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Revealing the Prevalence of Echinococcus Granulosus in Kirkuk City by Serological and Molecular Methods

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Annotation: The Echinococcus granulosus species complex is the source of the neglected parasite disease known as cystic echinococcosis (CE), which is found all over the world. Clinical and molecular epidemiological studies from endemic regions with little data, like the Middle East, are especially needed to better understand the paths of transmission of this parasite. The study's objectives were to determine the sociodemographic characteristics of the patients and the COX1 gene of the E. granulosus complex in Kirkuk, Iraqi people. Human blood samples (350), and hydatid cyst materials (HCF and germinal layer) were obtained from 20 HCs patients after surgical removal of the cysts and aspiration of the cyst fluid by sterile 20ml disposable syringe, in Kirkuk and Azadi teaching hospitals at October 2024 to February 2025. The results showed that out of 350 blood samples taken from patients for the purpose of detecting E. granulosus infection using the ELISA technique with anti-hydatid cyst antibodies (IgG), 27 (7.7%) were found to be positive. The findings found that females had the highest percentage, reaching 5.4%, while the percentage of males was 2.3%. As

for age, the age group 35-44 was the highest, reaching 11 (40.7%) out of a total of 27 positive samples, while the lowest age group was 15-24 years, reaching (14.8%) out of a total of 27 positive samples. Also, it was found that rural had the highest percentage, reaching 85.2%, while the percentage of urban was 14.8%. finally, the results showed the partial mt DNA of COX1 gene of all of the 20 samples that isolated from human were successfully amplified using conventional PCR, the expected band 443bps product were detected on 1.5% agarose gel after staining with Redsafe. Figure (1) showed the partial mt DNA of COX1 gene of all of the 17 samples that isolated from human were successfully amplified using conventional PCR, the expected band 443bps product were detected on 1.5% agarose gel after staining with Redsafe. It is concluded that the incidence of E. granulosus infection is related to sociodemographic factors. Furthermore, the COX1 gene may be considered a good indicator for detecting E. granulosus.

Keywords: Cystic echinococcosis, E. granulosus, COX1, IgG.

Introduction

A helminthic zoonotic disease called hydatid cyst (HC) or hydatidosis is caused by infection with the cyst stage of Echinococcus granulosus [1,2]. This chronic illness is quite common throughout the majority of the world's nations, particularly those in the Mediterranean region [3]. Dogs and other wild canid definitive hosts harbor the adult parasite stage (tapeworm) in their small intestines, where they release infectious eggs into the environment. Humans are among the several ungulate intermediary hosts that harbor the asexual larva (metacestode), which acts as a dead-end host because of the consumption of infectious eggs [3]. Although the liver and lungs are the primary sites of infection, cystic echinococcosis can develop in any human organ or tissue, including intermediate hosts [4]. Ten different genotypes (G1–G10) have been identified using genetic tools [5], and numerous researchers have examined morphological and biochemical criteria for strain identification [6, 7]. E. granulosus strains were adapted to a variety of

intermediate hosts, including cervids, sheep, pigs, cattle, horses, camels, and goats [8]. At least seven of these strains were infectious to humans [9]. Dogs and other wild canid definitive hosts harbor the adult parasite stage (tapeworm) in their small intestine, after which they release infectious eggs into the environment through their feces [10,11]. Many ungulate intermediate hosts, including humans (because they consume infectious eggs), serve as dead-end hosts for the asexual larva (metacestode) [12]. Although the majority of infections are detected in the liver and lungs, all human organs and tissues, as well as intermediate hosts, are susceptible to cystic echinococcosis [13]. Understanding the pathways of parasite transmission, implementing targeted control programs, and performing confirmatory diagnostics all depend on the genetic identification of the species and genotypes responsible for human cystic echinococcosis. E. granulosus, a complex of cryptic species, is the cause of CE. To date, E. granulosus has been identified with the genotypes [14,15]. Even though there is a dearth of published epidemiological data, cystic echinococcosis is a public health concern in Iraq and around the world. The study therefore sought to determine the sociodemographic characteristics of the patients as well as the COX1 gene of the E. granulosus complex in humans from Kirkuk, Iraq.

Martials & Methods

Sampling

Blood samples

Five milliliters of venous blood were extracted from 350 participants using a tourniquet and a disposable plastic syringe. The blood was then stored at 4°C for one to two hours before being centrifuged for five minutes at 3000 rpm. In order to prevent repeated freezing and thawing, the acquired serum was split into two portions in a screw-capped tiny vial and kept at -20 °C until it was needed. Sera were extracted from clotted blood using a micropipette.

Hydatid cysts

In the teaching hospitals of Kirkuk and Azadi, human hydatid cyst materials (HCF and germinal layer) were extracted from 20 HC patients following surgical cyst excision and aspiration of the cyst fluid using a sterile 20ml disposable syringe between October 2024 and February 2025. Separate samples were taken in sterile 50 ml centrifuge falcon tubes, and they were brought to the lab in a cold box for additional processing. [16].

E. granulosus IgG-ELISA

The ELISA kits, which had a sensitivity of 98% and a specificity of 93.5%, were supplied by SunLongbiotech/China and were used for the qualitative screening and detection of human blood IgG antibodies of E. granulosus

DNA extraction

Twenty samples of preserved germinal layers or protoscolices were used to create total E. granulosus genomic DNA. The Genomic DNA Extraction Kit Miniprep Tissue (Geneaid) was used to isolate the DNA in accordance with the manufacturer's tissue DNA isolation methodology.

Polymerase chain reaction (PCR)

As shown in Table (1), a sterile 1.5 ml Eppendorf tube was filled with the PCR reaction's necessary chemicals. After extracting DNA from the germinal layer of 20 hydatid cysts, the master reaction for each primer (COX1) table (2) was made, gently mixed in a 1.5 ml Eppendorf tube, and spun down for a short while. Next, 23 µl of the master reaction was added to a labelled 0.5 ml Eppendorf tube, and 2 µl of DNA (template) of each sample was added to each tube separately and gently mixed. The Eppendorf tubes were put in the thermocycler to do amplification after all of these procedures were completed on ice. According to Sanchez et al. [17], the PCR procedure included 45 cycles of denaturation at 95 ℃ for 60 seconds, annealing at 50℃ for 60 seconds, extension at 72 ℃ for 60 seconds, and a final extension phase at 72℃ for 5

minutes. The initial denaturation step lasted 3 minutes.

Table (1): PCR reagents required in 25µl volume.

Reagent	Volume µl	Concentration
Mastermix	12.5	1x
Forward primer	1	10pmol
Reverse primer	1	10pmol
Dnase Rnase free water	8.5	-
DNA template	2	30-50 ng
Total	25	-

Table (2): Primers used for genetic characterization of E. granulosus

Name	Sequences	Amplicon size	Ref.
COX1F	5'TTTTTTGGGCATCCTGAGGTTTAT-3′	443bp	[18]
COX1R	5'-TAAAGAAAGAACATAAGAAAATG- 3'		

Agarose gel electrophoresis

The agarose gel was prepared in a concentration of 1.5 % for detecting PC Rproduct [18].

Data analysis

Using the SPSS statistical analysis program (version 17) and Graph Pad, the data was statistically analyzed using one-way ANOVA, the t-test, and the Chi-square (X2) test to ascertain the probability value (p-value).

Results & Discussion

Prevalence of *E. granulosus*

Figure 1 shows that out of 350 blood samples taken from patients for the purpose of detecting *E. granulosus* infection using the ELISA technique with anti-hydatid cyst antibodies (IgG), 27 (7.7%) were found to be positive.

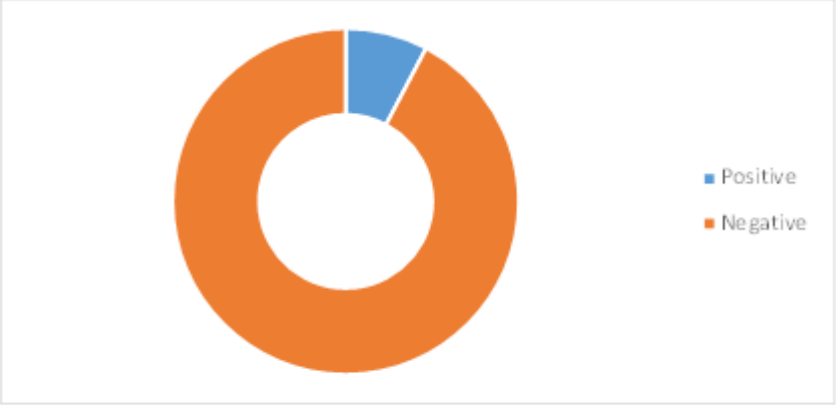

Figure (1): Seroprevalence rate of anti-hydatid cyst antibodies patients in Kirkuk city

Table (3) shows the relationship between gender and age in the infection rate of *E. granulosus*. It was found that females had the highest percentage, reaching 5.4%, while the percentage of males was 2.3%. As for age, the age group 35-44 was the highest, reaching 11 (40.7%) out of a total of 27 positive samples, while the lowest age group was 15-24 years, reaching (14.8%) out of a total of 27 positive samples.

Table (3): the relationship between gender and age in the infection rate of *E. granulosus*

Years	Sex				Total
	Positive		Negative		
	Male	Female	Male	Female	
15-24	2	2	58	65	127 (36.3%)
24-34	2	4	34	41	81 (23.1%)
35-44	3	8	31	27	69 (19.7%)
≥45	1	5	38	29	73 (20.9%)
Total	8 (2.3%)	19 (5.4%)	161 (46.0%)	162 (42.3%)	350

Similar to other studies conducted in the Kurdistan area and other parts of Iraq, including Theqar [19], Erbil [20], Baghdad [21], and Duhok [22], the rate of cystic echinococcosis was higher in females than in males. When gender data was provided, all of these studies found that infection rates were greater in females (compared to males): 58.3% versus 41.7%, 63.08% versus 37.58%, 60% versus 40%, and 64.6% versus 35.4%, respectively. Similarly, greater rates of CE among females were found in various bordering countries, including Iran [25], Jordan [24], and Turkey [23] (8.1%, 67.4%, and 57.95%, respectively). The increased frequency of CE among females in this study may be due to a number of factors, such as occupation and cultural habits, as was previously highlighted. Females also have a higher chance of coming into close contact with infection sources, like vegetables or soil that may contain viable E. granulosus eggs from dog feces [26]. However, living in rural places where pollution is endemic does not necessarily mean that being female is not a contributing factor, as large cohort studies on CE did not show any statistically significant difference between the male and female prevalence [27].

Table (4) shows the relationship between residency and infection rate of *E. granulosus*. It was found that rural had the highest percentage, reaching 85.2%, while the percentage of urban was 14.8%.

Table (4): the relationship between residency and infection rate of *E. granulosus*

Residency	Positive No.	Percentage
Urban	4	14.8%
Rural	23	85.2%
Total	27	100.0%

According to the current study, the seroprevalence rate of HC in rural areas was higher (85.2%) than in urban areas (14.8%). Although HC can be found anywhere, especially in places used for animal husbandry, Almufty [28] in Duhok concurred, saying that rural populations are more at risk due to the features associated with transmission, which include dogs and domestic animals including sheep, cattle, and goats. Relatively speaking, this result was likewise in line with the majority of other epidemiological investigations conducted throughout Iraq [29, 30]. According to all of them, the widespread prevalence of HC in rural populations may be caused by their intimate relationships with both domestic and wild animals. In a similar vein, Abdi [31] claimed that those living in rural areas were more likely than those in urban areas to have hydatid cyst infections. Actually, this parasite's life cycle is maintained in rural areas where a lot of stray dogs pollute the environment with Echinococcus eggs, raising the risk of human CE infection [32].

Genetic detection of COX1 gene

Figure (2) showed the partial mt DNA of COX1 gene of all of the 17 samples that isolated from human were successfully amplified using conventional PCR, the expected band 443bps product were detected on 1.5% agarose gel after staining with Redsafe stain.

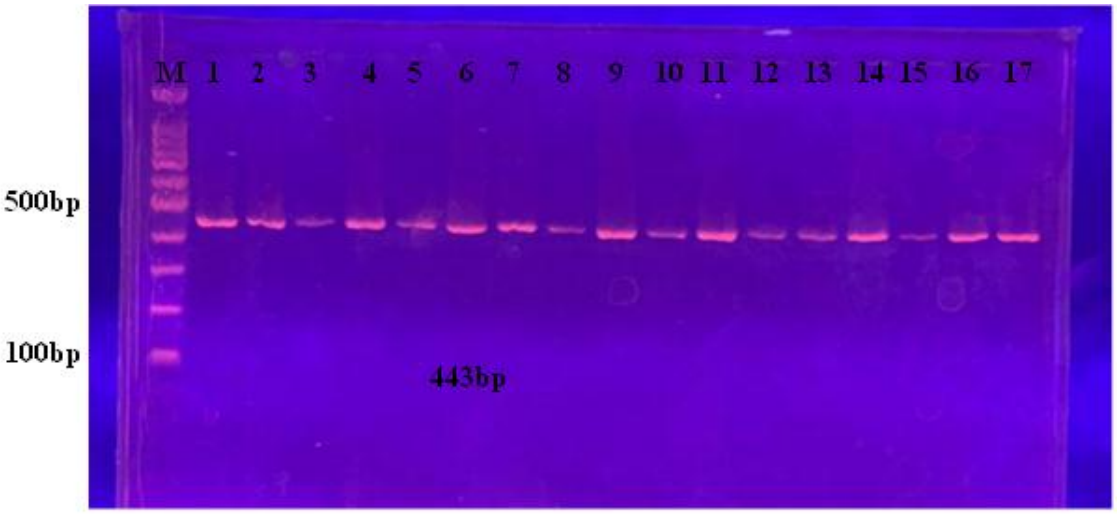

Figure (2): Electrophoresis of PCR products for COX1 gene 443bp at 75 voltages and 1.5% agarose. M= DNA ladder, 1-18= samples.

Hydatid cyst samples in all were identified as being caused by E. granulosus in the current study. A significant prevalence of E. granulosus was found, which is consistent with data previously reported in animals [39] and people (88.5%) [38]. The same is true in China, where E. granulosus (formerly the G1 strain) is responsible for 60% of human CE-positive cases [40] and 40.62% of infections recorded in India [41]. Cox1 gene sequencing was used to identify the sheep strain (G1) in human samples (n = 2) [9]. The high prevalence of E. granulosus found in this study may be due to the fact that it is currently the most common species in Iraq and its neighboring countries [9, 39, 40]. The most common cause of CE, even worldwide, is E. granulosus [38]. It is more prevalent in endemic areas due to its broad host range, even when it coexists in sympatry with other E. granulosus s.l. The fact that the majority of CE cases were found in rural areas, where people have a strong bond with dogs, may also be a contributing factor [41].

Conclusions

Based on the study results, it was found that the infection rate is higher in women than in men with E. granulosus, and that rural areas are more susceptible to infection due to the increase in livestock numbers. The COX1 gene can also be considered a good indicator for detecting E. granulosus infection and revealing the genetic diversity of the parasite.

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