Epidemiological And Morphological Comparative Study Of The
*Echinococcus granulosus* Parasite In Humans And Different
Animals in Al-Nasiriyah city, Thi-Qar province, South of Iraq

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Abstract

The current study aimed to find out the extent of the prevalence of hydatid cysts disease caused by the larval stage of the parasite *Echinococcus granulosus* that affects humans and different animals and measure the incidence among animals slaughtered in the slaughterhouses of the Al-Nasiriyah city, markets butchers and butchers working in markets selling livestock, in addition to human infected cases that taken from civil and government hospitals in Al-Nasiriyah city, and the epidemiology of the parasite in the final host (Canidae family) from loose dog feces to find the eggs of the parasite.

Where 910 cases were examined and found 42 cases of hydatid cysts distributed as follows (11 of cows, 3 of Buffalo, 6 of camels, 16 of sheep and 6 human cases) and different infection rates of *E. granulosus* in cow, buffalo,
camel, sheep, goats and human were found to be 3.5% (11/312), 4.5% (3/66), 16.7% (6/36), 4.6% (16/346), 0.0% (0/144) and 100% (6/6), respectively.

There are 12 samples are proved to contain eggs of the parasite from 89 samples was collected from loose dog feces that live and roam near the butchers working in the local market for selling sheep and goats in the north of Al-Nasiriyah city, Thi-Qar province.

Measurements of the small and large hooks of the protoscolices in the various infected organisms showed significant differences in the length and width of the hooks in addition to the length of the handle.

The current study found that all infected cases of intermediate hosts belong to strains of the genus *Echinococcus granulosus* after molecular diagnosis was completed using polymerase chain reaction technology (PCR) with the *CoxI* gene in the gel electrophoresis technique by using the primers JB3 and JB4.5.

The highest purity of the DNA used in the polymerase chain reaction technique was obtained by the using protoscolices as a source of DNA, where the extraction results showed that the use of protoscolices as a source of DNA is better than the use of germinal layer tissues because of the easy analysis of protoscolices in compared to germinal layer tissues.

**Introduction**

Hydatid cysts disease is an important and widespread disease in the world, where this disease is caused by the larval stage of the canine tapeworm *Echinococcus granulosus* in the intermediate hosts and is an endemic disease in Iraq that is transmitted from loose dogs infected with this tapeworm. (Saeed *et al.*, 2000; Alwan and Faleh, 2001).
Humans are accidentally infected with the parasite when eating food or drink contaminated with the parasite's eggs released with the feces of the final host, members of the Canidae family. (Bekçi, 2012; Cappello et al., 2013)

The parasite has several breeds, as indicated by Roratto et al., (2006) to differences within one type strains they differ from each other in many of facets, and these strains such as sheep strain, cows strain, Buffalo strain and camels strain.

Two medically important species are *E. granulosus* which causes cystic echinococcosis, and grilled or alveolar caused by genus *E. multilocularis* that causes alveolar echinococcosis. (Budke et al., 2006; Nunnari et al., 2012)

The parasite needs two types of host to complete its life cycle, the final host represented by members of the Canidae family such as dogs, wolves, foxes, jackals and hyenas, and intermediate host represented by livestock such as cows, sheep, goats, camels and Buffalo. Humans considered as an accidental host that the life cycle of the parasite ends in it. (Karyakarte and Damle, 2004).

The liver is the first candidate of the parasite, followed by the lungs, kidneys, spleen, brain, bone and heart, and the appearance of symptoms depends on the size and location of the cyst in the affected organ. (Karaman et al., 2002; Albayrak et al., 2008).

Investigations using mitochondrial DNA (mtDNA) sequences have characterized ten genotypes (G1–G10) within *E. granulosus sensu lato* (Scott et al., 1997; Lavikainen et al., 2003).

The dominant strain was the Buffalo strain (G3), and both the Buffalo strain (G3) and the sheep strain (G1) represented an actual source of human infection, and there is no host specificity to the genotypes detected. (Fadhil and A'aiz 2016).

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In cystic echinococcosis patients, four genotypes of *E. granulosus* were identified, with genotype G1 predominating followed by genotypes G3, G2, and G6. (Moradi *et al.*, 2019).

**Materials and methods**

**Study area**


**Sample collection**

Samples were collected for the period from mid-September 2020 until mid-March 2021 at a rate of three days a week.

The collection areas of human samples were from government and civil hospitals in Al-Nasiriyah city, Thi-Qar province. The collection of samples from the intermediate host (livestock) was from the massacres of Al-Nasiriyah districts, the butchers in the meat selling markets and the butchers in the livestock selling markets.
The samples taken from humans were in six human samples, while the animal samples were 36 samples distributed as (11 of cows, 3 of Buffalo, 6 of camels, 16 of sheep) in four organs (Liver, Lung, spleen and heart).

Also, 89 samples were collected from the loose dog feces (which are considered the final host of the parasite) that live and roam near the butchers working in the local market for selling sheep and goats in the north of Nasiriyah district, Thi-Qar province.

**Preparation of hydatid cyst samples for morphological study**

The hydatid cysts collected from different sources in the previous step were examined microscopically according to Lett (2013).

**Molecular study**

Genotyping including mitochondrial gene sequencing of partial mitochondrial cytochrome C oxidase subunit1 (CoxI) gene. (Bowles et al., 1992; Bowles and McManus, 1993).

**Preparation of hydatid cyst contents for DNA extraction**

The stored samples were centrifuged to get rid of the alcohol solution, and then washed with sterile distilled water, mixed by vortex thoroughly for one minute, then centrifuged at 14,000 rpm for three minute that repeated three times to remove the ethanol from the sample according to DNA extraction kit instructions.

**Genomic DNA extraction**

The hydatid cyst sample was prepared for DNA extraction by following the instructions in the protocol of DNA extraction kit (gSYNC™ DNA Extraction Kit, Geneaid, Korea) with some modification for some samples.

**Polymerase chain reaction (PCR)**

PCR of the purified DNA samples was performed using a master mix (the PreMix PCR kit component shown in table 2), then the DNA of *E. granulosus* was dissolved and the cumulative volume sufficient for the amplification mix was prepared as shown in table (1), and then the tubes was closed and mixed well by vortex.
Table 1: Components of master mix (Premix)

<table>
<thead>
<tr>
<th>Components</th>
<th>For 25µl reaction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top DNA polymerase</td>
<td>1 U</td>
</tr>
<tr>
<td>dNTPs (dATP, dTTP, dGTP, dCTP)</td>
<td>Each 250 µM</td>
</tr>
<tr>
<td>Reaction buffer with 1.5 mM MgCl2</td>
<td>1X</td>
</tr>
<tr>
<td>Stabilizer and tracking dye</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Mixture of PCR amplification for 25µl reaction volume

<table>
<thead>
<tr>
<th>Components</th>
<th>Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Premix</td>
<td>Vacuum dried pellet</td>
</tr>
<tr>
<td>Template DNA</td>
<td>5 µl</td>
</tr>
<tr>
<td>Foreword primer</td>
<td>2 µl</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>2 µl</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>16 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>25 µl</strong></td>
</tr>
</tbody>
</table>

PCR amplification cycles

PCR thermo-cycler program was designed up to manufacturer protocol:

1. Add template DNA and primers into AccuPower PCR PreMix tubes.
2. Add distilled water into tubes to total volume of 25µl.
3. Reaction mix as in (Table 3 – 9).
4. Dissolve the vacuum dried blue pellet by flick with finger or pipetting, and briefly spin down.
5. Perform the reaction under the following conditions:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Denaturation</td>
<td>95º C</td>
<td>5 minutes</td>
<td>1 Cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95º C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>50º C</td>
<td>45 seconds</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72º C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72º C</td>
<td>5 minutes</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>4º C</td>
<td>Forever</td>
<td>-</td>
</tr>
</tbody>
</table>
6. Maintain the reaction at 4º C after cycling.

7. Load samples on agarose gel without adding a loading-dye mixture, and perform electrophoresis.

**Preparation of Agarose gel and electrophoresis tray for PCR products:**

According to Bowles et al. (1992), the Agarose gel was preparing to the PCR products.

**Results**

**Epidemiology**

The current study included examination 910 of different cases as shown in table (3) were collected and found 42 cases of hydatid cysts distributed as (11 of cows, 3 of Buffalo, 6 of camels, 16 of sheep and 6 of humans) and different infection rates where the highest incidence in camels was 16.7% and the lowest incidence was in cows 3.5%. There is high significant difference in the infection among different animals at probability level ≤ 0.01. (Table 3)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Case</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Non infected</td>
</tr>
<tr>
<td>Cows</td>
<td>11</td>
<td>301</td>
</tr>
<tr>
<td>% within Animal</td>
<td>3.5%</td>
<td>96.5%</td>
</tr>
<tr>
<td>Buffalos</td>
<td>3</td>
<td>63</td>
</tr>
<tr>
<td>% within Animal</td>
<td>4.5%</td>
<td>95.5%</td>
</tr>
<tr>
<td>Camels</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>% within Animal</td>
<td>16.7%</td>
<td>83.3%</td>
</tr>
<tr>
<td>Sheep</td>
<td>16</td>
<td>330</td>
</tr>
<tr>
<td>% within Animal</td>
<td>4.6%</td>
<td>95.4%</td>
</tr>
<tr>
<td>Goats</td>
<td>0</td>
<td>144</td>
</tr>
<tr>
<td>% within Animal</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>% within Animal</td>
<td>100.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>868</td>
</tr>
<tr>
<td>% of Total</td>
<td>4.6%</td>
<td>95.4%</td>
</tr>
</tbody>
</table>

Cal.X²: 143.68  Tab.X²: 15.09  df: 5  P_value: 0.01
Percentage of infected organs

The table (4) shows that there were significant differences in the study totals according to the results shown in the variability of the infection in the study animals between infected organs at probability level ≤ 0.01 (Cal.X²: 48.30 to Tab.X²: 27.69).

Table (4): Distribution of E. granulosus parasite according to infected organ of animals in Al-Nasiriyah city

<table>
<thead>
<tr>
<th>Animal</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
<th>Heart</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>Count</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>20.7%</td>
<td>42.9%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Buffalos</td>
<td>Count</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>3.4%</td>
<td>0.0%</td>
<td>100.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Camels</td>
<td>Count</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>3.4%</td>
<td>23.8%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sheep</td>
<td>Count</td>
<td>15</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>51.7%</td>
<td>33.3%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human</td>
<td>Count</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>20.7%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>29</td>
<td>21</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>% within Organ</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>% of Total</td>
<td>54.7%</td>
<td>39.6%</td>
<td>3.8%</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

Cal.X²: 48.30  Tab.X²: 27.69  df: 12  P_value: 0.01

Morphological analysis

Eggs of E. granulosus parasite

After the completion of the microscopic examination, 12 from 89 samples (13.48%) are proved to contain eggs of the E. granulosus parasite.
Liver large hooks

The table (5) shows that the data are homogeneous (convergent) with high significant differences in the total lengths between cases, while in the total width there is high significant differences between sheep and cow as well as between cow and buffalo without significant differences between camel and human. There is no significant difference between cases in handle length between all cases.

Table (5): Measurements of liver large hooks of different animals

<table>
<thead>
<tr>
<th>Liver large hooks</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>L.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>100</td>
<td>20.02d</td>
<td>1.44</td>
<td>0.43</td>
</tr>
<tr>
<td>Camel</td>
<td>100</td>
<td>21.14c</td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>100</td>
<td>22.57b</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>23.80a</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Human</td>
<td>100</td>
<td>7.60c</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>100</td>
<td>7.60c</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>100</td>
<td>10.22a</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>8.86b</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Handle</td>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>Human</td>
<td>100</td>
<td>11.0a</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>100</td>
<td>11.04a</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>100</td>
<td>11.02a</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>11.0a</td>
<td>0.72</td>
<td></td>
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</table>

- The mean difference is significant at the 0.05 level.
- Results represent mean ± Standard deviation (S.D)
- Means having different letters in the same column differed significantly (P≤0.05).
- (L.S.D) least significant difference.

Liver small hooks

The table (6) shows that the data are homogeneous (convergent) with high significant differences in the total lengths between cases, there is high significant differences between cow and sheep as well as between sheep and camel without any significant differences between camel and human. While in the handle length there is high significant differences between all cases.

Table (6): Measurements of liver small hooks of different animals

<table>
<thead>
<tr>
<th>Liver small hooks</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>L.S.D</th>
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</thead>
</table>

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<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Camel</th>
<th>Cow</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>100</td>
<td>16.0(d)</td>
<td>17.50(e)</td>
<td>20.33(^a)</td>
</tr>
<tr>
<td>Width</td>
<td>100</td>
<td>5.68(^c)</td>
<td>5.85(^c)</td>
<td>7.52(^b)</td>
</tr>
<tr>
<td>Handle</td>
<td>100</td>
<td>8.39(^c)</td>
<td>8.04(^d)</td>
<td>11.24(^b)</td>
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The mean difference is significant at the 0.05 level. Results represent mean ± Standard deviation (S.D). Means having different letters in the same column differed significantly (P≤0.05). (L.S.D) least significant difference.

**PCR product and gel electrophoresis:**

In total 96 of different *E. granulosus* DNA isolates were studied by using PCR amplification of partial mitochondrial cytochrome C oxidase subunit1 (*Cox1*) with gene size of 440 bp, that by using the JB3 and JB4.5 primers. The PCR products of the isolates by using gel electrophoresis revealed major banding patterns. The isolates from sheep, cattle, camels, buffalo and humans produced similar patterns, so that's prove that all isolates belongs to *E. granulosus* strains. (Pictures 1-8)
Pic (1): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the Cox1 gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (1-8) represent the results of amplification of the gene at 440bp.

Pic (2): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the Cox1 gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (9-16) represent the results of amplification of the gene at 440bp.
Pic (3): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the \textit{Cox1} gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (17-28) represent the results of amplification of the gene at 440bp.

Pic (4): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the \textit{Cox1} gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (29-40) represent the results of amplification of the gene at 440bp.
Pic (5): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the Cox1 gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (41-52) represent the results of amplification of the gene at 440bp

Pic (6): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the Cox1 gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (53-64) represent the results of amplification of the gene at 440bp
Pic (7): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the *Cox1* gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (65-80) represent the results of amplification of the gene at 440bp.

Pic (8): Agarose gel electrophoresis containing the results of the polymerase chain reaction (PCR) products for the *Cox1* gene in the study samples. (L): Represents the Marker ladder (100-1500bp) and samples (81-96) represent the results of amplification of the gene at 440bp.
References


