



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

VET 2024; 9(6): 199-203

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www.veterinarypaper.com

Received: 10-10-2024

Accepted: 11-11-2024

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Molecular study of *Hydatid Cyst* isolated from human and domestic animal in Babylon province, Iraq

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DOI: <https://doi.org/10.22271/veterinary.2024.v9.i6d.1864>

Abstract

This study included collecting 50 cyst samples of human from different areas of in Babylon province in Iraq. The human hydatid cysts collected from patients infected by hydatidosis after surgical operation from hospitals and laboratory of Babylon province. Through a period from September 2023 to June 2024. Traditional methods and polymerase chain reaction testing have been used to detect genotyping of hydatid cyst in human. According to the geographical areas in human infections were higher in Al-mahaweel district which were (30%) and the lowest infection rate was in Al-hashimia district which was (4%). There was highly significant differences between the regions when analysis statistically at the probability level ($p < 0.05$). As for ages the age group (30-40 years) recorded highest infection rate (44%), while the lowest infection rate was recorded in the age group (< 40 years) which was (20%) with Significant difference at $p < 0.05$. The infection rate was higher in females (62%) than males which was (38%), and there was no significant difference recorded between the sex. The results showed, by using rapid PCR for detection genotyping, in human result eight genotype and It was a higher percentage G6 (16%), using conventional PCR amplification for results of cytochrome c oxidase subunit I (COX1) gene of *Echinococcus granulosus*, which had sent to the sequencer (6) samples gave good sequence that subjected to bioinformatics analysis (3) samples for human and there result confirmed that the samples were *Echinococcus granulosus*. These sequences were recorded in gene bank.

Keywords: *Hydatid cyst*, PCR, *Echinococcus granulosus*, COX1

Introduction

Echinococcus granulosus larval stage causes hydatidosis, a zoonotic disease that significantly impacts both human and domestic animal health, leading to various medical, veterinary, and economic challenges. Iraq is identified as one of the countries severely affected by this endemic condition (Alhadidi, Al-Hamairy *et al.* 2022) [6]. Hydatid disease is prevalent globally, largely due to the high population of stray dogs infected with adult worms. This disease is referred to by several names, including Hydatidosis, Echinococcosis, and Cystic Echinococcosis. Carnivorous animals serve as the definitive hosts for the parasite, with the canine family such as dogs, hyenas, wolves, leopards, and other carnivorous species playing a key role. Herbivorous animals, including sheep, cows, camels, buffaloes, and horses, act as intermediate hosts. The disease primarily affects organs such as the liver, lungs, spleen, brain, and other muscles in both humans and intermediate hosts, sparing areas like hair and nails (Al-Nuaimi and Al-Hassani 2023) [4]. Humans can become infected through contact with infected dogs that carry adult *E. granulosus* in their intestines, leading to the excretion of eggs in feces. Consequently, humans and other intermediate hosts may ingest eggs that contaminate food, water, or the environment. While the disease is often asymptomatic, it can present as a complicated cyst, with the most common complications being compression or rupture of pericystic structures. Additionally, hypersensitivity reactions may occur due to circulating immune complexes or activation of the alternative complement pathway, potentially resulting in glomerulonephritis or an anaphylactoid reaction. Furthermore, the cysts can become superinfected with bacterial or fungal pathogens (Baraak 2014) [8]. According to the WHO (2021), the incidence of human hydatidosis in endemic areas can exceed significant thresholds.

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The incidence rates of human hydatidosis in endemic areas can exceed 50 cases per 100,000 individuals annually, with prevalence figures ranging from 5% to 10% in certain regions of Peru, Argentina, China, Central Asia, and East Africa. Globally, the prevalence of echinococcosis varies between 9% and 66%. The cysts associated with this condition grow slowly, filled with clear fluid and numerous protoscolices. On average, they increase in size by about 1 cm each year and can attain diameters of up to 20 cm. These cysts primarily develop in the liver (70%) and lungs (20%), while the remaining 10% can manifest in various other locations, including the brain, skeletal muscles, kidneys, bones, heart wall, and eye socket.

2. Materials and Methods: The research involved the

collection of samples from 50 individuals in the Babylon province. These samples underwent molecular analysis using PCR assays to determine the prevalence of hydatid cyst infections in humans.

2.1 Sample Collection: Samples were gathered from 50 individuals of varying ages and both sexes across different regions of the Babylon province, including Al-Musayyab, Al-Qassim, Al-Mahaweli, and Al-Hilla, during the period from September 2023 to June 2024.

2.2 Primers Utilized in This Study: The primers and their sequences, along with the product size, pertain to the mitochondrial subunit 1 of the cytochrome c oxidase 1 (cox1) gene (refer to Table 1).

Table 1: Sequences of primers

No	(COX1) Primer sequence 5' to 3'	Amplicon size	Annealing TM	Ref
F	Tttttgggcatcctgaggtttat	444 Bp	54	Elyasi H, Golmohammadi R, (2020) [9].
R	TAAAGAAAGAACATAATGAAAATG			

3. Results and Discussion

3.1 Macroscopic appearance in human

The present study identified from 50 patients the isolated parasitic cysts 50 from different organs (Liver, lung, thorasic), while The characteristics macroscopic appearance of hydatid cyst sizes Large 12cm, Medium 10cm, Small 5cm and shape cyst Spherical, ovoid, color cyst White, Consistency cyst Soft as shown in Table 2. These results in agreement with results of (Issa *et al.* 2022; Mustafa, *et al.*, 2019) [16, 19]. The main reasons that control to size or diameter of hydatid cysts attributed to host species, physiological and histological nature of infected tissue, site of cystic development in organ (superficial or deep), host immune response that restricted cystic growth, early detection of the cyst and may be strains variation that infects inappropriate host.

Table 2: Macroscopic appearance of hydatid cyst in human

Macroscopic Appearance	Human
Organ	Liver, lung, thorasic
Shape	Spherical, ovoid
Color	White
Consistency	Soft
Size	5cm, 7cm, 10cm, 12cm



Fig 1: Hydatid cyst of human

3.2 Conventional PCR

DNA extraction was conducted on the samples, which included those that were microscopically positive, to confirm the findings. The results indicated successful PCR amplification of the cytochrome c oxidase subunit I (COX1)

gene of *Echinococcus granulosus* in human samples, as illustrated in Figure 2. The PCR product size was 444 bp.

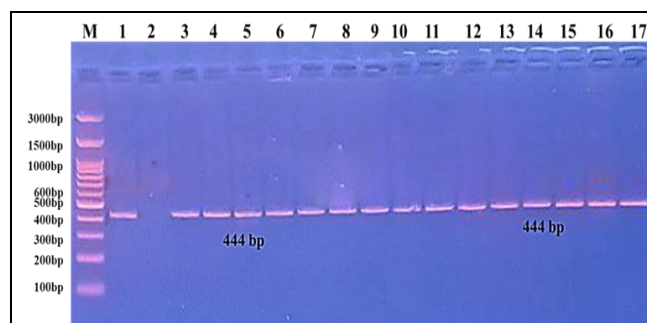


Fig 2: PCR amplification of (COX1) gene of *Echinococcus granulosus* in human samples

Table 3: Number of isolate of hydatid cyst in human according to the geographical are

Geographical Area	Human	
	No	%
Al-Musayyab	7	14a
Al-hashimia	2	4b
Al-Qassim	5	10ab
Al-Mahaweel	15	30c
Al-Hilla	12	24cd
Al-Eskndria	9	18ad
Total	50	100
X2	16.03	
P value	0.007 (HS)	

HS: Highly significant difference at $p < 0.05$

3.3 Number of isolate of hydatid cyst

3.3.1 Number of isolate of hydatid cyst in human according to the geographical area

The prevalence of infections in Hydatid Cysts this study was highest in Al-mahaweel districts which were (30c%). while the lower prevalence of Hydatid Cysts Al-hashimia district which was (4b%). The statistical analysis reveals that there was significant ($p > 0.05$) difference in the prevalence of parasite among geographical areas in (Table 3-2). The current study agreement with study in Punjab (32.45%) Muqaddas, Hira, *et al.* (2019) [19]. It was nearby the percentage that recorded in Karbala, Iraq (2.54%), India (10%) and Iran (3.84%) by Al-Gharawi, (2008) [2] consecutively. The

current study disagreement with rate infection Hydatid cyst was (54.2%) in Duhok, the present results contradict other studies in Iran as they reported higher infection rates, with 61% and 89.77%, respectively. These results are disagreement with Salem *et al.*, 2011) in Mauritania, (Al-Bosely 2013) ^[1] in Zakho (Kurdistan, Iraq), (Piccoli *et al.*, 2013) in Romania and (Khalf *et al.*, 2014) ^[14] in Baghdad (Iraq) as they reported rates of 76%, 90%, 92% and 47%, respectively (Table 3).

3.3.2 Number of isolate of hydatid cyst in human according to the age

In the present study the incidence of hydatid cysts was different according to age of the host. In human more prevalence rate (44a%) was recorded in (30-40 years) while little prevalence rate (20b%) was in (> 40) years people with a Significant difference at $p < 0.05$ as shown in (Table 3-3). These results in agreement with result of (Al-Marsomy 2021) ^[3] found to be the infection rate of hydatid cysts increases in the age group (31-40) years, while the lowest were at the age range (41-50) and (51-60) year. These result is disagreement with study that about (36.5%) of patients were at age 40 years (Fadhil and Baiee 2018) ^[10]. This finding disagreement with a previous study the highest rate of infection was found among the age class of 21-30 years (Issa *et al.* 2022) ^[16], (Table 4).

Table 4: Number of isolate of hydatid cyst in human according to the age

Age Interval	Human	
	No	%
20-30 years	18	36a
30-40 years	22	44a
<40 years	10	20b
Total	50	100
X ²	6.72	
P value	0.035(S)	

S: Significant difference at $p < 0.05$

3.3.3 Number of isolate of hydatid cyst in human according to the sex

The result of infection with hydatidosis according to sex revealed that females were exposed to infection more than males in human with No Significant difference at $P < 0.05$ (table 3-4). The results of the current agreement with study Al-Marsomy WA, (2021) ^[3] showed that the percentage of female infections (70%) was higher that of males (30%) and (Ismail *et al.*, 2023) ^[15]. The findings also revealed that females are infected at a higher rate than males. The rate of infection in females (61%) more than males (39%). Compared to males Mohamed *et al.*, 2021 ^[17]. may be due to females responsible for cooking and preparing food, which makes them in direct contact with the pathogens. These result is disagreement with studies (Gulsun, S *et al.*, 2010, Bakal *et al.*, 2015) ^[12, 7] there were higher rate males than females Table 5.

Table 5: Number of isolate of hydatid cyst in human according to the sex

Sex	Human	
	No	%
Male	19	38a
Female	31	62a
Total	50	100
X ²	2	
P-Value	0.157(NS)	

NS: No Significant difference at $p < 0.05$

3.3.4 Sequencing Phylogenetic tree analysis in human

According to the gene sequence analysis only 3 samples gave good sequence that which subjected to bioinformatics analysis, and their result revealed that of analyzed samples were *E. granulosus*, that which recorded in gene bank under accession numbers of PQ270266, PQ270267, PQ270268 (Figure 3), (Table 6).

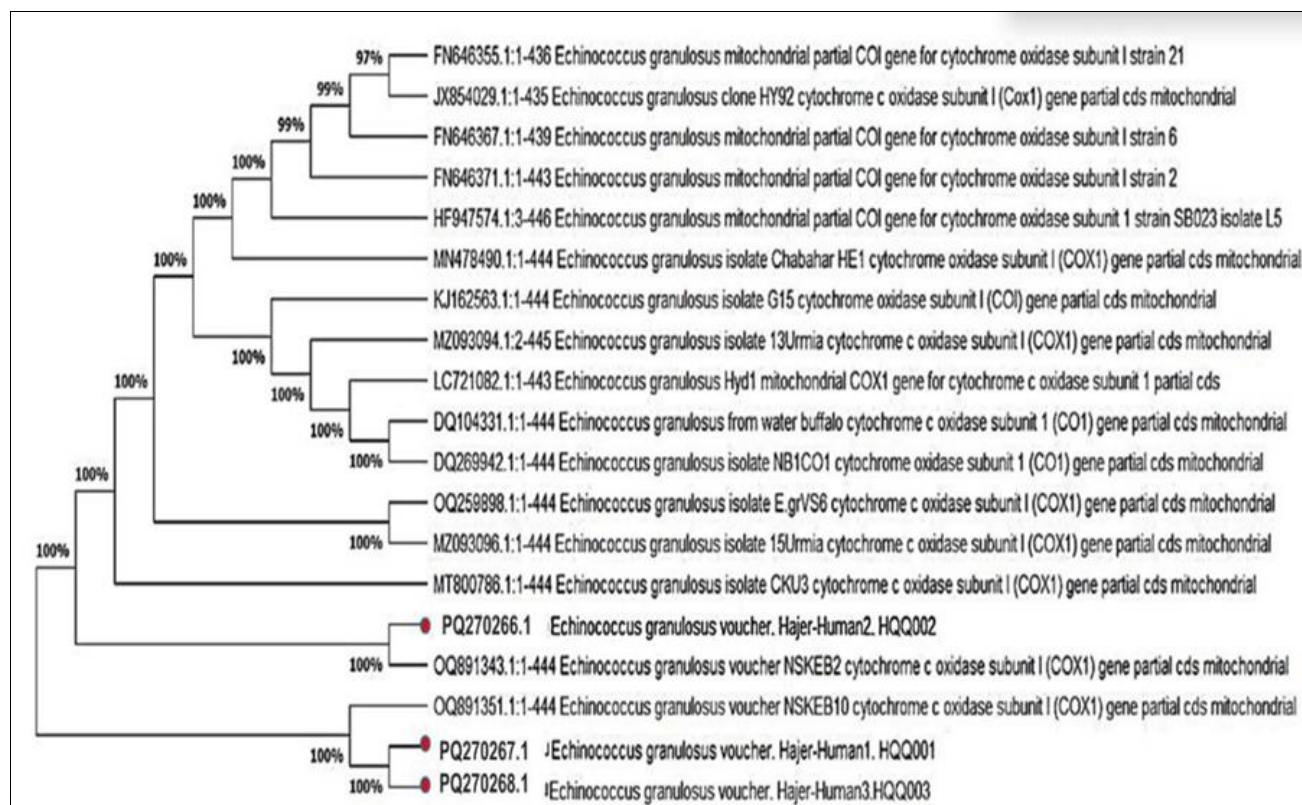


Fig 3: DNA sequences alignment of cestoda isolate for identical retrieved sequences at 444 bp amplicons of (ND4) site within the genomic DNA sequences of

Table 6: NCBI-BLAST Homology Sequence identity percentage between local *E. granulosus* human isolates and NCBI-BLAST submitted *E. granulosus* isolate

<i>E. Granulosus</i> Isolate	Accession Number	Homology Sequence Identity (%)		
		Country related NCBI	Accession number	Identity (%)
Human No 1 PQ270266-68		Iraq	OQ891351	100%
		Iraq	OQ891343	100%
		Iran	MT800786	100%
		Iran	MZ093069	100%
		Iran	OQ259898	100%
		Iran	MZ093094	100%
		Iran	KJ162563	100%
		Iran	MN 478490	100%
		India	DQ269942	100%
		India	DQ104331	100%
		India	IC721082	100%
		India	JX854029	97%
		Portugal	HF947574	100%
		Portugal	FN646371	99%
		Portugal	FN646367	99%
		Portugal	FN646355	97%

3.4 Rapid PCR examination of *E. granulosus* in human

3.4.1 Number hydatid cyst genotype in human according to the geographical area

In the present study, a total of 50 samples of human were liver and lung thoracicas were examined by rapid PCR detection, Hydatid Cyst, showed only eigher genotypes G2 and G9. The human strain G6 was the dominant 32% shown in Table 7. These study current agreement with (Nikmanesh *et al.*, 2014) [20] the isolates with G6 genotype was obtained from lung samples. Genotyping of *Echinococcus granulosus* Isolates from Human Clinical Samples in Iran, Tehran study

disagreement with study (Gholami *et al.*, 2012) [12] in Golestan province in Iran, of *E. granulosus* showed presence of G1 genotype in all 30 human samples. These study current disagreement with (Hedayati, *et al.*, 2019) [13] study in Mazandaran Province, North of Iran founded G1 and G2-G3 genotypes, respectively the Human Cystic Echinococcosis. The differences results between this study and others could be due to differences in animal management, nutrition, natural resistance, treatment programs, and local geo-climatic and international factors.

Table 7: Number hydatid cyst genotype in human according to the geographical area

Geographical Area	No	2 genotype	3 Genotype	4 Genotype	5 Genotype	6 Genotype	7 Genotype	8 Genotype	9 Genotype	X ²	P-Value
Al-Musayyab	7	1(14.28)	0(0)	0(0)	1(14.28)	2(28.57)	1(14.28)	1(14.28)	1(14.28)	3.75	0.808(NS)
Al-hashimia	2	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)	6.85	0.444 (NS)
Al-Qassim	5	0(0)	0(0)	1(20)	0(0)	3(60)	1(20)	0(0)	0(0)	14.4	0.045 (S)
Al-mahaweel	15	0(0)	2(13.33)	2(13.33)	3(20)	3(20)	0(0)	1(6.66)	4(26.6)	9.06	0.248 (NS)
Al-Hilla	12	0(0)	1(8.33)	1(8.33)	2(16.6)	5(41.6)	1(8.33)	1(8.33)	1(8.33)	12.19	0.094 (NS)
Al-eskndria	9	0(0)	0(0)	0(0)	4(44.44)	3(33.33)	0(0)	0(0)	2(22.22)	19.17	0.008 (HS)
Total	50	1(2)	3(6)	4(8)	11(22)	16(32)	3(6)	4(8)	8(16)		
X ²		6.26	3.01	3.12	5.44	4.29	4.36	6.42	3.40		
P-Value		0.281 (NS)	0.698 (NS)	0.681 (NS)	0.364 (NS)	0.508 (NS)	0.498 (NS)	0.267 (NS)	0.638 (NS)		

Conclusion

The study was concluded that the disease is zoonotic and widespread in Iraq and the results showed that the different strain in human and animal. Females in humans and animals are more susceptible to infection, and the reason is due to the direct contact of women with the handling and cooking of meat. Infections in rural areas are more than in urban areas due to the spread of dogs, the main carrier of the disease

Conflict of Interest: Not available

Financial Support: Not available

References

1. Al-Bosely ARI. Studies on epidemiology and some enzyme activities in laminated and germinal layers of hydatid cysts isolated from different intermediate hosts in Zakho, Duhok Province, and Kurdistan Region of Iraq. Master's Thesis, University of Zakho, Zakho, Iraq; c2013.
2. Al-Gharrawi AK. Epidemiological study of hydatid disease in patients underwent surgical treatment in Al-Hussain General Hospital/Karbala. Karbala Sci Uni J. 2008;6(3):87-93.
3. Al-Marsomy WA. Epidemiology of hydatid disease in Iraq: A study of hydatidosis patients in Baghdad Province. Indian Journal of Forensic Medicine & Toxicology. 2021;15(2):3525-3530.
4. Al-Nuaimi NZK, Al-Hassani SJM. Molecular investigation of *Echinococcus granulosus* in infected patients in Babylon province. Pakistan Heart Journal. 2023;56(2):20-28.
5. Al-Saeed ATM, Al-Mufti KSA. Human hydatidosis in Duhok-Kurdistan Region-North of Iraq. Med J Babylon. 2016;13:125-133.
6. Alhadidi RM, Al-Hamairy AK, Altameme HJM. Gene sequencing of hydatid cysts isolated from human and sheep in Central Euphrates Provinces, Iraq. Journal of University of Babylon for Pure and Applied Sciences. 2022;67-75.

7. Bakal U, Simsek S, Kazez A. Surgical and molecular evaluation of pediatric hydatid cyst cases in eastern Turkey. *Korean J Parasitol*. 2015;53(6):785.
8. Baraak M. Molecular study on cystic echinococcosis in some Iraqi patients. PhD Thesis, University of Baghdad, Iraq; c2014.
9. Elyasi H, Golmohammadi R. Genetic identification of *Echinococcus granulosus* isolates from domestic animals in Sabzevar, Northeast Iran. *Iranian Journal of Parasitology*. 2020;15(4):621-623. <https://doi.org/10.18502/ijpa.v15i4.4883>.
10. Fadhil AA, Baiee HA. Epidemiological and clinical features of hydatid cyst disease in Babylon Province during the years 2010-2015. *Journal of University of Babylon for Pure and Applied Sciences*. 2018;26(2):185-199.
11. Gholami Sh, Sosari M, Fakhar M, Sharif M, Daryani A, Hashemi M, *et al*. Molecular characterization of *Echinococcus granulosus* from hydatid cysts isolated from human and animals in Golestan Province, North of Iran. *Iranian J Parasitol*. 2012;7(4):8-16.
12. Gulsun S, Cakabay B, Kandemir MN, Aslan S, Atalay BASA, Sogutcu N, *et al*. Retrospective analysis of echinococcosis in an endemic region of Turkey: a review of 193 cases. *Iranian Journal of Parasitology*. 2010;5(3):20.
13. Hedayati Z, Daryani A, Sarvi S, Gholami S, Sharif M, Pirestani M, *et al*. Molecular genotyping of human cystic echinococcosis in Mazandaran Province, North of Iran. *Iranian Journal of Parasitology*. 2019;14(1):151.
14. Khalf MS, AlTaie LH, AlFaham MA. The incidence of hydatid cyst in humans in Baghdad governorate. *IOSR J Pharm Biol Sci*. 2014;9:11-14.
15. Ismail MA, Mohammed SJ, Hassan NH. The epidemiology of hydatid cysts liver diagnosis in Najaf governorate. *AIP Conference Proceedings*. 2023;2776(1):AIP Publishing.
16. Issa AR, Arif SH, Mohammed AA, Santolamazza F, Santoro A, Mero WMS, *et al*. Insights into human cystic echinococcosis in the Kurdistan Region, Iraq: characteristics and molecular identification of cysts. *Pathogens*. 2022;11(4):408.
17. Mohamed RJ, Alsaedi ANN, Taher JH. Epidemiology of hydatid disease in Najaf province/Iraq. *Journal of Physics: Conference Series*. 2021;1879(2):1.
18. Muqaddas H, Arshad M, Ahmed H, Mehmood N, Khan A, Simsek S. Retrospective study of cystic echinococcosis (CE) based on hospital records from five major metropolitan cities of Pakistan. *Acta Parasitol*. 2019;64:866-872.
19. Mustafa JY, Al-Azizz SA, Lazim AR. Molecular study of hydatid cysts in human, sheep and donkeys at Basra city, southern Iraq. *Biochem Cell Arch*. 2019;19(2).
20. Nikmanesh B, Mirhendi H, Ghalavand Z, Alebouyeh M, Sharbatkhori M, Beigom EKIA, *et al*. Genotyping of *Echinococcus granulosus* isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. *Iranian J Parasitol*. 2014;9(1):20.

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How to Cite This Article

Kaream HH, Amer QJ. Molecular study of *Hydatid Cyst* isolated from human and domestic animal in Babylon province, Iraq. *International Journal of Veterinary Sciences and Animal Husbandry*. 2024;9(6):199-203.