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Comparative Genotyping of *Echinococcus granulosus* Infecting Livestock in Turkey and Iran

Türkiye ve İran'da Çiftlik Hayvanlarına Bulaşan Echinococcus granulosus'un Karşılaştırmalı Genotiplenmesi

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ABSTRACT

Objective: *Echinococcus granulosus* contains a complex of different strains that represent diversity in the pattern of the life cycle and also their host types. So far 10 genotypes of this parasite have been identified, using molecular methods. The current study aimed to evaluate and compare the genotypic diversity of *E. granulosus* metacestodes from livestock of Turkey and Iran.

Methods: A total of 90 livestock liver and lung organs infected with hydatid cyst from industrial slaughterhouses of Bonab Province in the East Azerbaijan Province in Iran (60 samples, including 30 sheep and 30 cattle) and Van Province in Turkey (30 samples, including 15 sheep and 15 cattle) were collected. DNA was extracted from the protoscolices or germinal layers and polymerase chain reaction (PCR) were utilized, targeting the partial mitochondrial cytochrome *c oxidase subunit 1 (cox1)* and *NADH dehydrogenase 1 (nad1)* genes. PCR products were isolated from the electrophoresis gels and sequenced. The sequences were compared with each other, as well as with those related available sequences in the GenBank, using the BioEdit software and the BLAST algorithm. Finally, the phylogenetic trees were constructed by comparing sequences of cox1 and nad1 fragments, using the MEGA7 software and the maximum likelihood method.

Results: All samples sequenced from Iran corresponded to the genotype G1 (100%). Among the samples from Turkey, 15 samples (78.9%) were identified as G1 while only one sample (5.3%) corresponded to the genotype G3 and 3 isolates (15.8%) were defined as genotypes G1/G3. Five distinct haplotypes were determined within the examined isolates from sheep and cattle in both countries and all isolates clustered in one group. Phylogenetic analysis revealed that the intra-species genetic variations were 0.0-0.6% and 0.0-1.4% for cox1 and nad1, respectively.

Conclusion: The dominant genotype of *E. granulosus* sensu stricto of livestock in both countries was the G1 (sheep strain) genotype. Our findings indicate that the sheep-dog cycle is the leading cycle of *E. granulosus* in these two areas. Hence, adopting regional common policies and bilateral cooperation helps to control the disease in livestock as well as in human in these two regions. Further study is required to compare the genetic diversity of human isolates of *E. granulosus* in these two countries.

Keywords: Hydatid cyst, livestock, genotypes, Turkey, Iran

ÖZ

Amaç: *Echinococcus granulosus*, yaşam döngüsü paterni ve konak tiplerine göre çeşitlilik gösteren farklı suşlara sahiptir. Şimdiye kadar bu parazitin 10 genotipi, moleküler yöntemler kullanılarak tespit edilmiştir. Bu çalışmada, Türkiye ve İran'daki hayvanlarda *E. granulosus* metasestodlarının genotipik çeşitliliğinin değerlendirilmesi ve karşılaştırılması amaçlanmıştır.

Yöntemler: İran'ın Doğu Azerbaycan eyaletindeki Bonab şehrindeki (30 koyun ve 30 sığır dahil olmak üzere 60 örnek) ve Türkiye'nin Van şehrindeki (15 koyun ve 15 sığır dahil olmak üzere 30 örnek) endüstriyel kesimhanelerinden hidatik kist ile enfekte

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Türkiye PARAZİTOLOJİ Dergisi

toplam 90 hayvan karaciğeri ve akciğeri toplanmıştır. Protoskolekslerden veya germinal tabakalardan DNA çıkarıldı ve kısmi mitokondriyal sitokrom *C oksidaz subunit 1 (cox1)* ve *NADH dehidrojenaz 1 (nad1)* genlerini hedef alan polimeraz zincir reaksiyonu (PCR) uygulandı. PCR ürünleri elektroforez jellerinden izole edildi ve dizilendi. Diziler, BioEdit yazılımı ve BLAST algoritması kullanılarak Genbank'taki mevcut dizilerin yanı sıra birbirleriyle karşılaştırıldı. Son olarak, filogenetik ağaçlar, Mega 7 yazılımı ve maksimum olasılık yöntemi kullanılarak cox1 ve nad1 parçalarının dizilerini karşılaştırarak inşa edildi.

Bulgular: İran'dan alınan tüm örneklerde (%100) G1 genotipi tespit edildi. Türkiye'den alınan örneklerin 15'inde (%78,9) G1 genotipi, birinde (%5,3) G3 genotipi ve üçünde (%15,8) G1/G3 genotipi tespit edildi. Her iki ülkede de incelenen koyun ve sığır izolatlarında beş farklı haplotip belirlendi ve tüm izolatlar bir grupta kümelendi. Filogenetik analiz, tür içi genetik varyasyonların sırasıyla cox1 ve nad1 için %0,0-0,6 ve %0,0-1,4 olduğunu ortaya koydu.

Sonuç: Her iki ülkedeki hayvanlarda en sık saptanan *E. granulosus* genotipi G1 genotipi (koyun suşu) idi. Bulgularımız, koyun-köpek döngüsünün bu iki bölgede *E. granulosus*'un önde gelen döngüsü olduğunu göstermektedir. Bu nedenle, bölgesel ortak politikaların ve ikili işbirliğinin benimsenmesi, bu iki bölgede hayvanlarda olduğu kadar insanlarda da hastalığın kontrol edilmesine yardımcı olacaktır. Bu iki ülkede, *E. granulosus*'un insan izolatlarının genetik çeşitliliğini karşılaştırmak için daha fazla çalışma gereklidir.

Anahtar Kelimeler: Kist hidatik, hayvan, genotip, Türkiye, İran

INTRODUCTION

Cystic echinococcosis (CE) is one of the most important zoonotic parasitic diseases which is caused by the larval stage of *Echinococcus granulosus* (1). The adult form of this parasite lives in the intestinal tract of canidae as the definite hosts, and humans and herbivores act as intermediate hosts. The intermediate hosts become infected through ingestion of food contaminated with the eggs of these helminthes, passed in the dog's feces (2). Apart from the great morbidity and mortality of the disease in humans, the disease causes significant economic losses in the livestock (3).

E. granulosus contains a complex of different strains that represent diversity in the pattern of the life cycle and their host types. Up to now, 10 genotypes of this parasite have been identified, using molecular methods and in particular the sequencing of mitochondrial DNA (mtDNA) (4). *E. granulosus* has recently been classified in four main groups: sensu stricto (G1-G3 genotypes), *equinus* (G4), *ortleppi* (G5), and *canadensis* (G6-G10) (5). *Echinococcus felidis*, isolated from South African lions, are classified in a separate group (6). Apart from the G4 genotype, all other strains of *E. granulosus* have been identified as the cause of human CE. The genotypes G1 and G3 are the most common genotypes identified in livestock and human all over the world (7,8).

The infection has been reported from all of the Middle Eastern countries and in the meantime, Iran and Turkey are considered as hyper-endemic areas for human CE (9-11). About 1% of the surgeries performed in medical centers of Iran are due to hydatid cyst (12). Studies which have been conducted in different areas of Iran reported the seroprevalence rate of 1.2 to 21.4% for human CE and a prevalence of 1.7 to 70% for hydatid cyst among livestock (13). The main transmission pattern of the disease in Iran is involving dogs and sheep, whereas animals such as goats, cattle, wild boars, and camels are also contributing to different degrees to the life cycle of the parasite (14,15).

Both Turkey and Iran are located in the hyperendemic region of CE, and the disease is widespread in these two countries. There are some reports on the genotyping of *E. granulosus* in various intermediate hosts including humans in different geographical regions of Turkey and Iran. Utuk et al. (16) characterized different isolates of *E. granulosus* in East and Southeast regions of Turkey, using polymerase chain reaction-restriction fragment length polymorphism analysis of ribosomal ITS1 fragment and DNA sequencing of *cox1* gene. They came to the conclusion that the predominant genotype of *E. granulosus* in Turkey is the common sheep strain (G1 genotype) which is able to infect humans, cattle, sheep, goats, camels as well as the dog as the definitive host. In another molecular study in Turkey, Eryıldız et al. (17) after collecting 58 *E. granulosus* isolates from humans and animals in the province of Edirne, they used ITS1 fragments and *nad1* genes for characterization and DNA sequencing of *cox1* and *nad1* genes for genotyping of human and animal *E. granulosus* isolates. Their study indicated only two genotypes: G1 (sheep strain) and G7 (pig strain) with a predominance G1 strain. Based on their sequence analysis, they identified eight haplotypes of *Echinococcus* species in their study.

The prevalence of infection in cattle and sheep in Turkey has been reported to be 39.7% and 58.6%, respectively (18,19). During the 2001-2005, about 14.789 human cases of hydatidosis have been recorded by the Ministry of Health and Hospitals in Turkey (17). Eastern regions of Turkey are considered as a high-risk area for CE (20). In a study in Kars's slaughterhouse, an eastern province in Turkey in the neighborhood of Iran, the rate of infection with hydatid cysts was found to be 31.25%, 63.85% and 25.11% in cattle, sheep, and goats, respectively (21).

To integrate and incorporate information related to morphological taxonomy, molecular genetics, and evolutionary ecology of *E. granulosus*, the knowledge and a better understanding of biodiversity among different genotypes of this parasite are needed. Determination of the dominant genotypes of the parasite in different regions of the world would be necessary for providing an appropriate and effective prevention and controlling measurements (22).

Considering the fact that import and export of livestock have recently been increased between borders of two neighboring countries, Turkey and Iran, as two main foci of both human and animal CE in the Middle East, and given that there has not been a comparative genotyping study of *E. granulosus* in these regions, the current study aimed to find out and compare the genotypic diversity of *E. granulosus* of livestock in two neighboring areas, Van Province from Turkey and East Azerbaijan Province from Iran.

METHODS

Study Area

The study was conducted in two regions from two countries with almost similar climatic conditions; Van province from Turkey located in the east of Van Lake which is a part of the coldest region in Turkey, and East Azerbaijan province as a cold area located on the Sahand Mountain range of Iran in the southeast of Urmia Lake (Figure 1).

East Azerbaijan is located in Iranian Azerbaijan, bordering with Armenia and Republic of Azerbaijan with the geographical coordinates of 38° 28' 45.1020'' N and 47° 3' 50.9040'' Bonab city

is located in the Azerbaijan region. Because of its extensive and large pastures, its livestock numbers are significant compared to other cities in the province. It has a large industrial slaughterhouse and high daily intake capacity, which plays an important role in providing meat and livestock products of the region and also the country.

Van is one of the eastern provinces of Turkey located in neighboring Iran at latitude 38° 29' 40 N, longitude of 43° 22' 59 E and altitude of 1.725 meters in Turkey. Van has a harsh continental climate with cold, snowy winters and warm, dry summers. Rainfall occurs mostly during the spring and autumn. Because of Van Lake, the climate of this city can be changed between terrestrial and Mediterranean climate of Central Anatolia and Southeast Anatolia regions (23). Therefore, like the region introduced in Iran, it has similar climate conditions and, is an active and leading province in livestock breeding and production of livestock products in Turkey. A recently described rare sheep breed, Norduz, is mainly raised in a region of the same name in Gürpınar County of Van province (24). The study was approved by the Research Ethics Committee of Shiraz University of Medical Sciences (SUMS, Iran).

Sample Preparation

A total of 90 livestock liver and lung organs infected with hydatid cyst from two areas; Van city of Turkey (30 samples, including 15 sheep and 15 cattle) and Bonab city in East Azerbaijan Province of Iran (60 samples, including 30 sheep and 30 cattle) were obtained. The samples were collected from industrial slaughterhouses of Bonab and Van cities. Protoscolices (PSCs) were collected from the hydatid cyst fluid and after 3 time washes with phosphate buffered saline; the precipitated PSCs were frozen. Also, germinal layers of the cyst were carefully released from the outer host capsules, and were stored at -20 °C until use.

Extraction of Genomic DNA from Isolates

The genomic DNA from either germinal layers or PSCs were extracted, using a DNA extraction kit (YTA, Yekta Tajhiz Azma, Iran), based on the manufactures instructions and also modifications, previously introduced by the authors (25).

Polymerase Chain Reaction and Gel Electrophoresis

For all 90 samples collected from these two countries, polymerase chain reaction (PCR) was performed targeting a 450 bp and 550 bp fragments of *cox1* and *nad1* of the mitochondrial DNA respectively, using appropriate primers (26,27). The characteristics of the primers used and the genomic regions of the targets are presented in Table 1.

The cycling parameters for the amplification of both genomic pieces was: 1x (5′, 95 °C)+ 40x (45″, 94 °C+35″ 51 °C+45″ 72 °C)+ 1x (10′, 72 °C).

Table 1. The specific primers for amplification of *cox1* and *nad1* fragments

Genome	Primers	Sequences
cox1	JB3 (F)	5'-TTT TTT GGG CAT CCT GAG GTT TAT-3'
	JB4.5 (R)	5'-TAA AGA AAG AAC ATA ATG AAA ATG-3'
nad1	JB11 (F)	5'-AGATTCGTAAGGGGCCTAATA-3'
	JB12 (R)	5'-ACCACTAACTAATTCACTTTC-3'

PCR products were separated on a 1.5% agarose gel, and the obtained bands were visualized and recorded by a ultraviolet detector (Bio-Rad, USA).

DNA Sequencing

Of the total 90 available PCR products, 49 samples including 19 samples from Turkey (10 sheep and 9 cattle) and 30 samples from Iran (15 samples from each animal) were selected in terms of the quality of the resulting band on the electrophoresis gel and purified from the gel by EasyPure Quick Gel Extraction Kit (TRANS, TransGen Biotech, South Korea), based on the manufacturer's instructions. The purified products were sequenced for both *cox1* and *nad1* fragments from both directions using the same primers which were used in the PCR.

Phylogenetic Analysis

The sequences of *E. granulosus* isolates from both countries were aligned and compared, using BioEdit and also the BLAST program. Moreover, the obtained sequences were compared with those of available related sequences in the GenBank. Maximum likelihood tree was constructed based on the Tamura-Nei model, using the MEGA 7.0 software. *Taenia solium* (accession no: AB086256) was used as the out-group.

RESULTS

All gDNA isolates from collected 90 hydatid cysts from two countries were subjected to molecular analysis targeting both *cox1* and *nad1* genomic fragments and the resulting PCR product showed replication of the target genes. Figure 2 shows the PCR products of *cox1* and *nad1* genes in a few of the evaluated samples.

From all 90 evaluated samples, 49 of them with the highest quality in the resulting band on the electrophoresis gel were selected and sequenced, and the resulting sequences were deposited in the GenBank database with accession numbers which are shown in Table 2. All of the 30 samples (100%) from Iran were found to be the genotype G1 strain. Among the samples from Turkey, 15 samples (78.9%) were identified as G1 and only one sample (5.3%) corresponded to the genotype G3 strain.

Moreover, two samples had not any homologous to the related sequences in the GenBank, and one sample had similarity to the Echinococcus granulosus from Armenia (KX020349). Therefore, these three samples from Turkey were considered as genotypes G1/G3 strain. All isolates of sheep and cattle from both countries clustered in one group within 5 different haplotypes.

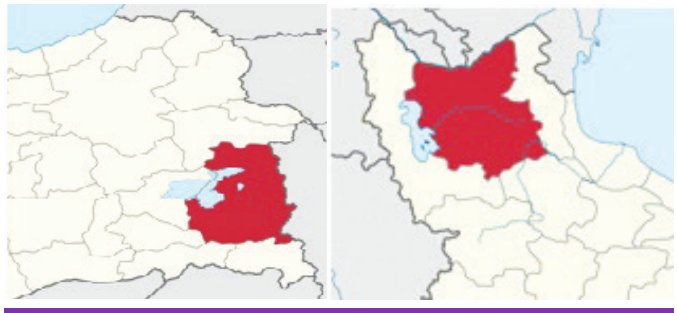

Figure 1. Geographic regions of Turkey (left) and Iran (right) where hydatid cyst samples were collected (red regions)

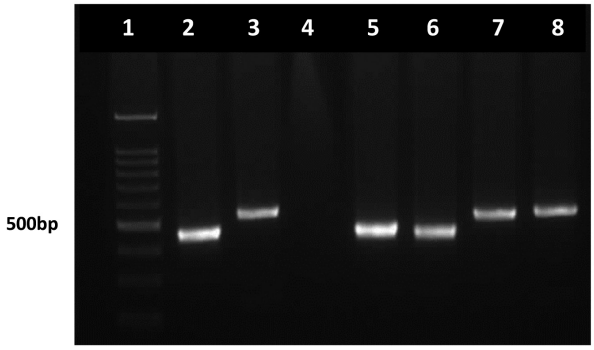

Figure 2. Electrophoresis of PCR products, using JB3 and JB4, 5 primers for *cox1* and JB11 and JB12 for *nad1*, on 1.5% agarose gel. Lane 1: Molecular weight marker; Lane 2: Positive control for *cox1*, DNA extracted from sheep isolate of Iran; Lane 3: Positive control for *nad1*, DNA extracted from sheep isolate of Iran; Lane 4: Negative control; Lanes 5, 6: Sheep isolates of Iran and Turkey in the current study, targeting the *cox1* gene; Lanes 7, 8: Sheep isolates of Iran and Turkey in the current study, targeting the *nad1* gene

PCR: Polymerase chain reaction

Table 2. Host origin of *Echinococcus granulosus* isolates from Turkey and Iran livestock and accession numbers deposited in GenBank, using *cox1* and *nad1* genomes

Sample no.	Code	Host	Origin	Accession no. (*cox1*)	Accession no. (*nad1*)
1	1SI	Sheep	Iran	MH542362	MH557949
2	2SI	Sheep	Iran	MH542363	MH557965
3	3SI	Sheep	Iran	MH542364	MH557950
4	4SI	Sheep	Iran	MH542365	MH557951
5	5SI	Sheep	Iran	MH542366	MH557966
6	6SI	Sheep	Iran	MH542367	MH557967
7	7SI	Sheep	Iran	MH542368	MH557952
8	8SI	Sheep	Iran	MH542369	MH557968
9	9SI	Sheep	Iran	MH542370	MH557969
10	10SI	Sheep	Iran	MH542371	MH557953
11	11SI	Sheep	Iran	MH542372	MH557954
12	12SI	Sheep	Iran	MH542373	MH557970
13	13SI	Sheep	Iran	MH542374	MH557971
14	14SI	Sheep	Iran	MH542375	MH557955
15	15SI	Sheep	Iran	MH542376	-
16	16CI	Cattle	Iran	MH542377	MH557972
17	17CI	Cattle	Iran	MH542378	MH557956
18	18CI	Cattle	Iran	MH542379	MH557973
19	19CI	Cattle	Iran	MH542380	MH557957
20	20CI	Cattle	Iran	MH542381	MH557958
21	21CI	Cattle	Iran	MH542382	-
22	22CI	Cattle	Iran	MH542383	MH557959
23	23CI	Cattle	Iran	MH542384	MH557960
24	24CI	Cattle	Iran	MH542385	-
25	25CI	Cattle	Iran	MH542386	MH557961
26	26CI	Cattle	Iran	MH542387	-
27	27CI	Cattle	Iran	MH542388	-
28	28CI	Cattle	Iran	MH542389	MH557962
29	29CI	Cattle	Iran	MH542390	MH557963
30	30CI	Cattle	Iran	MH542391	MH557964
31	1ST	Sheep	Turkey	MH542392	-
32	2ST	Sheep	Turkey	MH542393	-
33	3ST	Sheep	Turkey	MH542394	-
34	4ST	Sheep	Turkey	MH542395	---
35	5ST	Sheep	Turkey	MH542396	---
36	6ST	Sheep	Turkey	MH542397	---
37	7ST	Sheep	Turkey	MH542398	---
38	8ST	Sheep	Turkey	MH542399	---
39	9ST	Sheep	Turkey	MH542400	---
40	10ST	Sheep	Turkey	MH542401	---
41	11CT	Cattle	Turkey	MH542402	---
42	12CT	Cattle	Turkey	MH542403	---
43	14CT	Cattle	Turkey	MH542404	---
44	15CT	Cattle	Turkey	MH542405	---
45	16CT	Cattle	Turkey	MH542406	---
46	17CT	Cattle	Turkey	MH542407	---
47	18CT	Cattle	Turkey	MH542408	---
48	19CT	Cattle	Turkey	MH542409	---
49	20CT	Cattle	Turkey	MH542410	---

Forty-four samples from both countries were homologous to the *E. granulosus* sensu stricto G1 from Turkey (MF544127) and one sample from Iran was homologous to the *E. granulosus* G1 from Argentina (MG672258) described earlier. The third haplotype in our study was the only sample from Turkey (MH542404) which had similarity with the *E. granulosus* sensu stricto G3 from Turkey (MG682536) described earlier. Three of our samples from Turkey (MH542399, MH542406, and MH542395) were placed in two separate haplotypes compared to the rest of the samples (Table 3 and Figure 3, 4). The *nad1* sequences were not available for these three isolates and if available, they could be useful in the phylogenetic analyses.

Phylogenetic analysis of the sequences of two *cox1* and *nad1* genes and alignment of the sequences with available related sequences in the GenBank revealed that the intra-species genetic variation were 0.0-0.6% and 0.0-1.4% for *cox1* and *nad1*, respectively, while the polymorphism variation between the isolates or in other words, the isolates sharing the same haplotype was 0.0 (Figure 5, 6).

DISCUSSION

The Middle East countries have long been considered as important foci of both human and animal CE. The metacestodes of *E. granulosus* has been reported in almost all countries of the region, but its prevalence is higher in Iran, Turkey, and Iraq in comparison with the rest of the countries in the region (28).

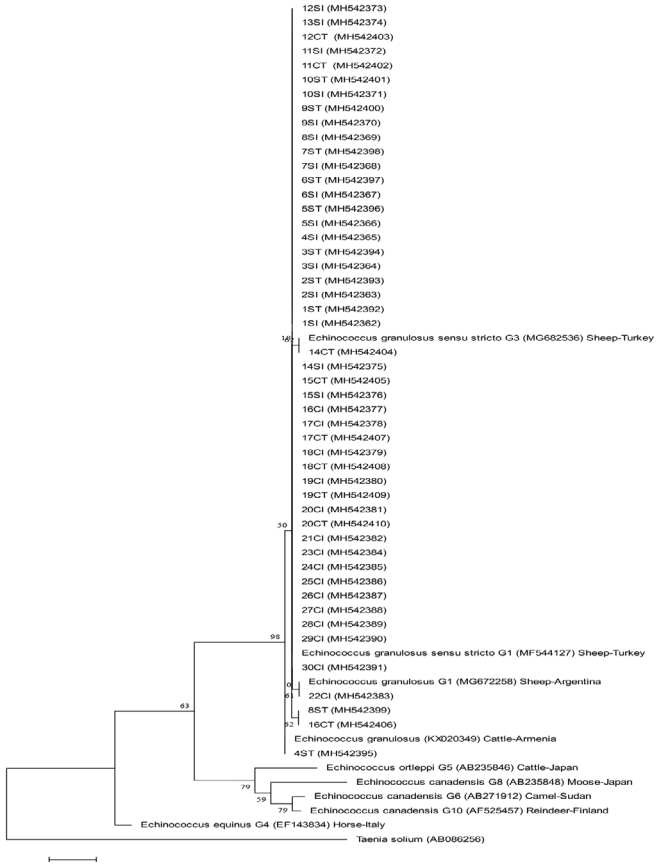

Figure 3. Phylogenetic tree of representative sequences of *Echinococcus granulosus* from Iran and Turkey and reference sequences of other genotypes, using the maximum likelihood method based on *cox1* gene. *Taenia solium* (AB086256) was used as the out-group sequence data

Intra-species genetic: 0-0.6%

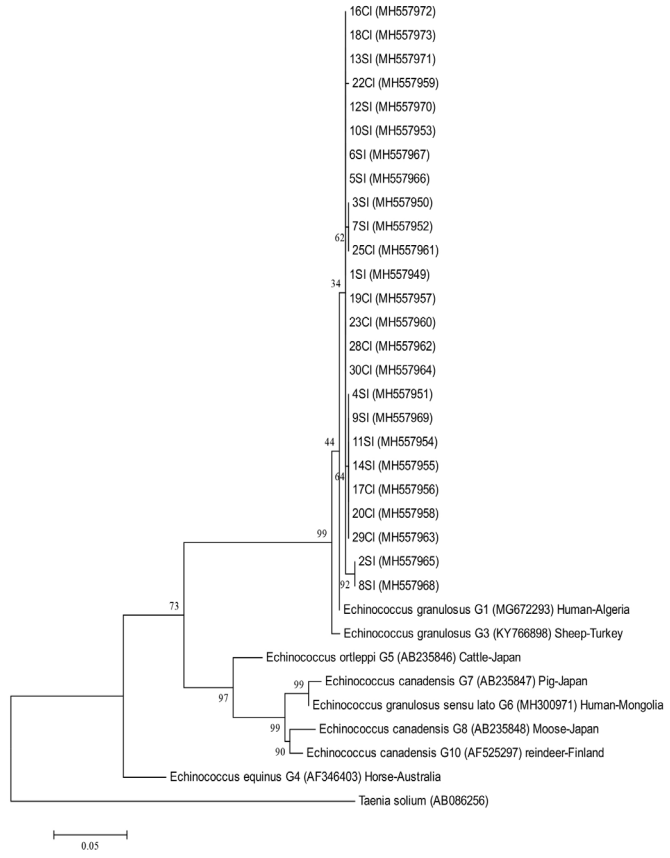

Figure 4. Phylogenetic tree of representative sequences of *Echinococcus granulosus* from Iran and reference sequences of other genotypes, using the maximum likelihood method based on *nad1* gene. *Taenia solium* (AB086256) was used as the out-group sequence data

Intra-species genetic: 0-1.4%

Table 3. *Echinococcus granulosus* haplotypes and genotypes detected in this study using *cox1* and *nad1* sequences

Haplotypes	Number	Origin/Host				Genotype	Homologous to
		Iran		Turkey			
		Sheep	Cattle	Sheep	Cattle		
1	44	15	14	8	7	G1	MF544127
2	1	-	1	-	-	G1	MG672258
3	1	-	-	-	1	G3	MG6822536
4	2	-	-	1	1	G1/G3	-
5	1	-	-	1	-	G1/G3	KX020349

Various mitochondrial and nuclei genomes have been used for molecular evaluation and to determine the genotype of *E. granulosus*. Regarding phylogenetic taxonomy of *E. granulosus* among closely related species, mtDNA has been reported more efficient than nuclear genomes due to the rapid sequence evolution and large datasets derived from mitochondrial genomes (29). The mitochondrial genes; including *cox1*, *nad1*, and *atp6*, as well as the fragment of the 12S rRNA gene have been used to identify the genotypes in different isolates. Findings of Rostami Nejad et al. (30) study on genetic diversity of *E. granulosus* in different hosts, revealed G1 and G6 genotypes in cattle, camels, sheep, buffalo and goats in different geographic areas of Iran. Likewise, the *internal transcribed spacer* (*ITS1*) gene region has also been utilized for genotypic analysis of this parasite (31). In two separate studies, Ahmadi and Dalimi (32) and Harandi et al. (33) used *ITS1* region gene to genotype the *E. granulosus* isolates. They found a similarity between strains in sheep and camel with cattle and humans.

However, *cox1* and *nad1* mitochondria genes, have been considered as the main and the best options for molecular characterization of CE. For distinction of intra- and interspecific variants, the gene *cox1* gene, can be used as a significant evolutionary marker (34).

Mahami-Oskouei et al. (35) used *cox1* and *nad1* genes to investigate the novel single-nucleotide polymorphism and reported that the G1 genotype with 27 haplotypes was the main strain in human, sheep, goat, cattle and dog isolates. Their study showed that cross transmission of sheep-dog strain is circulating among potential intermediate/definitive hosts with heterogeneity traits of *Echinococcus* in Iran and Turkey.

In the present study, we selected both *cox1* and *nad1* genomic fragments as the target and the resulting PCR product showed successful replication of the target genes. Recently, it has been reported that the differentiation between G1 and G3 genotypes for some cases is not possible and the identified genotypes has been reported as G1/G3 strain (36). In a recent study, carried out by Kinkar et al. (8), *nad5* fragment has been introduced for proper differentiation of *E. granulosus* sensu stricto genotypes G1 and G3.

Findings of the current study demonstrated the G1 strain as the dominant strain of *E. granulosus* in the livestock of the two studied regions; Azerbaijan from Iran and Van from Turkey. Only one case of G3 strain and 3 cases of G1/G3 strains were found in this study.

In general, *E. granulosus* sensu stricto (G1-G3) are the predominant strains in CE cases throughout the world (7,8). Findings of the current study are in accordance with other reports from Iran, Turkey, and also the Middle East countries. In some studies, conducted in different geographical areas of Iran, the G1 strain of *E. granulosus* was reported as the dominance genotype in intermediate hosts including cattle, sheep, human and camels (37,38). In one study conducted in Golestan province, northern Iran, G1 and G3 strains have been reported in 78.3% and 15% of CE cases respectively (39). The G1 strain also reported from the wild boar in Iran (14). This further emphasizes that the dominant strain of *E. granulosus* in Iran, not only in livestock but also in wild animals is the G1-G3 strains. In a study by Simsek et al. (20) on cattle and sheep isolates of *E. granulosus* metacestodes from eastern areas of Turkey, all of the 54 examined samples were found as G1-G3 strains. A study on the genetic characteristics of human and animals isolates of *E. granulosus* in the province of Edirne from Turkey, DNA sequencing of the *cox1* and *nad1* genes was performed and authors indicated that the sheep strain G1 was the most common genotype of *E. granulosus* affecting humans, sheep and cattle in the studied area. Moreover, 8 haplotypes of *Echinococcus* species were identified in the region (17). In another similar study for the molecular analysis of *E. granulosus* isolates from different regions of Turkey, the *cox1* gene was used for identification and molecular analysis of CE cases where all of the human hydatid cysts were belonged to the G1 (40). In 2008, Vural et al. (41) reported G1 strain of *E. granulosus* in 107 out of 112 samples whereas only 5 cases were determined as the G3 strain. The interesting point was that the parasites of the G3 genotype were identified only in the isolates derived from animals in the eastern regions of the country (41). This finding is fully consistent with our findings; where one of our samples derived from Van city (the eastern region in Turkey) was determined as the G3 strain and the rest of the isolates were identified as the G1 strain.

It seems obvious that two regions evaluated in the present study have very close similarity in genetic features of *E. granulosus* as there were no differences in terms of genotypes and also the diversity of isolates of the parasite in these two areas. Moreover, the isolates of both sheep and cattle from both countries were placed in the same cluster. It should be noted that only *cox1* gene was used for genotype analysis of Turkish isolates in this study and this should be considered as a limitation of the current study.

CONCLUSION

Findings of the current study revealed that the sheep strain G1 is the dominant strain of *E. granulosus* in livestock isolates in Turkey and Iran. The inter and intra heterogeneity of the isolates in the two countries were 0.0-0.6% and 0.0-1.4% for *cox1* and *nad1* genomes, respectively.

Findings of the study can be used for adopting the common policies and bilateral cooperation for prevention and also controlling the disease in these two countries. Further studies are needed to determine the dominant genotypes of *E. granulosus* in human cases in these two regions.

* Ethics

Ethics Committee Approval: The study was approved by the Research Ethics Committee of Shiraz University of Medical Sciences (SUMS, Iran).

Informed Consent: Patient consent was not obtained.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: A.B., B.S., G.S., Design: A.B., B.S., G.S., A.A., Data Collection or Processing: A.B., G.S., A.A., A.E., M.H., F.M., S.E., Analysis or Interpretation: A.B., B.S., F.M., Literature Search: A.B., B.S., F.M., Writing: A.B., B.S.

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