyah province.**

Organs involvement	No. of infection (%)		
	Total	Males	Females
<b>Liver</b>	<b>19 (45.23)</b>	<b>5(26.31)</b>	<b>14(73.68)</b>
<b>Ovary</b>	<b>16 (38.09)</b>	-	<b>16 (100)</b>
<b>Kidney</b>	<b>4 (9.52)</b>	-	<b>4(100)</b>
<b>Lung</b>	<b>1 (2.38)</b>	<b>1(100)</b>	-
<b>Intestines</b>	<b>1 (2.38)</b>	-	<b>1(100)</b>
<b>Breast</b>	<b>1 (2.38)</b>	-	<b>1(100)</b>

### PCR

The *AgB2* gene showed genetic variability with an important degree between different sampled hosts or between different-sampled-cystic-Echinococcosis (CE) endemic areas. The target amplified was at 400bp of length, figure 2.

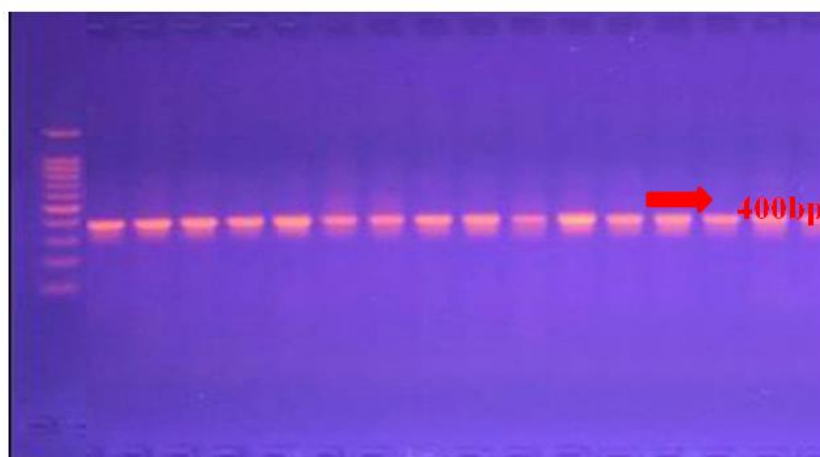


Figure 2: agarose gel electrophoresis of the *E. granulosus* PCR products for the AgB gene from human samples. Where M is the ladder.

### III. Discussion

The zoonotic disease, CE, is induced by the cestode *Echinococcus granulosus* which considered as an important infection affecting different parts of the world especially countries with poor veterinary and human health systems (1). The results of the microscopic visualization revealed characteristic presence of the hydatid cysts in humans. These results agree with the presence of this disease in some neighbor countries such as Iran showing endemic and hyperendemic epidemiology in different areas of this part of the world (11). The features of a typical cyst were shown to present hydatid sands with the protoscolex (12) who identified the presence of CE in an 8-year-old patient from Morocco. The use of Ryan and modified Baxby stains for microscopy were recommended by (13) for different techniques such as transmitted light. Microscopic identification of the CE is important as a feasible tool (14) who detected these cysts in patients from The Czech Republic .

**Table (1)** shows the percentage of infection in males and females of 42 patients according to their age groups. It was found that the age 21-30 years were 12(28.57%) being the highest rate compared to other age groups, While some other researchers have found high incidence in older patients such as study in Erbil province (15) which indicated that the peak age of incidence lay in the fifth decade (19,46%) of patients, as well as in Sulaimani Province (16) The seroprevalence rate significantly higher (7.3%) in the age group older than 35 years than the other age groups

,all these in Iraq, Also in Iran (17) they were found that the age 30-40 years were being the highest rate compared to other age groups, they suggested that the slow growth of the cyst, which is taken relatively 10 to 15 years or more according to the location of the cyst to develop the symptom of the disease. **Table(2)** shows the percentage of cystic hydatidosis among 42 patients according to their residence, the result showed that, 22(52.38%) were in areas around or outside the city (rural) while the others 20(47.61%) were lived in areas inside the city (urban), This result may be due to the many factors, including poor living conditions and lack of adequate health education in rural areas and economic instability and financial restrictions in control and prevention. This result is supported by many researches, (15, 17) where they found that the infection rate in rural are higher than that in urban and our study found the percentage of infection in female higher of male, this may due to the close contact of these women with the sources of infection, such as soil or vegetables contaminated with eggs of *E. granulosus* from dog feces. Wilson 1950 (18) explained that the high incidence of hydatidosis among Arab women is due to their domesticity, resulting in greater risk of infection, The finding of our study is supported by previous study (15) in Iraq they found that the women were more infected with cystic hydatidosis. And among different organs involved, liver had the highest rate of infection, 19 (45.23%) followed by 16 (38.09%) in Ovaryies (**Table 3**), Moreover the rate of infection in females was more than males, This result is agreement with researche in Erbil (15). Antigen B is a lipoprotein secreted by the *E. granulosus* metacestode and is involved in key host-parasite interactions during infection (19). The antigen is comprised of a group of subunit monomers of approximately 8 kDa in molecular size (20).

The *AgB2* gene is a good target for detecting the presence CE in human. The result of our study came in an agreement with (21,22) who mentioned variability in this gene family members. The PCR method was useful in identifying the current study CE cysts as was shown by (23) who used a restriction fragment length polymorphism (RFLP) based PCR technique revealing important information about the current etiology of the disease in Uganda and This is consistent with the results obtained from study in Iran which used *AgB2* gene for detecting the presence CE in human (24). In conclusion, Antigen B is an important diagnostic antigen of *Echinococcus* which has extensively been used for serodiagnosis of human cystic echinococcosis due to its

encouraging performance.

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