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RESEARCH ARTICLE OPEN ACCESS

Molecular Study Of Hydatid Disease In Iraqi Goats By using The Mitochondrial Cytochrome C Oxidase Subunit 1 Gene

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Abstract

The aim of the present study was to identify and recognize the genotype for cystic *Echinococcosis* that infects goats (n=19) in Iraq. The hydatid cyst was collected from different parts of the body, i.e. lungs, liver, heart, spleen and peritoneal cavity. The current study was conducted in five different regions of Iraq (Anbar, Baghdad, Saladdin, Karkuk, Babylon during October 2018 to July 2019. The mitochondria DNA was extracted and screened for the presence of (CO1) cytochrome C oxidase subunit 1 gene using polymer chain reactions (PCR). Amplification size was around 450bp. The amplicon was isolated and purified. The isolated CO1 amplicon was sequenced. The CO1 putative sequence was BLAST with available sequence from NCBI. The genetic tree was deduced. The genotype G1 is the most frequently spread strains and considered as a key source for infection in goats of Iraq.

Keywords: Mitochondria DNA, Cytochrome C oxidase, Genotype, Goats

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INTRODUCTION

E. granulosus causes Hydatid Cyst disease in goats.[1] The prevalence of disease spreading is significantly high worldwide. Hence, it is also considered as one of the significant zoonotic diseases.[1,2] The goats can be infected by consumption of infected water and food.[3] The contamination can be spread through infected dogs litter containing eggs of worms.[3]

The mutations in the *E. granulosus* strains can affect the characteristics, life circle, evolution rate of disease, drug sensitivity, drugs evolution against this disease in Iraq.[4] Cystic Echinococcosis is considered as one of the most significant pandemic diseases that have an important hazard for human beings and finally to animal health.[5-9] Recently, about 10 genotypes (range from G1 to G10) have been diagnosed internationally depending on nucleotide sequence using genes for ND1 and CO1. This disease was found to be infect various animals such as camels, pigs, sheep, cattle, horses, cervids, and goats.[10-15]

The genotype G1 reported to infect human beings as well as sheep and cattle.[16] For genotype recognisation, number of marker genes and various advanced techniques are commonly used in many countries.[17] The strain causing hydatid cyst disease in Iraqi Goats has been identified based on the ND1 gene sequences. Similar gene can be used to detect the G1 strain in goat.[19] The aim of the current study was to identify and recognize the cystic echinococcosis genotype that infects goats in Iraq. The PCR technique was used to recognize the minor strains which depending on CO1 gene amplification.

MATERIALS AND METHODS

Sample Collection

The current study was conducted in five different regions of Iraq. Anbar, Baghdad, Saladdin, Karkuk, Babylon during October 2018 to July 2019. The samples (n=19) were obtained through custom massacres and from veterinarians, who contributed to goats infected diagnosis with Hydatid cyst where the samples were collected by me in the field.

Sample Preparation and Protoscoleces Isolation

The isolated cyst was washed twice with normal saline to remove contamination. Further, the cyst was washed with 70% ethanol as per the protocol described by Mc-Manus and Smyth.[20] Each Hydatid Cyst was divided into two regions, viz. Outer cover and internal liquid containing protoscoleces. The protoscoleces were extracted using 10 ml sterile syringes into new sterile containers. The Hydatid Cyst was opened vertically and the liquids were separated. The protoscoleces were collected in a new sterilized container. The liquids was centrifuged at speed (3000) revolutions per minute (rpm), room temperature, for ten minutes. The whole protoscoleces pellet was collected. For isolation of germinal membrane, the procedure described by the Rishi and McManus[21] was followed. The germinal membrane was washed twice by using (pH 2) Hanks Saline which contains 0.2% Pepsin(W/V). Followed by centrifugation at speed (3000) revolutions per minute rpm RT for ten minutes. The remaining protoscoleces were collected as a pallet. The pallet was washed 3 times with normal saline and centrifuged to decant the liquid. The protoscoleces pallet was stored by using (70%) ethanol at 4°C for further processing.

DNA Extraction from Protoscoleces

The stored protoscoleces was washed with phosphate buffer saline to remove ethanol. The samples were subjected for the DNA extraction[21] using a Wizard purification DNA kit (USA).[22,23] Briefly, around 20 ng sample were used for the DNA extraction. The extracted DNA were evaluated for the presence of mitochondrial cytochrome C oxidase subunit 1 (CO1) gene. Amplified CO1 gene by using CO1 specific primers according to the procedure of PCR[24] (Table 1).

The PCR condition was as follows denaturation temperature at 94°C for 5 minutes

Table 1. The primers of (CO1) gene

Sequence of primer	Sense	Size
5' –TTT_TTT_GGG_CAT_CCT_GAG _GTT_TAT- 3'	Forwards	450 bp
5'-TAA_AGA_AAG_AAC_ATA_ATG_AAA_ATG-3'	Reverses	450 bp

then followed by 35 cycles of three steps. These three steps were denaturation at a temperature 94°C for 45 seconds, followed annealing step at temperature 58°C for 45 seconds and finally extension step at temperature 72°C for 45 seconds. These steps were followed by a final step for extension at temperature 72°C as extra time for 7 minutes.

***In silico* analysis of CO1 gene**

After CO1 gene amplification, the applicant was obtained and proceeded for extraction from the gel. The extracted amplicon was sequenced and BLAST with the reference CO1 sequences available in NCBI (www.ncbinlm.n.h.gov)

RESULTS

The extracted DNA from 19 Hydatid Cyst samples were screened for the amplification of the CO1 gene using PCR and CO1 specific primer. Fig. 1 showed CO1 gene amplification on agarose gel. Around 450bp amplicon was observed on the gel. The pieces of CO1 gene were amplified. The amplified amplicon was extracted and sequenced. The putative CO1 sequence was compared with the available CO1 sequences on NCBI. This putative sequence was corresponding with the Genotype G1 the main causative *E. granulosus* (Fig. 2).

In the present study, the registered sequences in Gene Bank have been accomplished by using Bioedit program which is considered as one of the dependent programs in analyzing the DNA. The isolated CO1 gene sequence showed 100% matching with the sheep strain G1 genotype (Accession no MN787561).[25]

DISCUSSION

In the present study, mitochondrial cytochrome C oxidase subunit 1 gene was amplified using available primers. These primers were found to be specific for the goat CO1 gene as all 19 samples showed positive results in PCR. The amplicon size was 450bp. Similar CO1 amplicon size was reported by various authors.[16,23,24] The genetic type G1 and particular sheep strain were affected by *E. granulosus*. This infection was proving to be a local host. It is worth to mention that the examined places are close and everlasting availability in the pastoral with these animals. This closeness and food sharing lead to increase the infection possibility of Hydatid Cyst disease.[15] This can be one of the major reasons behind the prevalence of infection.

It is worth referring that the remarkable spread of sheep strain G1 was common in the extreme inhabitance regions in Iraq. These results are in accordance with previous studies.[15, 18, 24, 26,27] It is the main reason of being such strain as more frequent and spread in the intermediate hosts for these reasons.[16,28] *E. granolosus* strain detection in domestic animals as well as in wild animal in this region will be epidemiologically significant. The genotype G1 is also considered as a disease

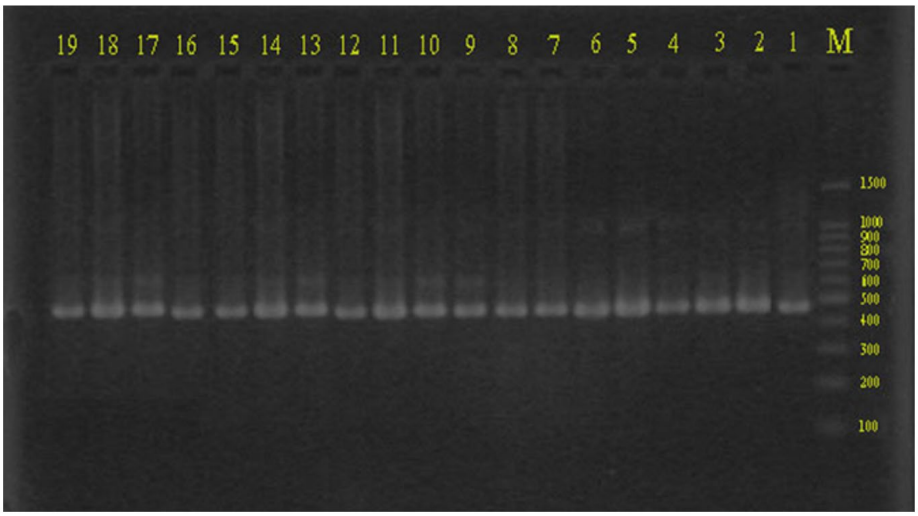

Fig. 1. PCR amplification of Co1 gene. Electrophoresed on 2 % agarose gel (80 V, 70 Amp) (M, 100 base pair, DNA ladder, lanes 19_1, *E. granulosus* isolates).

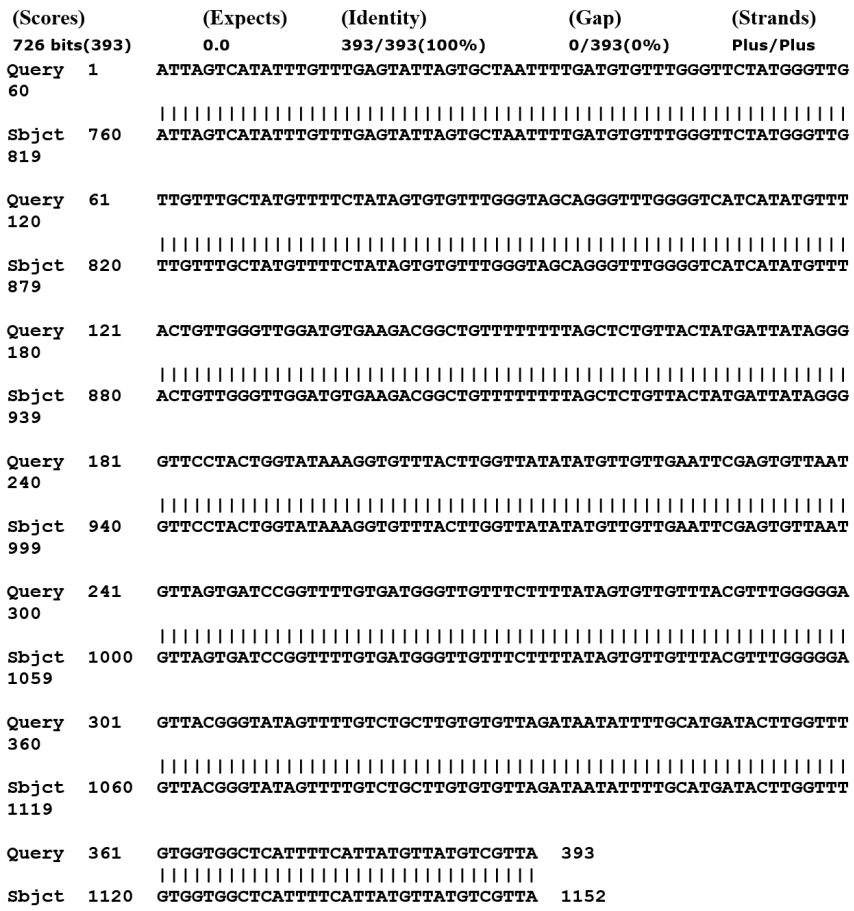

Fig. 2. Mitochondrial cytochrome C oxidase subunit 1 gene alignment of *Echinococcus granulosus,* by utilizing Gene bank.

that infects the other intermediate hosts such as goats.[29,30] From the aforementioned, the genotype G1 is considered as the most frequent type and the most effective strain in the Iraqi goats. In the present study, all 19 samples showed presence of genotype G1 i.e. CO1 gene, is a particular for sheep strain.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

ETHICS STATEMENT

Not applicable.

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