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Original Article

Molecular Characteristics of *Echinococcus granulosus* Strains Isolated from Iranian Camel Using High Resolution Melting Analysis of *atp6* and *cox1* Genes

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Abstract

Background: Cystic echinococcosis (CE) also known as hydatid disease, is a zoonotic helminthic disease caused by infection with the larval stage of a tapeworm *Echinococcus granulosus*. It is an important parasite regarding human health and is categorized into different genotypes. The present study aimed to identify different genotypes of *E. granulosus* metacestode isolated from Iranian camel.

Methods: In this cross-sectional study, 54 hydatid cysts were isolated from slaughtered Iranian camels (*Camelus dromedarius*) in Isfahan (33 samples) and Yazd (21 samples) province s' laughterhouses. The DNA was extracted from the isolated hydatid cysts and high resolution melting analysis (HRM) was performed. The curves were confirmed by sequencing and aligning with previously deposited sequences.

Results: Based on the results of the present study, 94.4%, 3.7%, and 1.9% of the studied isolates were identified as *E. granulosus* (G1), *E. granulosus* (G2), and *E. intermedius* (G6) in the two studied regions, respectively. Moreover, 85.18% of the cysts were isolated from lung and 5.82% of them were also isolated from the liver of the camels.

Conclusion: Based on the HRM analysis of *cox1* and *atp6* genes, *E. granulosus* (G1) accounts for the most cases of camelid cystic echinococcosis, and demonstrates camels as a source of human cystic echinococcosis.

Keywords: *Echinococcus granulosus*, genotype, real-time polymerase chain reaction, Iran, high resolution melting analysis

Background

Cystic echinococcosis (CE), also known as hydatid disease, is a zoonotic helminthic disease caused by infection with the larval stage of a tapeworm belonging to the family of Taeniidae, *Echinococcus granulosus*. The tapeworm is common in certain parts of the globe; with the exception of Antarctica, it is found on every continent. Thus, a large number of people are affected by CE. The prevalence rates are as high as 5%– 10% in the endemic areas and the relatively silent clinical aspect of CE causes challenges for accurate and early diagnoses. Cyst formation upon the infection mainly, about 70%, occurs in the liver (1,2).

Four species of the tapeworm have been recognized to cause public health problems. The most common species that causes the human disease is *E. granulosus*, the cause of CE in human and some animals. Although *Echinococcus multilocularis* is fairly rare, it is the most virulent one and causes alveolar echinococcosis in human and some animals. The 2 other species, *Echinococcus vogeli* and *Echinococcus oligarthrus* cause polycystic echinococcosis (2,3).

The CE is a major zoonotic disease with substantial public health and economic concerns (2, 4). It is endemic in many regions of the globe, especially the Mediterranean areas, Australia, Central Asia and the Tibetan Plateau, South America as well as Northern and Eastern Africa (5). There is the enormous socioeconomic impact of CE on the human populations, which is calculated as the global burden as 1 009 662 DALYs (disability-adjusted life years). It also stands for UU$763 980 979 (2,6).

Echinococcus granulosus is grouped in different genotypes or strains. In recent years, the usage of mitochondrial phylogenetic analysis allowed statists to classify some of the genotypes as new species (7). The latest classification indicates that *E. granulosus* includes the G1, G2, and G3 genotypes. The G1 strain is the most frequently reported genotype all over the globe, which produces fertile hydatid cysts mostly in sheep and it is also frequently isolated

from human cases. Lately, *E. granulosus* (G1) has been also identified in cats as well (7,8).

Echinococcus equinus (G4) has notable morphological and developmental differences compared to the G1 genotype and has only been reported in equines, yet no human cases have been reported (9). *E. ortleppi* (G5) produces fertile cysts most of the times in cattle and has been reported in few human cases (7, 10). The *E. intermedius* groups the G6 as well as G7, and E. canadensis relates to G8 and G10 genotypes. The main intermediate hosts for the G6 genotype are camels and goats; pigs are also the main intermediate hosts for G7. Besides, for the genotypes G8 and G10, cervids are considered as the main intermediate hosts. The G6, G7, and G8 genotypes have been isolated from humans as well. The human cases with the G9 genotype that was described in 1997 are now considered to be the G7 genotype (7,11).

Camels are important regarding the epidemiology *E. intermedius* (G6), which can be transmitted to humans (9). In Iranian context, few studies have been performed regarding the genetic characteristics of camelid echinococcosis (12, 13). Molecular studies on the *E. granulosus* genotypes in different parts of the world can produce a useful set of data about the parasites epidemiological, ecological and transmission as well as the sources of human infection. These data can be used for public health, prevention, and control programs for the disease not only in humans, but also in animals, in order to minimize its' socio-economic impacts.

The high resolution melting analysis (HRM) is a new approach by a single step with the closed tube method. It is reported to be appropriate especially for fast screening of a large number of isolates. The method is also fast, simple, accurate, and cost-effective, especially considering that it does not need post-PCR processes, thus it eliminates the hazards of ethidium bromide staining on the gel electrophoresis. In human research, this method has been vastly used for identification of single nucleotide polymorphisms in genetic disorders and also for detection of some infectious germs (14-17).

Objective

The present study aimed at identifying different molecular characteristics of *E. granulosus* metacestode using HRM.

Methods

Sampling and Sample Preservation

In the present cross-sectional study, 54 hydatid cysts were isolated from slaughtered Iranian camels (*Camelus dromedarius*) in Isfahan (33 samples) and Yazd (21 samples) provinces' slaughterhouses; the involved organ was recorded as well. The studied regions (Yazd and Isfahan) are located in the central part of Iran. The samples were transferred to the Applied Physiology Research Center, Isfahan University of Medical Sciences. The hydatid fluid was examined for the presence of protoscoleces, centrifuged at 800 rpm for 1 minute. The sedated protoscoleces and a piece of hydatid cysts wall preserved in 70% ethyl alcohol for DNA extraction.

DNA Extraction

Samples were washed in phosphate-buffered saline (PBS) prior to DNA extraction in order to remove residual ethanol from the parasite tissue. DNA extraction kit from tissue (PrimePrepTM, Genet Bio, Korea) was used for genomic DNA extraction. The procedure of the DNA extracting was performed according to the manufacturer's manual. The extracted DNA samples were preserved at -20°C for later application.

Real-Time Polymerase Chain Reaction and High Resolution Melting Analysis

The 2 pairs of primers, *cox1*; F: TTTTTTGGGCATCCTGAGGTTTAT, R: TAAAGAAAGAACATAATGAAAATG (18) and *atp6* F: GCATCAATTTGAAGAGTTGGGGATAAC, R: CCAAATAATCTATCAACTACACAACAC (19) were applied for the real-time implication of partial sequence of *cox1* and *apt6* genes. The real-time PCR detection system (Rotor-Gene 6000, Germany) was used for 25 µL reactions. The master mix contained, 10 µL master mix premix (Type-it® HRM™, Qiagen, Germany), 10 µL distilled nuclease-free water, 1 µL from each primer (10 pmol/uL), and 4 µL template DNA.

The reaction condition was as follows, *cox1*: 10 minutes at 95°C (initial denaturation) followed by 40 cycles of 95°C for 10 seconds (denaturation), 55°C for 40 seconds (annealing) and 72°C for 27 seconds (extension), and a final extension step at 72°C for 5 min, *atp6*: 10 minutes at 95°C (initial denaturation) followed by 40 cycles of 95°C for 17 seconds (denaturation), 60°C for 30 seconds (annealing) and 72°C for 28 seconds (extension), and a final extension step at 72°C for 5 minutes. The melting curve was drawn by increasing the temperature from 65°C to 90°C at intervals of 0.2°C/2 seconds (20). The curves were interpreted according to the simultaneously tested known genotypes.

Three previously known isolates regarding genotypes with the identical sequences to the accession numbers, namely, G1: KC660075, G3: KU697314, and G6: KC415063 were obtained from the first author of the previous study in the same laboratory (20). Control and sample isolate were tested simultaneously. The identity of the curves was confirmed by sequencing of the amplified genes. From each distinct curve of the 2 studied genes, the amplicons of 3 isolates were purified and directly sequenced by ABI 3730xl DNA analyzer (Applied Biosystem®, USA). The sequences were compared to GenBank using basic local alignment search tool (BLAST). Melting curves were interpreted according to the control and sequenced isolates.

Results

Totally, 54 hydatid cyst samples (8 from liver and 46 from lungs) were collected and analyzed. The results illustrated that, 32 out of 33 (97%) isolates from Isfahan province were *E. granulosus* (G1) and one out of 33 (3%) was *E. intermedius* (G6). In Yazd province, 2 out of 21 (9.6%) isolates were *E. granulosus* (G3) and 19 out of 21(90.4%) isolates were *E. granulosus* (G1). The involved organ and city distribution of the 54 studied *E. granulosus* isolates are available in Table 1. The mean temperature (°C), SD, as well as intra- and inter-assay coefficient of variations calculated for *E. granulosus* G1, G3, and G6 genotype are available in Table 2. The normalized HRM curves of the standard known genotypes and the studied samples are available in Figure 1 and 2.

Based on the results of the present study, 94.4%, 3.7%, and 1.9% of the studied isolates were identified as *E. granulosus* (G1), *E. granulosus* (G3), and *E. intermedius* (G6) in the 2 studied regions, respectively.

The sequences of the isolates were identical to the following accession numbers, *atp6*; G1: KU925413, G1: KU925413, G1: KX039965, G3: KJ559023, G3: KJ559023, G6:AB208063 and *cox1*; G1: KM100575, G1: KM100575, G1: HF947559, G3: HF947568, G3: KJ559023, and G6: KU359038.

Discussion

The results of the present study showed that *E. granulosus* G1 accounts for the majority of camel CE in Isfahan and Yazd provinces.

The HRM approach is increasingly used for identification and diagnosis of some parasitic infections recently. The method is optimized for molecular characterization and genotyping of some parasitic organisms, e.g., *Cryptosporidium* spp., *Leishmania* spp., *Giardia* spp., *Toxoplasma* sp., and *Plasmodium* spp. The HRM is also being practiced for a quick and reliable identification of some helminthic parasites, such as *E. granulosus* (17). A temperature-based real-time PCR method has been first used for genotyping of *E. granulosus* in 2009 (17,21).

The latest molecular classification based on the mitochondrial genes' sequences of *E. granulosus* categorized it in different species and genotypes as *E. granulosus* (G1, G2, and G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. intermedius* (G6, G7), and *E. canadensis* (G8, G10) (22). The G1 and G2 are considered as sheep strains and G3 and G5 as bovid strains; in addition, G4 and G6 belong to horse and camel strains, respectively. Besides, the G7 is regarded as pig strain, and the G8 and G10 as cervid strains (23). Moreover, *E. felidis* is the lion strain (7).

Based on the results of the present study, 94.4%, 3.7%, and 1.9% of the studied camel isolates were identified as *E. granulosus* (G1), *E. granulosus* (G2), and *E. intermedius* (G6) in the 2 studied regions, respectively. The vast majority of the isolates belonged to the G1 genotype, yet one G6 strain was also identified. In the previously published studies on animals and humans, the presence of G1, G3 as well as G6 genotypes has been reported in Isfahan area (23,24). In addition, other genotypes such as G5, G7, and G2 have been reported from animal cases in Iran (25,26). Only genotypes of G1, G2, G3, and G6 are reported from human cases in the country (27).

Echinococcus granulosus (G1) which is reported 88.44%, is responsible for the large majority of human CE all over the globe, and has the most worldwide distribution; it is

Table 1. The Involved Organ and City Distribution of the 54 Studied *Echinococcus granulosus* Isolates

		Isfahan No. (%)	Yazd No. (%)	Total No. (%)
Organ				
	Lungs	28 (84.8)	18 (85.7)	46 (85.18)
	Liver	5 (15.2)	3 (14.3)	8 (14.82)
Fertility				
	Fertile	25 (75.8)	19 (90.5)	44 (81.48)
	Non-fertile	8(24.2)	2(9.5)	10 (18.52)

Table 2. Mean Temperature (°C), SD and Intra- and Inter-assay Coefficient Of Variations Calculated for *Echinococcus granulosus* G1, G3, and, G6 Genotypes

		G1		G3		G6	
		Cox1	*Atp6*	*Cox1*	*Atp6*	*Cox1*	*Atp6*
N		51	51	2	2	1	1
Mean		79.83	77.95	80.31	78.92	79.09	77.4
SD		0.077	0.1698	0.046	0.3577	0.0827	0.2071
Coefficient of variation%	Inter-assay	0.097	0.21	0.05	0.45	0.1	0.267
	Intera-assay	0.090	0.2	0.06	0.35	0.06	0.25
Minimum	Temperature	79.7	77.63	80.2	78.52	79.24	77.2
Maximum	Temperature	80	78.25	80.39	79.38	79	77.7
Total				54			
%		94.45	94.45	3.7	3.7	1.85	1.85

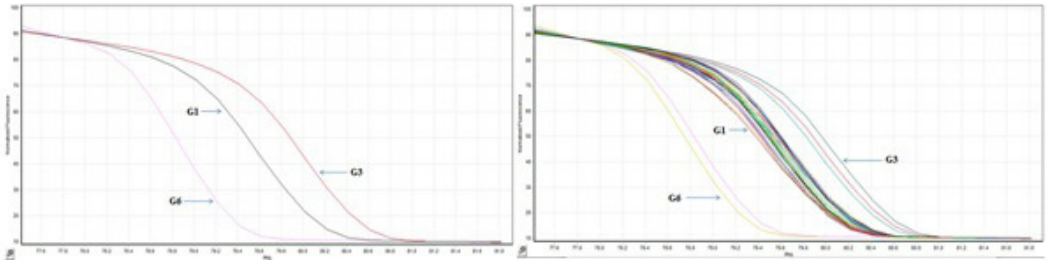

Figure 1. Normalized HRM Curves of the *cox1* Gene Among the Studied Samples and the Standard Isolates. HRM based analysis of the gene with the previously sequenced controls is shown. The HRM curves of the G1, G3, and G6 genotypes are used as the standard for determination of the samples. The different genotypes are located close to the known standard.

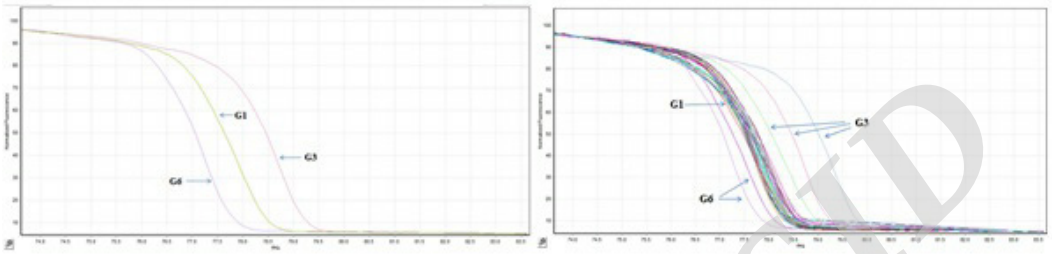

Figure 2. Normalized HRM Curves of the *atp6* Gene Among the Studied Samples and the Standard Isolates. HRM based analysis of the gene with the controls is demonstrated. The HRM curves of the G1, G3, and G6 genotypes are used as the standard for determination of the samples. The different genotypes are located close to the known standard.

often associated with sheep as intermediate hosts (11). In the present study using HRM approach, 94.4% of the camel isolates of *E. granulosus* were identified as G1 genotype, which shows that even in camel, G1 is the most frequent strain. There are quite a few studies on the genetic characteristics of camelid CE in Iran, some of which are mentioned below.

Sharbatkhori et al studied the genotypes of *E. granulosus* in sheep, cattle, camels, and humans in Golestan province, Iran, using *cox1* and *nad1* gene analyses (13). They found 4 *E. granulosus* genotypes among the 74 studied CE isolates. Their reported isolates from animals and humans were G1 (78.3%), G2 (2.7%), G3 (15%), and G6 (4%). Considering camel as an intermediate host, 44.5%, 22.5%, and 33.3% were reported to be G1, G3, and G6, respectively. Similar to the results of the present study, G1 was the most prevalently found genotype. The G1-G3 complex was reported to be found in all of the sheep, goat, cattle, and buffalo isolates of their study. All those four human CE isolates belonged to *E. granulosus* G1. Sharbatkhori et al found more *E. intermedius* (G6) in camels (3 out of nine isolates, 33.3%) compared to findings of the present study (one out of 54 isolates, 1.9%). Similar to the findings of the present study, they identified G1, G3, and G6 genotypes from the camel CE in Golestan province.

Moghaddas et al studied the molecular characteristics of 50 *E. granulosus* isolates from the camel in eastern Iran, using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of *cox1* and *ITS1* genes. Their results showed that 27 out of 50 (54%) *E. granulosus* isolates belonged to G1 strain and 46% were of G6 genotype (12). They reported the fairly high prevalence of G6 genotype compared to the findings of the present study. Their results indicate the high rate of G6 genotype in eastern Iran, yet not in central part of Iran.

Sharifiyazdi et al explored the genotypic characterization of camel isolates of *E. granulosus* using *cox1* and *nad1* gene sequencing in Mashhad, North West of Iran. Similar to the findings of the present study, they reported G3 and G6 genotypes, yet not G1 strain among 85 studied camels. They also reported the lungs as the most-affected organs (95.0%) followed by the liver (26.5%) and spleen (2.4%); these results are in line with the findings of the present study in which 85.18% of the cysts were isolated from the lung and 5.82% from the liver (28).

In a recent study, Jafari et al investigated the genetic characteristics of the human isolates of *E. granulosus* by the cox1 and nad1 gene analyses in Isfahan. The same genotypes from human cases of CE (86% G1, 6% G3, and 8% G6) were reported by the researchers in Isfahan (24).

In the present study, the three previously reported genotypes were isolated from Iranian camel and the results indicated that *E. granulosus* (G1) was the predominant genotype in camels in central parts of Iran and considering the high rate of human infections with G1 strain, camel can be one of the major sources of human infections in

this area.

Conclusions

Based on the HRM analysis of *cox1* and *atp6* genes, *E. granulosus* (G1) accounts for the most cases of camelid CE, which represents camels as a source of human CE.

Conflict of interest

The authors declare that they have no conflict of interest

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References

1. Pakala T, Molina M, Wu GY. Hepatic echinococcal cysts: A review. J Clin Transl Hepatol. 2016;4(1):39-46. doi: 10.14218/jcth.2015.00036.
2. Sarkar M, Pathania R, Jhobta A, Thakur BR, Chopra R. Cystic pulmonary hydatidosis. Lung India. 2016;33(2):179-91. doi: 10.4103/0970-2113.177449.
3. Mandal S, Mandal MD. Human cystic echinococcosis: epidemiologic, zoonotic, clinical, diagnostic and therapeutic aspects. Asian Pac J Trop Med. 2012;5(4):253-60. doi: 10.1016/s1995-7645(12)60035-2.
4. Craig PS, McManus DP, Lightowlers MW, Chabalgoity JA, Garcia HH, Gavidia CM, et al. Prevention and control of cystic echinococcosis. Lancet Infect Dis. 2007;7(6):385-94. doi: 10.1016/s1473-3099(07)70134-2.
5. Wahlers K, Menezes CN, Wong ML, Zeyhle E, Ahmed ME, Ocaido M, et al. Cystic echinococcosis in sub-Saharan Africa. Lancet Infect Dis. 2012;12(11):871-80. doi: 10.1016/s1473-3099(12)70155-x.
6. Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis. 2006;12(2):296-303. doi: 10.3201/eid1202.050499.
7. Cucher MA, Macchiaroli N, Baldi G, Camicia F, Prada L, Maldonado L, et al. Cystic echinococcosis in South America: systematic review of species and genotypes of *Echinococcus granulosus* sensu lato in humans and natural domestic hosts. Trop Med Int Health. 2016;21(2):166-75. doi: 10.1111/tmi.12647.
8. Armua-Fernandez MT, Castro OF, Crampet A, Bartzabal A, Hofmann-Lehmann R, Grimm F, et al. First case of peritoneal cystic echinococcosis in a domestic cat caused by *Echinococcus granulosus* sensu stricto (genotype 1) associated to feline immunodeficiency virus infection. Parasitol Int. 2014;63(2):300-2. doi: 10.1016/j.parint.2013.11.005.
9. Thompson RC. The taxonomy, phylogeny and transmission of *Echinococcus*. Exp Parasitol. 2008;119(4):439-46. doi: 10.1016/j.exppara.2008.04.016.
10. Eckert J, Thompson RC. Intraspecific variation of Echinococcus granulosus and related species with emphasis on their infectivity to humans. Acta Trop. 1997;64(1-2):19-34.
11. Alvarez Rojas CA, Romig T, Lightowlers MW. *Echinococcus granulosus* sensu lato genotypes infecting humans--review of current knowledge. Int J Parasitol. 2014;44(1):9-18. doi: 10.1016/j.ijpara.2013.08.008.
12. Moghaddas E, Borji H, Naghibi A, Shayan P, Razmi GR. Molecular genotyping of *Echinococcus granulosus* from dromedaries (*Camelus dromedarius*) in eastern Iran. J Helminthol. 2015;89(1):100-4. doi: 10.1017/s0022149x13000631.
13. Sharbatkhori M, Tanzifi A, Rostami S, Rostami M, Fasihi Harandi M. *Echinococcus granulosus* sensu lato genotypes in domestic livestock and humans in Golestan province, Iran. Rev Inst Med Trop Sao Paulo. 2016;58:38. doi: 10.1590/s1678-9946201658038.
14. Bastien R, Lewis TB, Hawkes JE, Quackenbush JF, Robbins TC, Palazzo J, et al. High-throughput amplicon scanning of the *TP53* gene in breast cancer using high-resolution fluorescent melting curve analyses and automatic mutation calling. Hum Mutat. 2008;29(5):757-64. doi: 10.1002/humu.20726.
15. Krypuy M, Ahmed AA, Etemadmoghadam D, Hyland SJ, DeFazio A, Fox SB, et al. High resolution melting for mutation scanning of *TP53* exons 5-8. BMC Cancer. 2007;7:168. doi: 10.1186/1471-2407-7-168.
16. Millat G, Chanavat V, Rodriguez-Lafrasse C, Rousson R. Rapid, sensitive and inexpensive detection of SCN5A genetic variations by high resolution melting analysis. Clin Biochem. 2009;42(6):491-9. doi: 10.1016/j.clinbiochem.2008.10.014.
17. Rostami S, Talebi S, Babaei Z, Sharbatkhori M, Ziaali N, Rostami H, et al. High resolution melting technique for molecular epidemiological studies of cystic echinococcosis: differentiating G1, G3, and G6 genotypes of *Echinococcus granulosus* sensu lato. Parasitol Res. 2013;112(10):3441-7. doi: 10.1007/s00436-013-3523-z.
18. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol. 1992;54(2):165-73.
19. Snabel V, Altintas N, D'Amelio S, Nakao M, Romig T, Yolasigmaz A, et al. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. Parasitol Res. 2009;105(1):145-54. doi: 10.1007/s00436-009-1376-2.
20. Safa AH, Harandi MF, Tajaddini M, Rostami-Nejad M, Mohtashami-Pour M, Pestehchian N. Rapid Identification of *Echinococcus granulosus* and *E. canadensis* Using High-Resolution Melting (HRM) Analysis by Focusing on a Single Nucleotide Polymorphism. Jpn J Infect Dis. 2016;69(4):300-5. doi: 10.7883/yoken.JJID.2015.161.
21. Maurelli MP, Rinaldi L, Capuano F, Perugini AG, Cringoli G. Development of a real-time PCR for the differentiation of the G1 and G2/G3 genotypes of *Echinococcus granulosus*. Parasitol Res. 2009;105(1):255-9. doi: 10.1007/s00436-009-1388-y.
22. Thompson RC. Biology and systematics of *Echinococcus*. Adv Parasitol. 2017;95:65-109. doi: 10.1016/bs.apar.2016.07.001.
23. Sharafi SM, Rostami-Nejad M, Moazeni M, Yousefi M, Saneie B, Hosseini-Safa A, et al. *Echinococcus granulosus* genotypes in Iran. Gastroenterol Hepatol Bed Bench. 2014;7(2):82-8.
24. Jafari R, Sanei B, Baradaran A, Spotin A, Bagherpour B, Darani HY. Genetic characterization of *Echinococcus granulosus* strains isolated from humans based on nad1 and cox1 gene analysis in Isfahan, central Iran. J Helminthol. 2017:1-7. doi: 10.1017/s0022149x17000967.
25. Spotin A, Mahami-Oskouei M, Harandi MF, Baratchian M, Bordbar A, Ahmadpour E, et al. Genetic variability of *Echinococcus granulosus* complex in various geographical

populations of Iran inferred by mitochondrial DNA sequences. Acta Trop. 2017;165:10-6. doi: 10.1016/j.actatropica.2016.03.002.

26. Fadakar B, Tabatabaei N, Borji H, Naghibi A. Genotyping of *Echinococcus granulosus* from goats and sheep indicating G7 genotype in goats in the Northeast of Iran. Vet Parasitol. 2015;214(1-2):204-7. doi: 10.1016/j.vetpar.2015.09.029.
27. Rostami S, Shariat Torbaghan S, Dabiri S, Babaei Z, Ali Mohammadi M, Sharbatkhori M, et al. Genetic characterization of *Echinococcus granulosus* from a large number of formalin-fixed, paraffin-embedded tissue samples of human isolates in Iran. Am J Trop Med Hyg. 2015;92(3):588-94. doi: 10.4269/ajtmh.14-0585.
28. Sharifiyazdi H, Oryan A, Ahmadnia S, Valinezhad A. Genotypic characterization of Iranian camel (*Camelus dromedarius*) isolates of *Echinoccocus granulosus*. J Parasitol. 2011;97(2):251-5. doi: 10.1645/ge-2642.1.