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Short Communication

Genetic Characterization of Hydatid Cysts Isolated from Domestic Animals in Lorestan Province, Western Iran

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Abstract

Background: Regarding Hydatid cyst (cystic echinococcosis, CE) as a human public health problem in the West of Iran, molecular data related to the genotypes of *Echinococcus granulosus* in cattle and sheep in these regions are still insufficient. Here, we evaluated the genotypes of *E. granulosus* infecting sheep and cattle in western Iran.

Methods: Totally, 36 hydatid cysts including 18 hydatid cysts of sheep and 18 hydatid cysts of cattle were collected from Khorramabad slaughterhouse (Lorestan Province), Western Iran between May to September 2014. Protoscoleces or germinal layers were collected from cysts, DNA was extracted, and genotyping was performed by sequencing and analyzing mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene.

Results: In sequencing analysis, all of sheep isolates belonged to genotype G1 (sheep strain). Among cattle hydatid cyst isolates, 16/18 (88.9%) were belonged to genotype G1 and 2/18 (11.1%) were belonged to G3 genotype. The phylogenetic analysis showed two clusters; one of the clusters includes cattle G3 genotype and the other cluster represents sheep and cattle G1 genotype that were isolated during in this study.

Conclusion: The common sheep strain/G1 is predominant genotype in the western part of Iran, followed by G3 genotype, circulating among the animal hosts in this region. Further studies to find more isolates may need to be understood if there are other genotypes in this region.

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Introduction

Cystic echinococcosis (CE), is the larval cystic form (called hydatid cyst) of a dog tapeworm (*Echinococcus granulosus*) that may cause disease in intermediate hosts, generally domestic livestock as well as human [1]. In the usual life cycle, tapeworm eggs are passed in the feces of the definitive host (dog) and might be ingested by intermediate hosts such as sheep; the released embryos penetrate the intestinal wall, and then transferred via blood system throughout the body mainly liver and/or lungs; where the hydatid cysts grow up [2]. In CE, clinical manifestations are mostly related to the localization, number of cysts, and size. There is a possible association between the genotypes and the size of cysts; whereas liver cysts in the patients with G1 genotype were notably bigger than those infected with G7 genotype [3]. Molecular epidemiological data have recognized 10 distinct genotypes of *E. granulosus* (G1–G10): *E. granulosus sensu stricto* (G1, G2, G3), *E. equines* (G4), *E. ortleppi* (G5), *E. intermedius* (G6, G7, G8, G10) [4]. In Iran, to date, three genotypes have been found including G1 (Sheep), G3 (buffalo), and G6 (camels) genotypes in humans and animals in different regions [5, 6]. G1 strain is the most predominant genotype in Iran [6] as well as various regions of the world [7]. The mitochondrial markers such as cytochrome c oxidase subunit 1 (*cox1*) and NADH dehydrogenase 1 (*nad1*) genes, because of the ability to eliminate some limitations in taxonomy [8] are suitable molecular tools for studying genetic variation in *E. granulosus* isolates from various hosts in various geographical parts of Iran [5, 7].

Recently, Parsa et al. have demonstrated three genotypes G1, G2 and G3 are the main genotypes of the *E. granulosus* isolates of stray dogs, from Lorestan province, western Iran using DNA sequencing of the partial mitochondrial *cox1* and *nad1* [9]. Nevertheless,

genotyping data do not exist about *E. granulosus* isolates of domestic livestock in this province using DNA sequencing of *cox1* and *nad1*.

Therefore, this study aimed to molecularly recognize and genotype hydatid cysts of some livestock (sheep, and cattle) in Lorestan Province by sequencing and analyzing mitochondrial *cox1* gene, to guess on possible transmission patterns of *E. granulosus* by homology analysis, and to recognize the phylogeny of genotypes of this cestode by constructing neighbor-joining trees.

Materials and Methods

Ethics statement

This study was confirmed by the Ethics Committee of Animal and Human Experiments of Lorestan University of Medical Science, Khorramabad, Iran (Permit Number: 89/6).

Collection of hydatid cyst samples

A total of 36 hydatid cysts including 18 hydatid cysts of sheep and 18 hydatid cysts of cattle were collected from Khorramabad slaughterhouse (Lorestan Province), Western Iran between May to September 2014 and carried to the Parasitology Laboratory at the, Lorestan University of Medical Sciences, (Khorramabad, Iran).

Microscopic examination of protoscoleces

In order to have or not the protoscoleces, the contents of hydatid cysts were subjected to the microscopic examination. The collected protoscoleces washed several times with phosphate-buffered saline (PBS). The remaining pellets were fixed in 70% ethanol and stored at 4 °C until use.

Extraction of genome DNA

To extract the genomic DNA (gDNA) of hydatid cyst samples, the fixed samples from cysts were washed by sterile-distilled water to

discard the ethanol and extracted by High Pure PCR Template preparation kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. In order to assess of the DNA extraction accuracy, concentration of the extracted DNA samples were evaluated by NanoDrop. The extracted DNAs were stored at -20°C until PCR.

Mitochondrial PCR amplification

The specific primers JB3 (5'-TTT TTT GGG CAT CCT GAG GTT TAT -3') / JB4.5 (5'-TAA AGA AAG AAC ATA ATG AAA ATG -3') were used to amplify of the mitochondrial *cox1* gene [10]. PCR was performed in a 25 µl final volume containing 1 X PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 0.2 µM of each primer and 1.5 µM AmpliTaq Polymerase. Two primers, JB3 (forward) and JB4.5 (reverse) (10), were used to amplify a 450 bp fragment of *cox1* gene under the following conditions: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 45 s at 72 °C. Final extension was done at 72 °C for 10 min. The PCR product was electrophoresed on 1.5% agar gel. The non-template water control was used as the negative control.

Sequencing analysis

Products of PCR were sequenced by means of dideoxy chain termination technique. The electropherogram of each sequence was checked by eye, and compared with each other using the software BioEdit. The sequencing in both directions was performed to confirm the sequencing data accuracy. Nucleotide sequences obtained in this research were subjected to BLAST searches (<http://www.ncbi.nlm.nih.gov/blast/>), and then aligned and analyzed with each other and *E. granulosus* reference sequences obtained from GenBank using Clustal X 1.83.

Phylogenetic analysis

All sequences were aligned via the CLUSTALW software package (www.ebi.ac.uk/clustalw) and analyzed using the Neighbor-Joining (NJ) method provided in the MUST software package. The phylogenetic tree was run by sequences obtained in present research as well as reference sequences available for *E. granulosus* G1 (accession number, KT200223.1) and G3 (accession number, HM563022.1) genotypes. Some of G1 (DQ062857.1, JX878690.1) and G3 (KT074949.1, JN604105.1, KF731907, JX854031.1) genotypes in the gene bank was used for comparison. *E. multilocularis* COX1 (KT318128.1) was used as an out-group in the model.

Results

Totally, 36 hydatid cyst isolates from domestic animals from Lorestan Province were tested for the molecular analysis. All these specimens were analyzed using mitochondrial *cox1* primers. For all of isolates, fragment of about 450 bp were successfully PCR-amplified within *cox1* gene. In sequencing analysis, the alignments of the sequences determined in this study with those of known genotypes of *E. granulosus* demonstrated all of sheep isolates belonged to genotype G1 (sheep strain). Among cattle hydatid cyst isolates, 16/18 (88.9%) were belonged to genotype G1 and 2/18 (11.1%) were belonged to G3 genotype. All the hydatid cysts belonged to G1 were confirmed to be fertile (with protoscoleces) by microscopy (Fig.1); whereas both hydatid cysts of belonged to G3 were observed to be fertile.

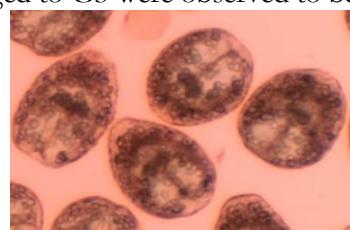


Fig. 1: Live (colorless) protoscoleces of hydatid cysts with 0.1% eosin

Nucleotide sequences obtained in present research were deposited in the GenBank database under the accession numbers LC068914.1 and LC068958.1 for G1 genotype (for both sheep and cattle) and G3 genotype (cattle) respectively. The phylogenetic-

ic analysis showed two clusters; one of the clusters includes cattle G3 genotype and the other cluster represents sheep and cattle G1 genotype (Fig. 2).

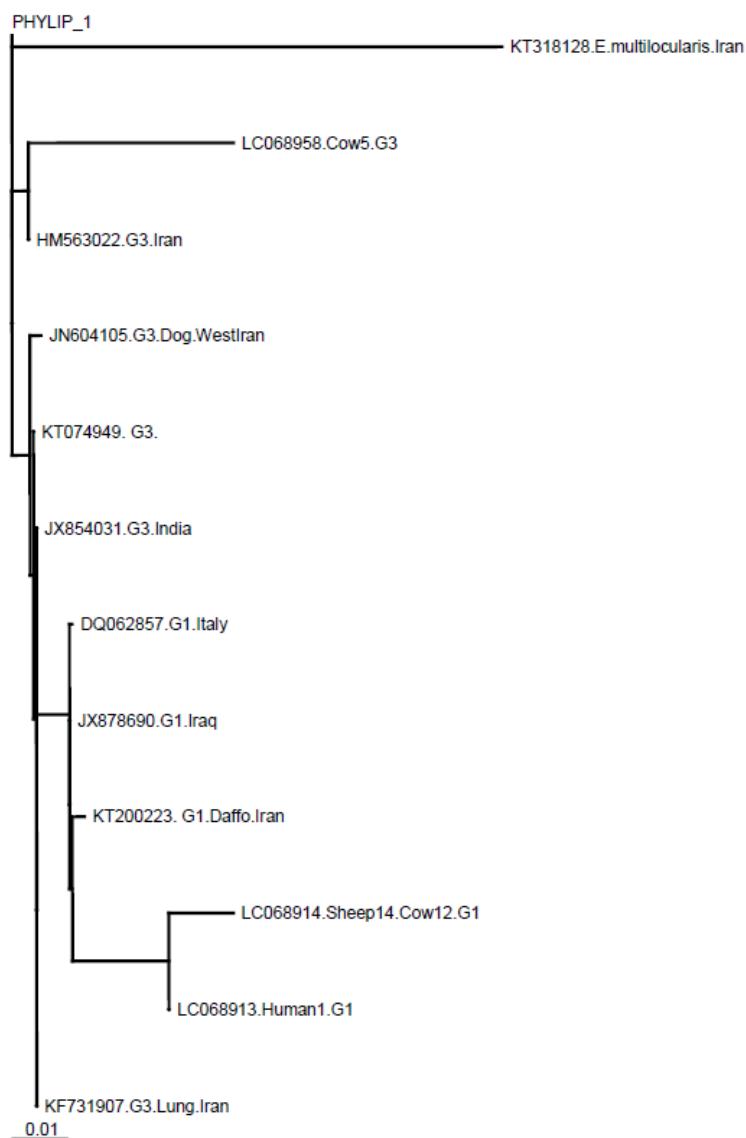


Fig. 2: Molecular phylogenetic tree of 3 *E. granulosus* isolates of sheep, cattle, and human along with reference isolates based on CO1 gene sequence. Accession numbers of KT200223 and HM563022 represent reference sequences of *Echinococcus granulosus* genotypes G1 and G3, respectively. Some of G1 (DQ062857, JX878690) and G3 (KT074949, JN604105, KF731907, JX854031) genotypes was used for comparison. *E. multilocularis* KT318128 was used as out group sequence data

Discussion

Lorestan Province due to favorable weather conditions and the spread of livestock is one of the prone provinces in Iran for prevalence of parasites [11-16]. At present, WHO categorized the hydatidosis as one of the main selected Neglected Tropical Diseases (NTDs) that must be considered among international plans for control of NTDs [1].

In the present study, 36 hydatid cysts including 18 hydatid cysts of sheep and 18 hydatid cysts of cattle were tested for the molecular analysis. Overall, 34 (94.4%) and 2 (5.6%) of isolates were identified as G1 and G3 genotype of *E. granulosus*, respectively. The outcomes obtained confirmed that G1 was the dominant genotype of CE in some livestock animals such as sheep and cattle in Western Iran. All the hydatid cysts of isolated from sheep and 16/18 (88.8%) of cattle isolates were belonged to genotype G1. The G1 genotype is able to grow fertile cysts in sheep, other than it can also infect goats, cattle, camel, pigs, and humans. We found only two isolates (3.6%) belonged to genotype G3. These isolates belonged to the cattle samples (2/18, 11.1%). The presence of G3 genotype producing fertile cysts in cattle, suggests the correlation between G3 genotype and producing fertile cysts in cattle and its high host specificity.

G1 genotype of *E. granulosus* is the main responsible genotype in animals and humans worldwide. For example, Utuk et al. [17] performed a research in Turkey on 208 isolates (179 sheep, 19 cattle, seven goats, one camel, one dog, and a single human sample) and detected only the G1 genotype. Reviews have announced a similar result with different G1 to G3 proportions: for example, 95.5 % G1 vs. 4.5 % G3 in 112 sheep and cattle in Turkey [18]; 78.75 % G1 vs. 12.5 % G3 in 80 cattle and water buffaloes in Italy [19]; 93.3 % G1 vs. 6.7 % G3 in 30 ovine, bovine, and humans in Tunisia [20]; 77.8 % G1 vs. 11.1 % G3 in 18 humans and dogs in southern Brazil [21]; and 73.7 % G1 vs. 13.2 % G3 in 38 different in-

termediate hosts in southeastern Iran [22]. Unlikely, Pednekar et al. [23] have demonstrated G3 in 63 % as the predominant genotype, whereas the G1 genotype was found only in six (13%) isolates from 46 domestic livestock in India.

In line with our findings, Yoosefi et al have demonstrated that dominant strain of *E. granulosus* in Chaharmahal-va-Bakhtyari Province, Central Iran, was G1 [24]. Other investigation conducted in Isfahan Province, demonstrated that G1 genotype was the main strain among hydatid cysts isolated from human and some livestock including cattle, sheep, goat [25]. Among 86 isolates *E. granulosus* from humans and domestic animals from Zanjan Province by the mitochondrial cox1 gene, 82 (95.35 %) isolates were G1 genotype, and the remaining 4 (4.65 %), were G3 genotype [26]. All samples isolated in definitive and intermediate hosts of the *E. granulosus* belonged to G1 genotype (sheep strain) [27]. In another study, G1, G3, and G6 genotypes were announced from human, sheep, cattle, goats, and camels in Kerman Province, southeastern Iran [22]. Sadri, et al. demonstrated that 93 isolated hydatid cysts from slaughtered livestock of Yasuj City were G1 genotype [28]. Although, Pezeshki, et al. announced the first G3 genotype in human isolates of Ardabil Province, but they also indicated that genotype G1 was the foremost strain of *E. granulosus* among hydatid cysts isolated from domestic animals of this province [29].

Based on the study conducted by Parsa et al that showed three genotypes (G1 [75%], G2 [10%] and G3 [15%]) from the *E. granulosus* isolates of 71 stray dogs, from Lorestan Province using DNA sequencing of the partial mitochondrial cox1 and nad1 genes [9], show sheep to be a main animal intermediate host for *E. granulosus* in this region.

In other studies on human and domestic herbivores of this province, the results showed G1 as the dominant strain indicating the main intermediate host and biological maintenance in the nature are sheep and goat (30, 31). In

Lorestan Province, the livestock (sheep and cattle) industry is a most important economic component. Once these domestic animals are infected with *E. granulosus*, the feeding habit raw offal of animals to dogs will easily cause *E. granulosus* infection in dogs. Therefore, *E. granulosus* can complete its life cycle in this region.

Conclusion

The common sheep strain/G1 is predominant genotype in the western part of Iran, followed by G3 genotype, circulating among the animal hosts in this area. The presence of G1 genotype as dominant sheep strain in sheep and cattle of Lorestan Province shows that these animals probably infected of the same origin. This is because of the popularity of traditional livestock in the area that cattle and sheep use from one pasture.

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Declaration of Interest

The authors report no conflicts of interest.

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