

## Molecular Detection of *Echinococcus granulosus* from Visceral Organs of Cattle Abattoirs-Kerbala Province

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The aim of this study was to choose the optimal PCR technique with specific designing primer design of cystic fluid of *Echinococcus granulosus* hydatid cyst in cattle have slaughtered in kerbala abattoirs / kerbala governorate, the techniques have allowed to reduce the time consumption ,Nucleotide sequences of *Echinococcus granulosus* in Iraq cattle was obtained from NCBI websites with other researchers concerned in this topic, numerous visceral organs Livers , lungs and liver with lung suspected have hydatid cysts were isolated from 35 cow's slaughter, In PCR the highest number of positive results have 22(62.8%) were detected as *Echinococcus granulosus* hydatid cyst, on the other organs, the parasite isolation ratio was calculated from internal organs the percentage of hydatid cysts were significant increases as 12 (63.6%), 6 (27.2%)and 2(18.1%) for Liver, lung and liver with lung, respectively.

**Keywords:** *Echinococcus granulosus* , hydatid cyst in cow and PCR.

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Echinococcosis is a zoonotic disease of man and domestic animals with world wide importance<sup>1</sup> caused by larval stage of *Echinococcus granulosus* , the common site for infections are liver , lung and other organs<sup>2</sup>.

The life cycle of this parasite is indirect , it has dogs and other canids as a final host and Omnivorous with herbivorous as intermediate host<sup>3</sup>.

The disease transmission , when food or water that contains the eggs of parasite is eaten or close contact with infected.

Animals<sup>4</sup> , in the human and intermediate host via infected handling soil or dirt animal hair with eggs<sup>5</sup>, but there are not biological or mechanical vectors for larval stage except birds and arthropods can act a mechanical vectors for eggs<sup>6</sup>. The disease occurs without symptoms and signs but these appear depend on the cyst's size and location<sup>7</sup>, the disease has in both human and animals health

considerable impact with economic loss in animal production<sup>8</sup>.

There are ten genotypes of *E. granulosus* (G1-G10), (G1) in sheep , (G2 – G3) in buffalo , (G4) in equine , (G5) in cattle , (G6) in camel , (G7) in pigs and (G8 – G10) are cervid strains<sup>9,10</sup> , the strains have a biological and molecular aspects<sup>11,12</sup> with different in regions distribution and the pathological identification is difficult in the case of aberrant forms<sup>13</sup> also different in host specificity , pathogenicity life cycle pattern , transmission and rates of development finally response to the chemotherapeutic drugs<sup>14</sup>.

Polymerase chain reaction (PCR) based other methods has been vastly used for genotyping of *E. granulosus*<sup>15,16</sup> , now the clinical sample are taken biopsy to PCR for amplified fragments of mitochondrial and nuclear DNA are subsequently sequenced<sup>17</sup>

### The aim of this study

The aim of this study were to choose the optimal PCR technique with specific designing primer for cystic fluid to detect *Echinococcus*

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*granulosus* hydatid cyst in internal visceral organs of cow abattoirs-kerbala governorate.

## MATERIALS AND METHODS

An internal organ of cow which includes Liver, lung and liver with lung were sent to parasitological laboratory in the kerbala Veterinary medicine for parasite testing. For this, all cysts with organs were cut into one centimeter pieces and left in warm water for 20 minutes. Then, removed by compression, and the sediments were obtained<sup>9</sup>. DNA extraction were used for all cystic fluid and pieces by Tissue/Blood DNA Mini Kit (Geneaid, Korea), by following of manufacturer's instructions, The primer pair was used to amplify the partial mitochondrial NADH dehydrogenase subunit 1 (ND1) gene of *Echinococcus granulosus*, The sequence used in this study was from the National Center for Biotechnology ( <http://www.ncbi.nlm.nih.gov/>) websites have accession numbers as (AY386216.1, KY766890.1, AF297617.1 and KX039956.1) the primer was designed from Primer3 plus ( <http://primer3.wi.19.edu/> ) website, The sequence of the primers are designed for the following. PCR amplification was completed using conventional PCR conditions in a final volume of twenty five microne : five microne of DNAtemplate (20 ng/  $\mu$ l), 12 $\frac{1}{2}$ lMilliQ water and 1.5 $\mu$ l for both primer forward and reverse primer (10  $\mu$ M),all mixer

were collected on each PCR tubes containing 5 $\mu$ lof AccuPower® ProFiTaq PCR PreMix (Bioneer, korea). The PCR reactivity was hold out in a Techne TC-512 thermocycler with the following cycling conditions: initial denaturation(95 °C, 15 min), succeed by 35 cycles of denaturation (95 °C, for thirty seconds), annealing (56 °C, 40 s) and sixty second for extension have (72 °C), and a last extension step (72 °C, 10 min). All the amplification of single fragment of the expected size was establish by gel electrophoresison a 1.5 % agarose gel (TBE, 1 %) stained with ethidium bromide. followed by 30 cycles using 1 min denaturation at 94°C, I min annealing at 55°C and 2 min extension at 72°C, PCR reactions were assayed on 1.5% agarose gels in the presence of 5 g/ml ethidium bromide.(B).

## RESULTS AND DISCUSSION

*Echinocossosis* in Iraqi is major endom disease<sup>20</sup> and enzootic<sup>21</sup>, a total number of 35 slaughtered cattle were inspected in this study. The overall prevalence of hydatidosis in slaughtered livestock was 22 (62.8%). In total, 12 (63.6%), 6 (27.2%) and 2(18.1%) Liver, lung and liver with lung, were condemned in slaughterhouses due to *E. granulosus* hydatid cysts in cattle, respectively. Additionally, PCR technique were used to detect these parasites in hydatid cysts in both organs. Figure 1 .

Table 1. Showed the primer instruments of this study

Name of primers	sequences	Base pair	Melting temperature	G-C contents
Cox1F	5'- CGAACACGTGGTAATGTCGC -3	20 bp	59.83 C°	50.0%
Cox1R	5'- TCCGTGTTGTGGTTTTGGC -3'	20 bp	59.91 C°	50.0%

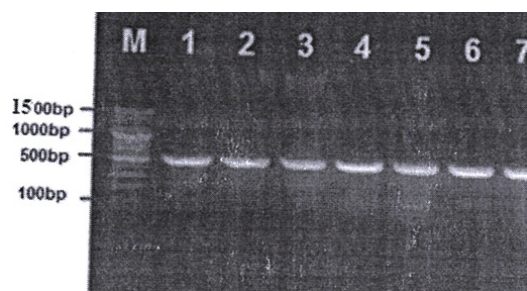


Fig .1.

1 to 74.4 % in dogs and sheep, respectively, 3.5 to 38.3 % in cattle 1.7 to 20 Vorin. L goats, (Estaim—and Hosseint1998; Ahmacti 2005; AnsarirLari\_2 05; gsakiaL20\_091 Shahb-azi—eral72171:4).

The application of polymerase chain reaction (PCR) is based on studies involved large number of *Echinococcus granulosus* were limited due to cumbersome methods access to the genomes of these parasities (@) lytic Enzymes and protein

Extraction to purified DNA parasites in particular *Echinococcus granulosus* involve weakening of the membrane of hydatid cyst by the presence of followed by enzymatic treatment and ionic detergents.

As detergents such as (sodium dodecyl sulfate) SDS have a deleterious effect on the protein denaturation of membrane of hydatid cysts (@) it was necessary to modify a designing primer such procedures to use in PCR, the selection of appropriate primers for maximal specificity and efficiency. Primer specificity was affected by a number of factors, including sequence, primer location and the PCR system used. General primer – designing rules for polymerase chain reaction were also applicable in PCR to avoid primer – dimer mispriming formation.

### CONCLUSION

There are many studies reviewed the importance in of echinococcosis but the program control against hydatid cyst cannot be successful unless the farmers education, charged people about this disease and controlled slaughters of livestock must be effective.

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