Kufa Journal for Veterinary Medical Sciences Vol.(6). No.(1) 2015

Kufa Journal for Veterinary Medical Sciences
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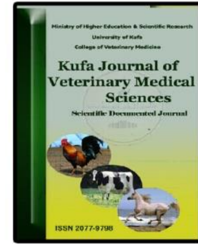

Molecular detection of Hydatid cyst isolated from Goats in AL-Qadisiyia province by Polymerase Chain Reaction

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Abstract

The present study was conducted during the period from August 2013 to April 2014 in AL-Qadisiyia province to detect the infection of hydatidiosis in Goat using Molecular and conventional methods. One hundred and seventy nine Goat were examined at slaughters in Aldewanyia abattoir. Caprine hydatid cyst were removed from infected liver and lung after visceral inspection of these organs then investigated the viability of protoscolices by staining with eosin stain then examining under light microscope of 40x.The results showed 45 hydatid cyst were found from 179 goat , the prevalence rate of infection (25.13%) . 20 cyst were found fertile only whereas 25 cyst were sterile. Regarding to infection site in organs, out of 45 hydatid cyst collected , 28(62.22%) cyst were isolated from liver whereas 17 (37.77%) isolated from lung with significant differences at p<0.05. Out of 45 hydatid cyst samples were taken from infected goats, and examined by conventional PCR , the PCR product demonstrated an expected fragment of 440 bp in the length , 10samples were given positive cases , 7(25%) samples from liver whereas only 3(12%) from lung samples gave positive PCR result, with statistically significant difference (P<0.05) between two organs.

Key words: Echinococcsus granulosus , Hydatidosis , Hydatid cyst, Goat,PCR

الخلاصة:

أجريت الدراسة الحاليه خلال الفترة من اب 2013 إلى نيسان 2014 في محافظة القادسية للكشف عن الإصابة بالأكياس العدرية في الماعز باستخدام الطرق الجزيئية والتقليدية.

فحصت مائة وتسعة وسبعون عينة من الماعز المذبوح في مجزرة الديوانية. أزيلت الاكياس العدريه من الكبد المصاب والرئة بعد اجراء الفحص العياني لأحشاء الذبائح . قييست حيوية الرؤوس الاولية باستخدام صبغة الايوسين تحت المجهر الضوئي باستخدام القوه X40. أظهرت النتائج وجود 45 كيس عدري بين الحيوانات المفحوصة ،حيث بلغت نسبة الإصابة (25.13٪). بينما كانت النسب اعتمادا على نوع الاكياس 20 كيس مائي خصب و25 كيس مائي عقيم . وفي ما يتعلق بموقع الإصابة , بينت الدراسة ان اعلى نسبه للإصابة كانت في الكبد وبنسبه 62.22% بينما كان نسبة اصابة الرئة اقل حيث بلغت 37.77% مع وجود اختلاف معنوي كبير P<0.05. فيما يتعلق باختبار تفاعل السلسله المتبلمره, عندما جزء من (400 زوج قواعد) كان يتضخم من(CO1) جين, أثبتت النتائج وجود 10 عينات موجبه فقط ، حيث كانت 7 (25٪) عينة من الكبد بينما

3(12٪) عينات فقط من عينات الرئة أعطت نتيجة إيجابية بهذا الاختبار، مع فروق ذات دلالة إحصائية (P
<0.05) بين الاعضاء .

Introduction

Echinococcosis (Hydatidosis) is a cosmopolitan, hyper endemic zoonotic disease caused by infection with (larval stage) of *Echinococcus granulosus*. Its one of the most important parasitic diseases in under developed countries especially rural communities, where man in close contact with the dogs (final hosts) and various domestic animals which acts as intermediate hosts (1).

The genus Echinococcsus has six important species these includes *E. granulosus, E. multilocularis, E. oligarthus, E. Vogeli, E. shiquicus and E. felidis*. Among these six species four are of medical and public health importance and widely prevalent and may cause severe disease in humans (2).

Hydatidosis is diagnosed by different ways as X- ray, CT scan, other immunological and serological tests including modern technique Polymerase Chain Reaction (PCR) which have high sensitivity and specificity in detection of hydatidosis infection added to that used in genotyping of *E. granulosus* to facilitate treatment and vaccination (3) .

E. granulosus occur as several different genetically distinct forms known as strain or genotypes. Based on two mitochondrial cytochrome C oxidase subunit I (COI) and NADH dehydrogenase subunit I (nadI) genes, at least ten distinct genetic types (G1-G10) have been identified with in the *E.granulosus* complex infected intermediate host species including man, which have a degree of host adaptation some of which exhibit marked biological and morphological differences, such genotypes were recently proposed to merit species status. The G1(sheep strain), G2(Tasmanian sheep strain) and G3(buffalo strain) are grouped together in species namely *E.granulosussensustricto* (G1-G3), *E-equinus* G4 (horse strain), *E.orteppi* G5 (cattle strain), *E.canodensis* G6(camel strain), G7(pig strain),G8 (cervid strain),G9 (human strain) and G10 (fennoscandian strain) (4,5). in this study using of conventional PCR as a tool to determine the CO1 genes extracted from the Hydatid cyst samples taken from different goats hosts .

Materials and Methods

Sample collection

During period of the study from August 2013 to April 2014 , totally 179Goat were examined at slaughters in Aldewanyia abattoir. Caprine hydatid cyst were removed from infected liver and lung after visceral inspection of these organs and then transported by ice boxes with normal saline to parasitology laboratory College of veterinary medicine ,University of Al-Qadisiya for examination.

Assessment the cyst fertility

Hydatid fluid was aspirated from cyst by syringe and protoscolices were scraped from sides of germinal layer and putting in test tubes then centrifuged at 2500 rpm for 5 minute, the supernatant discharged and sediment used for measuring viability of protoscolices by staining with eosin stain then examining under light microscope of 40x (6).

DNA extraction

Each cyst was separated into membrane and fluid with protoscoleces. The germinal layer was washed many times in ethanol to remove any contaminant . then both membrane and protoscoleces were preserved in 70% ethanol and stored at −20°C. then the genomic DNA extracted after the protoscolices were washed with distill water two times, by extraction kit (Bioneer Company, South Korea).

Polymerase chain reaction (PCR).

The fragment of 440 bp of mitochondrial cytochrome C oxidase subunit 1(CO1) gene specific to *E. granulosus* was amplified from each isolate using the following primer pair: forward primer (J3-´5: TTTTTTGGCCATCCTGAGGTTTAT-3´) and reverse primer (J4.5-´5: TAACGACATAACATAATGAAAATG-3´) which were designed based on the mitochondrial G1 genome sequence(7). The PCRprogram was performed by one cycle primary denaturation (5min at 95 °C), followed by 35 cycles of denaturation step (45 s at° 94C), annealing step (45 s at 56 °C) and final extension (72 °C for10 min).

Statistical analysis :

The results of present study were analyzed by SPSS program (version 18) software 2010 , using Chi-square test and a P values of p ≤0.05 were considered to record statistical significance (8).

Results

A total of 45 hydatid cyst were isolated from 179 examined goat , the prevalence rate of infection (25.13%) . 20 cyst were found fertile only whereas 25 cyst were sterile .

Table (1) The prevalence rate of hydatid cysts in Goat

No. of examined	No. of infected	Percentage of infection
179	45	25.13

Regarding to infection site in organs, out of 45 hydatid cyst collected , 28(62.22%) cyst were isolated from liver whereas 17 (37.77%) isolated from lung with significant differences at p<0.05.

Table (2) The site of infection of hydatid cysts in Goat.

Origin	No. of infected	Liver	Percentage	Lung	Percentage
Goats	45	28	62.22a	17	37.77b

The different letters refers to the significant differences in percentage of infection according to the site of infection at p<0.05

Molecular detection

Out of 45 hydatid cyst samples were taken from infected goats, and examined by conventional PCR , the PCR product demonstrated an expected fragment of 440 bp in the length , 10samples were given positive results , 7 samples from liver whereas only 3 from lung samples gave positive PCR result, with statistically significant difference (P<0.05) between two organs . (Table 3, Fiqure 1).

Table (3) The site of infection of hydatid cysts in Goat according to PCR result.

Samples	Examined No.	Positive No.	Percentage of infection
liver	28	7	25%a
lung	17	3	12%b

The different letters refers to the significant differences in percentage of infection according to the PCR result at p<0.05

Figure : 1 Agarose gel electrophoresis image that shown the PCR product analysis of CO1 gene of *Echinococcus granulosus* at 440bp PCR product in hydatid cyst Goat samples. Where L: Ladder 1000bp, lane (1-10) positive samples.

Discussion

Hydatidosis is one of the most important diseases in human and animals distributed in many countries all over the world and in Iraq too. A total of 45 hydatid cyst were found in 179 of examined Goat, investigating a high prevalence rate of infection (25.13%) . Regarding to type of cysts, 20(44.44%) cysts were found fertile .

The rate of infection in this study was nearly similar to certain studies did in Iraq by (9) when he recorded the prevalence of hydatid infection rate with hydatid cysts were 18.37%, 23% and 11.25% in Cattle ,Sheep and Goat respectively.

Regarding to site of infection , The liver was more frequently predominant infected than lungs, the result of this study agreement with the result of (10and 11) mentioned that cystic larva develops mainly in liver (70%),then lungs (20%), and 10% of cysts in anywhere of the body such as heart, bone, muscle, nervous system and the abdominal cavity. All body organs and tissues are considered to be exposed to hydatid cysts infection except the hair, nails and teeth (12). Also the results disagreed with several results of different regions that indicated by (13) in Iran which recorded the prevalence rate of infection 38.46% were found in liver and 61.54% in lung out of Ninety five sheep cysts, also (14) in Morocco who found the lung was more infected than liver .

Since several years extensive literature on the application of molecular biological methods has been published in order to discrimination *Echinococcus* strain/species. PCR is one of the methods used for molecular characterization of *Echinococcus* isolates (4). This research was conducted to identify and determine *E. granulosus* larval stage in Goat of Aldewanyia abattoir through mitochondrial cytochrome C oxidase subunit 1 fragment was amplified by PCR and PCR products were eletrophoresed. 440 bp bands were observed from amplification of (CO1) in ten samples only out of 20 samples. The DNA were not detected in other samples may be attributed to the different of DNA extraction methods , or due to the different in quantity of parasite's DNA or the condition and prolonged period of storage which lead to degradation of the DNA and produce negative PCR results(15).

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