Gastroenterology and Hepatology From Bed to Bench.

REVIEW ARTICLE

Echinococcus granulosus genotypes in Iran

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ABSTRACT

Hydatidosis, caused by *Echinococcus granulosus* is one of the most important zoonotic diseases, throughout most parts of the world. Hydatidosis is endemic in Iran and responsible for approximately 1% of admission to surgical wards. There are extensive genetic variations within *E. granulosus* and 10 different genotypes (G1–G10) within this parasite have been reported. Identification of strains is important for improvement of control and prevention of the disease. No new review article presented the situation of *Echinococcus granulosus* genotypes in Iran in the recent years; therefore in this paper we reviewed the different studies regarding *Echinococcus granulosus* genotypes in Iran.

Keywords: *Echinococcus granulosus*, Hydatid cyst, Genotype, Iran.

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Introduction

Hydatid cyst caused by larval stage of *Echinococcus granulosus* is a worldwide spread zoonosis. The parasite is an important health problem and also causes economic burden in domestic animals especially in developing countries (1). Hydatid cysts are mainly located in liver or lungs and may cause pathological damages in these tissues. *Echinococcus granulosus* is widespread through many regions of Asia including Middle East countries. Cystic hydatid disease is endemic in most parts of Iran (2) and is hyperendemic in some areas (3, 4). Therefore, this disease is one of the most important zoonotic diseases prevalent in different parts of this country and responsible for approximately 1% of admission to surgical wards (5).

Previous studies revealed that extensive genetic variations exist within *E. granulosus* genus. To date, molecular analysis based on mitochondrial and nuclear genetic markers have identified ten different genotypes (G1–G10) within *E. granulosus*, including G1 and G2 as sheep strains, G3 and G5 as bovid strains, G4 and G6 as horse and camel strains, respectively, G7 as a pig strain, and G8 and G10 as cervid strains. These genotypes differ in criteria affecting host specificity, pathogenicity, life-cycle patterns of the parasite, transmission dynamics developmental

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rates, biochemistry, infectivity to humans, and sensitivity to chemotherapeutic agents (6). Since *E. granulosus* has a number of genetically distinct strains, which are known to differ morphologically and epidemiologically from each other, the identification of the strains is considered a major requirement in the control and prevention of hydatid disease (7).

The identification of *E. granulosus* strains or variants has been carried out in different laboratories using various analytical methods (morphology, physiology, biochemistry and molecular genetics), all of which have proved to be useful, particularly when used together. Therefore, using both morphological and molecular approaches together could provide more accurate and reliable information about the nature and extent of variation within *E. granulosus* (8).

Various techniques such as polymerase chain reaction based on restriction fragment length polymorphism (PCR-RFLP) were used to determine variation within *E. granulosus* (9). PCR-based methods, have been extensively used to characterize strain grouping within *E. granulosus* (10).

In Iran, molecular characterization of the *E. granulosus* strains was previously performed based on mitochondrial and nuclear DNA markers (11) using both molecular (PCR-RFLP of ITS1) and morphological analysis (12). Due to prevalence of hydatid cyst in Iran and also importance of identification of strains for improvement of control and prevention of this disease, in this paper different genotypes of hydatid cyst isolates in Iran has been reviewed.

Echinococcus granulosus genotyping using ITS1 region

In hydatid cyst investigation performed by Ahmadi et al. (2006), isolates were collected from human, sheep and camel and characterized based on protoscoleces hook morphology and PCR-RFLP. Morphological study of all isolates showed the presence of two different strains including sheep and camels strains. Rostellar hook of sheep isolates were significantly different from those of camel ones. Moreover, human isolates were found to be morphologically more similar to those isolated from sheep. Results of molecular analysis of the ITS1 region of rDNA were in agreement with the morphological results. PCR-RFLP method results revealed the sheep and human isolates related to the same genotype and the camel isolates were related to a different genotype (8).

In the study by Parsa et al. (2011), 140 hydatid cyst isolates were collected from sheep, goat and cattle from the slaughterhouse of the Lorestan province. DNA of protoscoleces was extracted and subjected to PCR-RLFP analysis using TaqI, HpaII, RsaI and AluI enzymes. The amplified PCR product for all isolates was identified as sheep strain. (1).

In another study by Buxton et al. (1995), hydatid cyst isolates were collected from human in Isfahan, Iran. By amplification of internal transcribed spacer-1 region of ribosomal DNA and RFLP using AluI and MspI enzymes, the genotypes of 30 samples were determined (13). The results of this investigation also confirmed that G1 was the dominant genotype of hydatid cyst extracted from different organs including liver, lung, and brain in Isfahan (6).

In another study performed by Yousofi Darani et al. (2008) 30 sheep hydatid cysts samples were collected from Chaharmahal va Bakhtiari province. DNA was extracted from preserved protoscoleces and nested PCR was performed on the DNA samples. The rDNA-ITS fragment was amplified and the products were digested by four enzymes including, Taq1, HpaII, Rsa1and Alu1. The authors confirmed the presence of sheep strain in Chaharmahal va Bakhtiari (14). According to the results of this investigation human hydatid cyst strain in this province was different from sheep ones (14).

On the other hand, Dousti et al. (2013) collected 30 animal and four human hydatid cysts from different slaughterhouses and hospitals of the Ilam province. DNA genome of protoscoleces was extracted and rDNA-ITS1 of each isolated samples was amplified. PCR products were then subjected to RLFP-PCR using TaqI, HpaII, RsaI and AluI restriction enzymes. According to the results of this investigation, genotypes G1 and G3 were present in Ilam province (15).

***Echinococcus granulosus* genotyping using mitochondrial genes**

Sharbatkhori et al. (2011) collected 19 camel hydatid cyst isolates from central Iran. The isolates were then subjected to sequence analysis of NADH dehydrogenase subunit 1 (*nad1*) and mitochondrial cytochrome c oxidase subunit 1 (*cox1*) genes. In these camel isolates five different sequences in cox1 and nine in nad1 genes were recognized. The results of sequence analysis revealed that the isolates belonged to G1, G3, and G6 genotypes and G3 (buffalo strain) of hydatid cyst was the dominant genotype in camels (16).

In Rostami-Nejad et al. (2008) investigation, thirty livers and lungs isolates of cattle, sheep and goats naturally infected with *hydatid cyst were* collected from abattoirs in northern and western Iran and characterized using DNA sequences of the mitochondrial 12S rRNA gene. Two new primer pairs that specifically amplify portions of the mitochondrial 12S rRNA gene of the two strains (G1 and G6) of *hydatid cyst* were used. One primer pair amplified a fragment of 259 base pairs (bp) from only the G1 strain. The second pair amplified a fragment of 676 bp from the G6 strain. The results of this study showed presence of G1 genotype in whole samples (17).

Karimi et al. (2008) used molecular and morphological analyses to study phenotypic and genotypic characteristics of two common sheep and camel isolates of hydadtid cyst in Fars province. According to the morphology of hooks and PCR-RFLP results G1 and G6 strains were identified (18).

On the other side, Pour et al. (2011) collected 25 isolates of hydatid cyst protoscoleces from 25 buffaloes in five different provinces of Iran. DNA was extracted and amplified using specific primers derived from *cox1* gene and then the samples were sequenced. Twenty-three isolates were identified as G1 and two isolates were identified as G3 genotype (19).

In an investigation performed by Parsa et al. (2012), *E. granulosus* adult worms were collected from 71 dogs from western Iran. The samples were then genetically characterized using *cox1* and *nad1* genes. Three genotypes including G1 (75%), G2 (10%) and G3 (15%) were identified from the isolates (20).

In another study by Rajabloo et al. (2012), 20 isolates of goat were characterised by mitochondrial DNA sequencing and morphology of the hooks. The mitochondrial cytochrome oxidase 1 sequences were tested, and the sequence analysis indicated two genotypes G1 and G6 within the isolates. The results of the morphological studies were in agreement with of the molecular results. Type 1 hooks were morphologically similar to sheep strains, whereas the morphology of the hooks in type 2 was similar to those in the camel strain (21).

Rostami et al. (2013) collected 218 *E. granulosus* isolates from sheep, cattle, and camel from different parts of Iran. PCR coupled with high-resolution melting curve (HRM) were used for discriminating common genotypes of hydatid cyst in these samples. According to the results of this investigation the isolates were categorized as G1, G3 and G6 for sheep, cattle, and camel, respectively. HRM results were completely compatible with the results of sequencing and rostellar hook measurement (22).

Table 1. Different genotypes of *Echinococcus granulosus* in Iran

Parasite stage	Source of isolate	Sample tested	Fragment	Method applied	Reported genotype	Area
Protoscoleces	human, sheep and camel	Lung and Liver	ITS1	PCR-RFLP	G1, G6	Whole country
Protoscoleces	sheep, goat and cattle	Lung and Liver	ITS1	PCR-RLFP	G6	Lorestan
Protoscoleces	Human	Lung,Liver and brain	ITS1	PCR-RLFP	G1	Isfahan
Protoscoleces	camel	liver, lung	*cox1* and *nad1*	PCR-sequencing	G1-G3-G6	Isfahan
fertile cysts	cattle, sheep and goats	Lung and Liver	*12S rRNA*	PCR-sequencing	G1 and G6	northern and western Iran
morphology of protoscoleces	sheep and camel	liver, lung	*nad1*	PCR-RLFP	G1 and G6	Fars
Protoscoleces	sheep	Lung and Liver	ITS1	PCR-RFLP	G1	Chaharmahal va Bakhtiari
Protoscoleces	buffaloes	Lung and Liver	mitochondrial genes	PCR	G1 (G1α, G1β, G1γ and G1δ), and G3	Northwest, North and Southwest
adult worms	dog	intestines of dogs	*cox1*and *nad1*	PCR	G1, G2, G3	Lorestan
Protoscoleces	goat	Lung and Liver	mitochondrial genes	PCR-sequencing	G1, G6	Isfahan
Protoscoleces	sheep, cattle, camel	Lung and Liver	*cox1*	HRM& real-time PCR	G1, G3, and G6	different parts of Iran
Protoscoleces	human and sheep and cow		ITS1	PCR-RFLP	G1,G3	Ilam
Protoscoleces	Cattle, sheep and goats	Lung and Liver	*atp6*	PCR-sequencing	G1	Varamin
protoscoleces and/ or germinal layers	sheep, goat and cattle		*cox1*	Real Time PCR	G1, G3 and G6	Isfahan
protoscoleces	human sheep, camel, cattle and goat	Lung and Liver	ITS1	PCR-RFLP	G1and G6	Isfahan
Protoscoleces	human and sheep	Lung and Liver	ITS1	Nested PCR PCR-RFLP	G5	Chaharmahal va Bakhtiari

In another investigation by Pestechian et al. (2013), 71 hydatid cysts samples were collected from infected sheep, goat and cattle slaughtered in Fasaran, Khomeinishahr and Najafabad in Isfahan during 2013. For each sample DNA was extracted from protoscoleces and/or germinal layers and *cox1* gene (420 bp) was amplified using real time PCR. Overall, in 66 isolates the partial sequences of *cox1* gene of *E. granulosus* strains showed the presence of genotypes G1, G3 and G6 in 74.24, 22.72 and 3.03 percent of the collected samples respectively (23).

Shahnazi et al. (2011) collected hydatid cysts from the liver and lungs of patients and also from domestic animals. DNA was extracted from the protoscoleces and rDNA internal transcribed spacer1 (ITS1) segment examined using PCR and PCR-RFLP. In addition, fragments of the genes

(*cox1*) and (*nad1*) were sequenced. Based on results of this work two different strains/genotypes (sheep and camel) were identified. It was shown that the sheep strain was the most common genotype of *E. granulosus* affecting humans, sheep, cattle and goats (24). Also, about 35% camel samples were infected with sheep strain. Moreover, the camel genotype was observed in cattle, human and camels strains. About 65% of camel isolates, 19% of human and 36% of cattle samples were infected with the camel genotype. A PCR and RFLP-PCR pattern of camel genotype was different from the pattern of other isolates. According to the results of this investigation it seems that the 'camel' strain was a source of human infection (24).

Research studies about *E. granulosus* genotypes in Iran, which had been published by the end of the year 2013, have been summarized in Table 1. Also the genotypes isolated from human, cattle, sheep or camel hosts have been presented in Table 2.

Table 2. *Echinococcus granulosus* genotypes in Iran in different hosts

Host	Genotypes
Human	G1, G3
camel	G1-G3-G6
sheep	G1 , G6 , G3, Sheep strain
buffaloes	G1 (G1α, G1β, G1γ and G1δ), and G3
dog	G1 , G2 , G3
goat	G1 , G3,G6, Sheep strain
cattle	G1, G3, and G6

Conclusion

Echinococcosis/hydatidosis is one of the most important zoonotic diseases prevalent in different parts of Iran (5). Identification of strains is important for improvement of control and prevention of disease (25). In this article the situation of hydatid cyst genotypes have been reviewed. Based on studies performed in different regions of Iran, presence of G1 (6, 16-26), G2 (21), G3 (16, 20-22, 25) and G6 (16-18, 21-23) genotypes were reported.

Molecular studies using mitochondrial DNA sequences have identified 10 different genotypes (G1—10) within hydatid cyst in different parts of the world (27, 28). In China it has been indicated that the common sheep strain is the most predominant in the northwest region of this country (29, 30).

In Kenya, hydatid cyst is hyperendemic between two pastoral communities; the Turkana in the northwest and the Massai in the southwest. Molecular studies indicated the presence of two strains (sheep and camel) in this country. Also, it has been shown that the camel strain appeared restricted to the Turkana region, where camels are kept as livestock. Intermediate hosts for both strains appeared to be the same (sheep, cattle and camel) except that in human cases the camel strain was not isolated (31).

Hydatid cyst is also a major public health problem in Argentina and many human cases have been reported (32). Molecular studies demonstrated the presence of several genotypes including sheep strain (G1) in sheep and human, Tasmanian strain (G2) in sheep and humans, the pig strain (G7) in pigs, and the camel strain (G6) in humans (33).

In Nepal, where hydatid cyst is a significant public health and environmental problem, three strains including sheep (G1), cattle (G5) and camel (G6) have been reported from buffalo, sheep, goat and human hosts (34).

In comparison of hydatid cyst genotypes in Iran with other countries, 4 strains have been reported from Iran whereas in china, Kenya, Nepal and Argentina genotypes 2, 2, 3 and 4 have been reported respectively. Therefore, it is obvious that Iran is a country, which contains more variation of these parasite genotypes.

In conclusion, it should be emphasized that hydatid cyst exists with genotype variation in Iran and the majority of *E. granulosus* infected

domestic animals can potentially act as reservoirs of human infection. Therefore, this diversity should be considered in prevention programs.

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