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Molecular Detection for Genotype In Sheep Infection with Echinococcus Granulosus at Holy Kerbala Province

Rana A. Jawad[1*], Riyadh Hatim Haddawee[2], Eman Jawad Jabber[3], Fatema Ali AL kafhage[4], Ahmed F. Farhood[5]

[1,4] College of Veterinary Medicine, Kerbala University
[2] College Applied Medical Sciences, Kerbala University
[3,5] Veterinary Teaching Hospital in Babylon.
*Corresponding author: Email: rana.a@uokerbala.edu.iq

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Abstract
Hydatidosis due to cystic Echinococcosis is one of the most important public health and economic problems in different countries including Iraq". The study was conducted the prevalence of Hydatid cysts disease in sheep in slaughter houses in the holy city of Kerbala "and to detect the rates of infection with E. granulosus genotype using optimal (PCR) technique with specific designing primer for cystic fluid" . The study was carried out between October 2021 and April 2022 . A total number of 93 sheep were examined via autopsy for hydatid cysts in slaughterhouses in Kerbala , including both sexes and different ages , then we used molecular genotype sequencing technique , specific PCR primer design . "Our PCR results of the study showed that cystic echinococcosis genotypes was 24 (53.33%) were detected as Echinococcus granulosus isolates which isolated from internal organs and the percentage were significantly increases as 49 (52.68%), 33 (35.48 %), 11 (11.82 %) for liver, lung and liver with lung respectively" .

***Keywords**:* "PCR , Hydatid cysts, Echinococcus Granulosus, Kerbala"

Introduction:

There is a global concern for echinococcosis because it is a zoonotic disease that can be transmitted to humans "and domestic animals". [1], "Infections caused by the larval stage of the worm Echinococcus granulosus typically manifest in the liver, lungs, and other organs" ; the adult worm resides in the small intestine of carnivorous animals. [2] This parasite has a complex life cycle that involves omnivorous and herbivorous hosts as well as the final host, dogs and other candies. [3] disease spread through ingesting parasite eggs from contaminated food or water or through close contact with animals that have the disease [4], humans and other intermediate hosts via direct animal hair infected with eggs or dirt contaminated with infected hands [5] Although birds and arthropods can serve as mechanical carriers for eggs, neither of these categories applies to the larval stage [6].
Symptoms and indicators of the condition might arise at any time, depending on how large and where the cyst is located. [7] The disease has serious consequences for human and animal health as well as for the economy due to the loss of animal products. [8]

. Sheep carry genotype G1, buffalo G2, cattle G5, horses G4, swine G7, camels G6, pigs G7, and cervid G8-G10 carry genotypes G4-G10. [9-10], Both the biological and molecular characteristics of the strains have been studied. [11-12] The prevalence of abnormal forms varies from place to place, making pathological identification challenging. [13], "In addition, there are variations in host specificity, pathogenicity, life cycle pattern, transmission, progression, and sensitivity to chemotherapeutic treatments" [14]. For the most part, different approaches based on the polymerase chain reaction (PCR) have been utilized for E. granulosus genotyping. [15][16], presently we sequence the amplified mitochondrial and nuclear DNA fragments from the clinical samples obtained via biopsy and PCR [17].

Methods

Samples collection

Samples from liver and lungs were collected from sheep under 5 years old from both sexes suffering from Hydatidosis in slaughterhouses in kerbala city abattoir and Al-Hussenyia sheep farmers in kerbala during the period from October 2021 to April 2022. A total number of 93 sheep was examined via autopsy for hydatid cysts in slaughterhouses 49 samples from liver , 33 for lungs and 11 was liver with lung .

Samples were transported in sterile container to the laboratory. In addition, a 5-milliliter TSB-CV injection was made with a little stool sample and sent off to the lab. (Promega, USA)

DNA extraction and purification

"To isolate the cells, we placed 60 mg of lung and liver tissue to a 1.5 ml Eppendroff tube, centrifuged at 16000 rpm for 2 minutes, and discarded the supernatant",

The cells were then suspended by adding 600 l of a nuclei lysis solution and gently pipetting the mixture. "Cells were lysed by being heated to 80 °C in a water bath for 5 minutes before being cooled to room temperature" .

Cell lysate was combined with 200 l of protein precipitation solution using a vortex mixer for 20 seconds. After 5 minutes, centrifuge the sample at 16000 rpm for 3 minutes while keeping it on ice. "The DNA-containing supernatant was transferred to a clean 1.5 ml micro centrifuge tube containing 600 l of isopropanol at room temperature". "The DNA was inverted gently to combine the threadlike strands into a visible mass". The supernatant was separated after being centrifuged at 16000 rpm for 2 minutes. "To dispose of its contents, the tube was placed on fresh absorbent paper. Afterwards, 100 l of DNA rehydration solution was added, and the DNA was rehydrated by incubating the tube at 65 °C for an hour while gently tapping the tube to mix the solution at regular intervals". The DNA was then stored at 2-8 °C. Electrophoresis on 1% agarose gels allowed for the detection of the purified products.

Laboratory protocol

The following reagent was added for each tube on ice.

Green master mix	25 µl
Upstream primer	2.5 µl
Downstream primer	2.5 µl

(a light overtaxing was used) The mixture was mixed thoroughly by shaking & spin.

Five µl of DNA sample was added by using specified pipette for sampling of DNA.

Complete the volume to50µl with nuclease-free water, mix for 3-5 seconds. One drop (20-25 µl) of mineral oil was added to each tube.

After getting the thermocycler hot, we added the PCR tubes and began the reaction.

"The comb was placed at one end of the casting tray after the agarose 2% gel was assembled, the gel was then poured into the tray and let to cool for 30 minutes at room temperature, Once the comb was removed, the electrophoresis chamber was reassembled with gel". TBE-

electrophoresis buffer was poured into the chamber until it was between 3 and 5 millimeters (mm) above the gel's surface.

"Loading and running DNA in agarose gel"

The 2% agarose gel had DNA (10 µl) and bromophenol blue (3 µl) injected into its wells, "with the cathode connected to the well side of the unit and the anode on the opposite side". Bromophenol blue tracking dye was run through the gel at 70 V till it reached the gel's far end. "Gels were stained with ethidium bromide and examined under a UV trans illuminator to reveal the DNA".

Results

In Iraq, *Echinococcosis* was both endemic and enzootic [12]. There was a total of 93 inspected sheep before they were slaughtered for this study, making the prevalence of hydatid cysts in animals at the time of slaughter 53.33 percent. The presence of an E. granulosus hydatid cyst resulted in the condemnation of 49 (52.68%), 33 (35.48%), and 11 (11.82%) sheep at the slaughterhouse. In addition, this parasite was discovered in various organs using the polymerase chain reaction (fig.3).

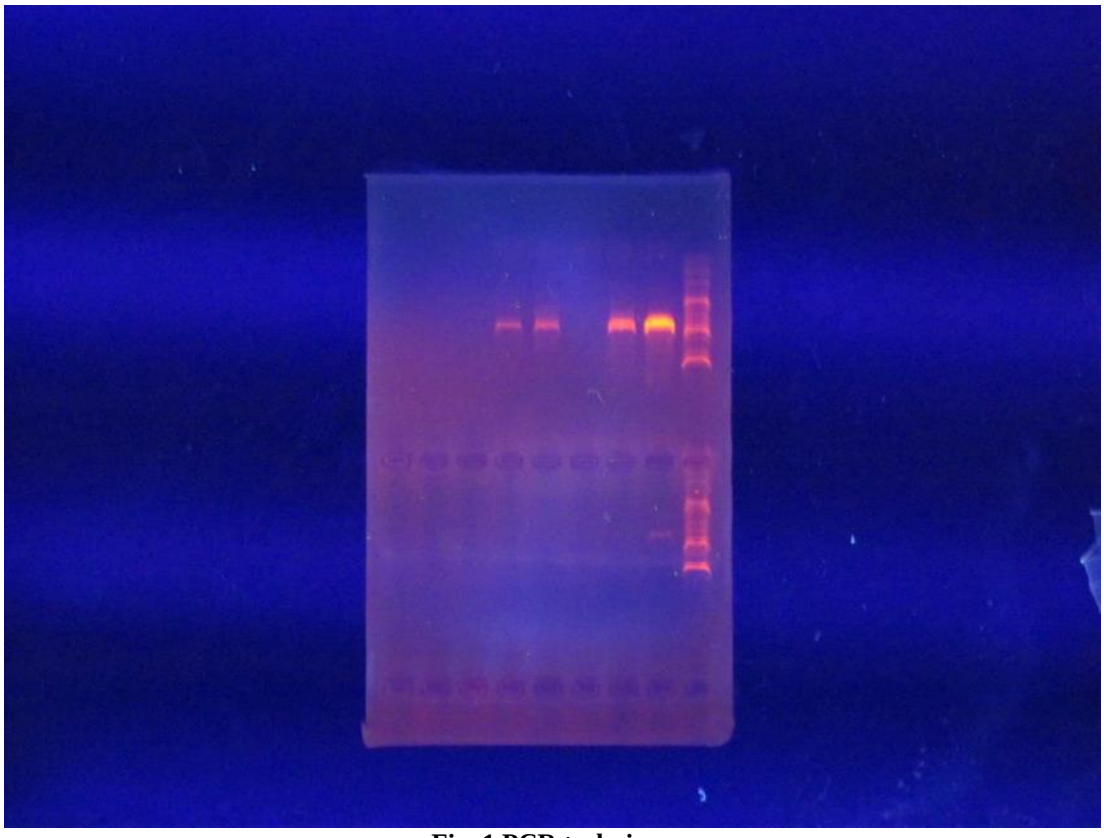

Fig. 1 PCR technique

Table (4-1): "showed the primer instruments of this study."

Name of primers	Sequences
COX1F	5-TTGAATTTGCCACGTTTGAATGC-3
COX1R	5-GAACCTAACGACATAACATAATGA-3

"These primers used to amplification of a fragment of approximately 800 bp corresponding to the atrial COI gene" [21]

Discussion

Hydatid cyst, which is caused by many species of tapeworm, is one of the most significant zoonotic diseases from a medical and economic perspective. There is a global epidemic of Echinococcus, which affects both humans and animals. Ingestion of canine faces, which contain infected eggs, causes an infection that spreads to different tissues, causing obvious health problem. [34].

Parasite strains are found in many different locations, and the ten primary genotypes of EC (G1-G2), each with their own unique biological and molecular characteristics, have been identified. "Understanding the diversity of parasitic organisms requires a synthesis of morphological taxonomy, molecular genetics, and evolutionary ecology in order to gain insight into the phenotypic and genotypic characteristics of these organisms, which is crucial for the prevention and treatment of parasitic diseases in humans and other animals". [35]

It is now possible for non-morphologists to identify parasites thanks to advances in detection made possible "by the amplification of DNA fragments using the polymerase chain reaction (PCR) technology". [36] The diagnosis of echinococcosis carried out by clinical signs pathological detection for causative agent &molecular diagnosis [37] In Iraq this disease is major endemic & zoonotic [38] And prevalence of infection has been highlighted among animals commonly in sheep and cattle are widely raised outdoor & high rate due to distribution the stray dogs which are the final host for this parasite. In this study the sheep strain (G1) is the predominating of echinococcosis in Kerbala city different internal organ (lung, liver, lung with liver), there are many molecular genetics studies which have been carried out these genetic characters in sheep echinococcosis by using DNA fingerprints and PCR yielded the similar amplified DNA band .

There are many studies which are repeated that at least seven strains in human, "but the most common one in sheep strain in north of Iraq and Basrah city some of strains and many regions in world (England, Australia, Russia, China, Kenya, Switzerland and Iran" [39]

The results of this study showed common strains of sheep in Kerbala city is (G1) strain because it's the most common strain one also it was wide spread among inter mediate host [40] So, this study showed the (G1) strain in sheep, It condemned in slaughter house in toted number of (45) samples in internal organs (lives, lung, lung with liver) lay using PCR technique to detected this parasite in the organs .The results obtained in the present *E.granulosus* showed that by PCR, and showed G1 strain specific in sheep which is infested with this cyst and it's represent direct or indirect source of infection to human, is the most reliable indicator for potential risk factor for human echinococcosis, in particular because of the effect that poor extraction methods have on PCR sensitivity .

Therefore, it is necessary to avoid the health risks and economic losses resulting from infection with hydatid cyst disease, whether in humans or animals, by adopting health principles and activating the role of veterinary health control over red meat, focusing on pre-slaughter and post-slaughter examination, in addition to the necessity of a health coalition. For organs infected with hydatid cysts in order to interrupt the parasite's life cycle.

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