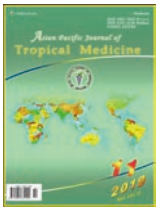

Original Article
Asian Pacific Journal of Tropical Medicine
journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.271290
Impact factor: 1.77

Molecular characterization of *Echinococcus granulosus* in paraffin-embedded human tissues from Southwest Iran

Elham Yousefi[1], Abdollah Rafiei[1], Iran Rashidi[2], Shahram Khademvatan[3]✉, Masoud Foroutan[4]

[1]*Department of Medical Parasitology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2]*Department of Pathology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[3]*Cellular and Molecular Research Center and Department of Medical Parasitology, Urmia University of Medical Sciences, Urmia, Iran; Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Iran*

[4]*Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

ARTICLE INFO

Article history:
Received 18 November 2018
Revised 29 May 2019
Accepted 2 June 2019
Available online 26 November 2019

Keywords:
Echinococcus granulosus
Human
Genotype
Iran

ABSTRACT

Objective: To investigate *Echinococcus (E.) granulosus* genotypes as the causative agents of hydatidosis in humans in the southwest of Iran (Khuzestan province).

Methods: In this study, isolates of 80 archived human paraffin embedded hydatid cysts were collected from pathology laboratories in Ahvaz city, Khuzestan province. DNA was extracted and examined by nested-PCR of ribosomal DNA (rDNA) internal transcribed spacer 1 (ITS1), and PCR-RFLP. In addition, the sequences of fragments of genes coding for Cox space1 and NADH dehydrogenase 1 (ND1) were also examined.

Results: Of the 80 paraffin samples, 44 (55.0%) were from the liver, 27 (33.8%) from the lung, and the rest from other organs. The amplified hydatid genomic DNA showed that the cysts were *E. granulosus* strains. The results of PCR-RFLP and sequencing analysis revealed the presence of G1 genotype (sheep strain) in all human isolates. Furthermore, no camel strain (G6) was detected among all samples in the regions studied.

Conclusions: The molecular findings indicate that the predominant genotype involved in *E. granulosus* transmission in southwest of Iran is the common sheep strain (G1), which occurs in human populations. These results may have important implications for hydatid disease control in the studied areas.

1. Introduction

Hydatidosis is a disease caused by the larval stage of *Echinococcus (E.) granulosus*, and is considered as a zoonotic disease. This disease spreads globally, and many cases of human infections are reported annually. In addition to physical harms, huge sums are spent on treatment of patients. In Iran, contamination with this worm is also of huge importance. Dogs are considered the final hosts and sheep are intermediate hosts of this parasite[1-3]. Larval stage of this parasite is seen in animals such as livestock and humans. Iran is considered an endemic region for this parasite, and 1% of surgeries annually concern hydatid cysts in Iran[1]. This parasite has various strains, and so far 10 strains (G_1-G_{10}) have been identified for this parasite. In regions where the disease is endemic, there is usually a relatively vast diversity among *E. granulosus* strains in genetic and biological terms[4-6]. Several studies have demonstrated that in endemic areas, *E. granulosus* sensu lato exists as a complex of different strains, and this diversity affects epidemiology and

✉Corresponding author: Shahram Khademvatan, Cellular and Molecular Research Center and Department of Medical Parasitology, Urmia University of Medical Sciences, Urmia, Iran; Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Iran.
Tel: (+98 44) 3367543-50
Fax: (+98 44) 3332036
E-mail: khademvatan@yahoo.com
Foundation project: This study was supported by the Research Project of the Cellular and Molecular Research Center of Ahvaz Jundishapur University of Medical Science (grant No. CMRC-1000).

How to cite this article: Yousefi E, Rafiei A, Rashidi I, Khademvatan S, Foroutan M. Molecular characterization of *Echinococcus granulosus* in paraffin-embedded human tissues from Southwest Iran. Asian Pac J Trop Med 2019; 12(11): 507-511.

pathogenicity of hydatid cyst[5].

Today, the best way to identify parasite strains is to use molecular-based techniques. Although characteristics of genus *Echinococcus* species in livestock and humans have also been studied using morphological methods, molecular study of parasite genotypes in humans has rarely been performed in Iran[4,5,7,8]. There are also many studies that have demonstrated DNA extracted from parasite protoscoleces have important effects on PCR results[9]. The aim of this study was to characterized *E. granulosus* sensu lato strains from paraffin embedded hydatic cyst isolated from human tissue in south west Iran (Khuzestan province).

2. Materials and methods

2.1. Sample collection

A total of 80 paraffin block samples were collected from the archives of the teaching hospitals of the south west Iran; Khuzestan province (Imam Khomaini and Shafa hospitals) and the city's private laboratories (Figure 1). All suspicious paraffin samples were excluded and only confirmed samples were included in the study. Information such as age, sex and infected organ were registered. Two thin sections (8-10 micron) were prepared from each paraffin block, and placed into 1.5 mL microtubes. In the next step, thin sections were de- paraffinized using Schneider *et al*. 2008 method, by pouring 1 mL of *xylol* on each sample, incubating at 37 ℃ for 10 min, and centrifuging at 1 500 *g* for 5 min. This stage was performed twice, and samples were then placed in 70%, 80%, 90%, and 100% alcohol, for re-hydration and preparation for molecular methods respectively[10].

Figure 1. Location of Ahvaz city in Iran.

2.2. DNA isolation and nested PCR

Genomic DNA from all 80 samples were extracted applying a procedure using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions with modification, including: increasing time of incubation to 48 h and amount of proteinase K to 40 μL in 56 ℃, respectively. DNA concentration was measured by spectrophotometric determination at A260.

An ITS1 fragment was amplified from each sample by Nested PCR. First step PCR amplification was performed using primer pair EGF 1 (5' CCA AAC TTG ATC ATT TAG AGG AAG 3') and EGR 2 (5' TAT GGG CCA AAT TCA CTC ATT ACC 3') as outer primers and for second PCR, internal primers were as follows: EgF: (5'GTC GTA ACA AGG TTT CCG TAGG 3') EgR: (5' TAG ATG CGT TCG AAG TGT CG 3')[11]. The thermo cycler used and PCR conditions were set as previously described for amplification of first and second step PCR as follows: One cycle of 95 ℃ for 2 min, 94 ℃ for 30 sec, 55 ℃ for 45 sec, and 72 ℃ for 1 min, all steps repeating for 30 cycles. For final extension 72 ℃ for 5 min was used[11,12]. Electrophoresis was performed by adding 7 μL sample of the second PCR to 1.5% (w/w) agarose gel, stained with ethidium bromide for 45 min at 100 V. The bands were observed by ultraviolet trans-illumination.

2.3. PCR–RFLP

All PCR products were digested for 6-8 h with the restriction endonucleases *Alu*I, *Taq*I and *Rsa*I using buffers recommended by the manufacturer (Fermentas, Vilnius, Lithuania) in a final volume of 20 μL including 5 μL of PCR product, 5 units of the restriction enzymes, 2 μL of the supplied buffer and 8 μL of molecular grade water. Restriction fragments of amplicons were electrophoresed using a 2% (W/V) agarose gel at 100V for 180 min.

2.4. Sequencing method and phylogenetic analysis

Fragments of mitochondrial genes amplified with specific primers that previously described as JB3/JB4.5 primers (5´-TTTTTTGGGCATCCTGAGGTTTAT-3´/5´ TAAAGAAAGAACATAATGAAAATG-3´) for *COX1* and JB11/JB12 primers (5´-AGATTCGTAAGGGGCCTAATA-3´/5´ ACCACTAACTAATTCACTTTC-3´) for *NAD1*, respectively[4] and *NAD1* band was found 450 bp, and *COX1* band was 400 bp respectively. PCR product of the *COX1* and *NAD1* genes from 17 randomly selected samples was sequenced by MWG (Germany) and the resulting data were analyzed using Chromas software (http://www.technelysium.com.au/Chromas.html). Data submitted in the GenBank database were obtained with accession numbers: Cox1 LC060667.1-LC060676.1 and NAD1 LC060691-LC060697, respectively.

GenBank database was searched for similar sequences using BLAST (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov/BLAST/) and output was then analyzed to find a significant homology. DNA sequences were aligned with ClustalW and the sequences were entered in PAUP* to generate phylograms for each dataset.

3. Results

The present study was conducted on 80 paraffin-embedded samples of human hydatid cysts, collected from Khuzestan province. Examination of 80 paraffin-embedded isolated during 2005 to 2014 showed that among the 80 samples, the highest frequency related to the age group of 21-30 years with prevalence of 21.25% (17/80), and the lowest frequency related to the over 60-year-old age group. Of the 80 isolates, 52 were collected from female (29 from liver, 17 from lung, 2 from spleen, and 4 from others) and 28 from male (15 from liver, 10 from lung, 1 from spleen, and 2 from peritoneum) respectively. Women's and men's mean age were (29±2) and (31±1) years, respectively. Of the 80 paraffin samples, 44 (55.00%) were from liver, 27 (33.75%) from lung, and the rest from other organs [(Spleen, 3 (3.75%); peritoneum, 3 (3.75%), kidney, 1 (1.25%), parotid1 (1.25%), pelvis1 (1.25%)]. About 55.00% of the samples were from the liver, and 33.75% from lung. Following De-paraffinization, samples' genomes were extracted and results showed that, 74 samples of the 80 paraffin isolates examined had suitable DNAs for the next stage. Nested-PCR was performed with *ITS-1* gene specific primers (internal and external primers), that amplified genomic DNA showed sharp bands (1 000 bp) that this rang associated with *E. granulosus* sens lato (Figure 2)

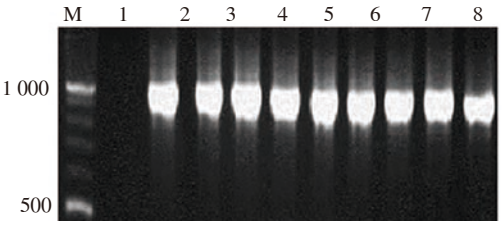

Figure 2. Internal transcribed spacer 1 (ITS1) nested-PCR results of extracted DNA of *Echinococcus granulosus* sens lato isolates. Nested PCR using inner primers. M: DNA marker (100 bp); N: negative control; Lane 1: positive control; Lane 2-9: isolates of liver 1, liver 2, lung 1, lung 2, spleen, parotid glands, kidney, and the peritoneum.

Results of genotyping hydatid cyst paraffin isolates showed that enzyme pattern of all samples were related to *E. granulosus* sensu stricto strain (G1 or sheep strain). Results obtained from enzyme-digested PCR products with *ALU-1* enzyme showed 180 bp and 700 double-band pattern in all samples (Figure 3A). Results of enzyme digested PCR products with *Taq-1* enzyme showed 1 000 bp single band pattern in all samples, similar to the sheep strain pattern (Figure 3B). Results obtained from enzyme-digested PCR products with RSA enzyme showed the same 320 bp and 680 double-band pattern in all samples, similar to sheep pattern (Figure 3C).

The multiple sequence alignment was done in Mega software

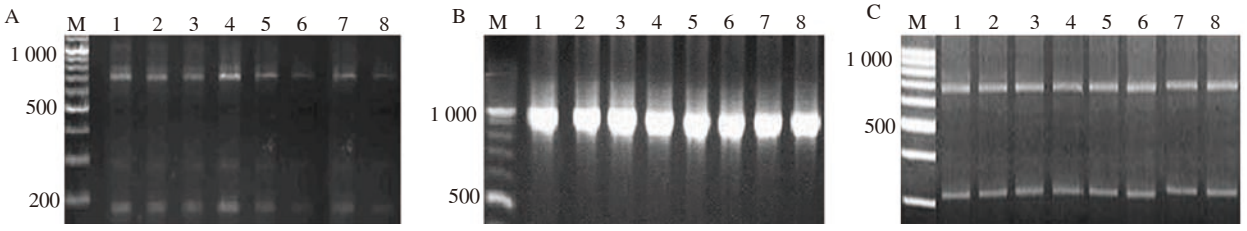

Figure 3. PCR-RFLP digests with: A: *Alu* enzyme; B: *Taq-1* enzyme and C: *RsaI* enzyme respectivly; M: DNA size marker (100 bp); Lane 1-8: isolates of liver 1 , liver 2, lung 1, lung 2, spleen, parotid glands, kidney and the peritoneum.

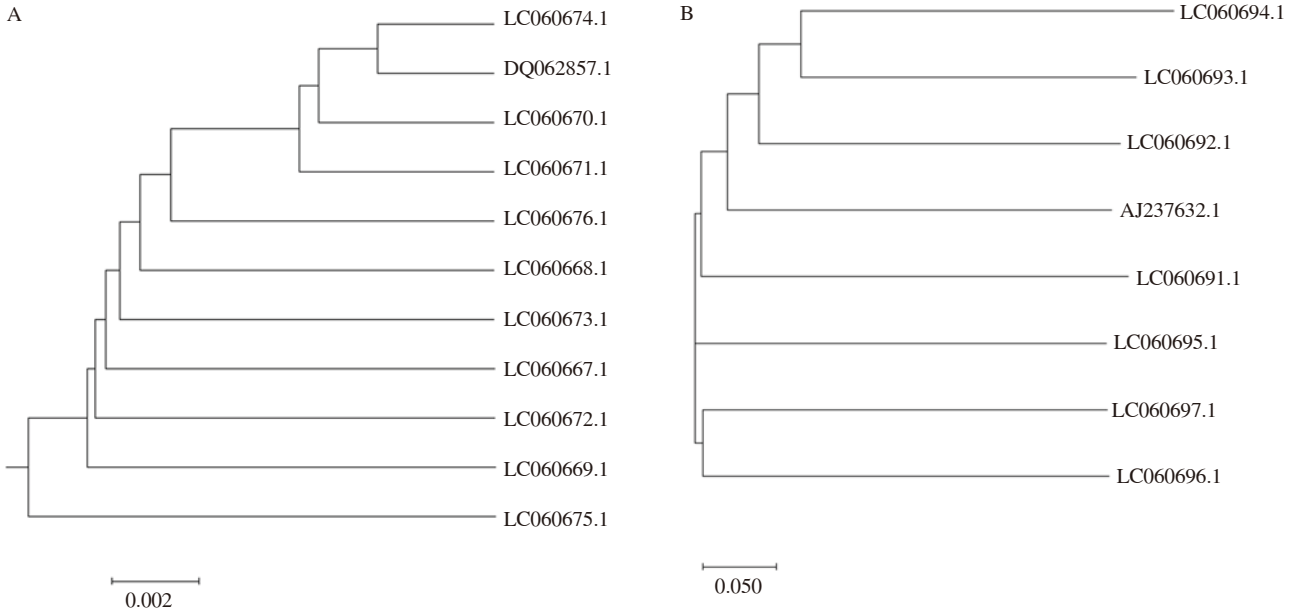

Figure 4. Dendrogram obtained for *Echinococcus granulosus* isolates by the maximum likelihood analysis at the *COX1* (A) and *NAD1* (B) genes in comparison with reference (DQ620857.1 & AJ237632.1) respectively . The result strongly supports they are supposedly an anamorph/teleomorph pair. A: *COX1* rooted tree and B: *NAD1* rooted tree.

and results were compared between the obtained sequences. Phylogenetic trees were generated by comparing submitted sequences with reference sequences in GeneBank: DQ062857.1 for *COX1* and AJ237632 for *NAD1*. The sequences were highly homologous with few differences, corresponding to punctual base substitution. Phylogenetic analyses using character method like Maximum Parsimony showed that the topology was similar among the trees obtained with significant bootstrap support for the clades which confirmed *E. granulosus* sensu stricto strain (G1 or sheep strain) (Figure 4 A and 4B).

4. Discussion

Hydatidosis is a zoonotic disease, and is commonly found in many areas of the world, especially in the Mediterranean region[13,14]. Identifying various genotypes of *E. granulosus* sensu lato, as hydatid cyst agent in endemic regions, can have a significant effect on disease control programs, especially in human[15-17]. The present study conducted on 80 paraffin block samples showed that the highest level of infection in humans occurred in the liver (55.00%), followed by the lung (33.75%). In addition, our results showed that the infection is more prevalent in 21-31 years old which is in concordance with the study of Rokni *et al* which mentioned the overall range of 20-40 years old as the age group of the highest cases[1].

The present study findings showed that liver is more affected compared to other organs. This are similar with studies conducted in Iran and other parts. In Iran, liver infection has been reported from 90.5% by Ahmadi *et al*. to 71.6% by Yazdi *et al*[1,18-20].

DNA extraction methods are important for genotyping of the larval stage of *E. granulosus* and researchers have proposed various techniques to extract tissue DNA[9,10,21,22]. In the present study, extraction of DNA from paraffin blocks faced with some difficulties. After trying various extraction techniques, finally, Schneider *et al*. method (with slight modification) was employed to extract parasite genome[10]. These modifications included: increased incubation time at 65 ℃, and use of large amounts of proteinase K. DNA extraction and PCR results showed 74 positive isolates out of 80 samples. This may have been due to cyst tissue calcification and extended length of time elapsed since the preparation of blocks, as well as inappropriate paraffin block preparation techniques, which in the long term causes DNA damage and lack of response in molecular studies.

In this study, ITS-1 and rDNA examination results using Nested-PCR specific method showed that all 74 human isolates had the same pattern, weighing approximately 1 000 bp. This pattern is similar to that found in studies of Rahimi *et al*.[11] and Mc Manus *et al*.[13] and is indicative of hydatid cyst infection in people. Shahnazi *et al*. reported *E. granulosus* DNA open pair 1 000 and 1 100 sizes in human hydatid cysts. In a study similar to the present one, Kia *et al*.[21] determined genotypes of 30 human hydatic cyst samples using PCR method and RFLP with EGF1/EGR2 primers, and obtained 1 000 bp bands. In a study of Zhang *et al*.[8] in Iran, conducted on 4 human isolates and 16 animal isolates of *E. granulosus* using molecular methods on COX-1, various sheep strains and camel strain from different parts of Iran were reported.

In the present study, RFLP results obtained from all separated human isolates using restriction enzymes: *ALU-1*, *Taq-1*, and *Msp-1* showed similar patterns to sheep isolate pattern with G1 genotype, and no other strain, such as *E. canadensis* G6 or buffalo G3 strains were found in Khuzestan province. *E. granulosus* sensu stricto *G1* strain, as the dominant strain has been reported in many studies in different parts of Iran, Middle Eastern countries, and North Africa[7,12,23,24]. The present study results are similar to previous studies in southwest Iran, in which out of 329 isolates separated from slaughtered animals in the province, such as: cattle, sheep, goat and 5 human isolates, but only sheep strain was found[12]. In two similar studies conducted by Parsa *et al*. in Lorestan province, adjacent to Khuzestan on animals, the dominant strain in Lorestan was senso stricto, which is the same sheep strain[23,25]. In another adjacent province, Ilam, animal studies also indicated frequent G1 strain in the region[25]. Interactions and constant movement of nomadic livestock between these three provinces, hugely helps uniformity of a dominant strain in these adjacent provinces[26]. The present study results are also similar to Zhang *et al*. study conducted in 1998 from north China and Iran[8].

In an extensive study conducted in 2005 in Tunisia on 50 human, 166 cattle, 150 sheep, and 3 camel isolates, all sheep, human, and cattle isolates were identified as sheep strain, and camel strain was only found in camel isolates[27]. Parsa *et al*[23], conducted a study on animal and human samples in Khorramabad city, and reported all as sheep strain, which is similar to the results of the present study. Perhaps, a reason for not obtaining camel isolates in Iran's west and south western areas is that camel is not slaughtered in Khuzestan abattoirs, while G3 and G6 strains have been reported in central Iran[7,28] .

In the present study, obtained *COX1* gene subunit (400 bp) was compared with reference sequence DQ062857 for G1, and results showed significant homology results. Moreover, an *NAD1* gene subunit (450 bp) was also compared with a reference sequence registered at Genebank, such as AJ237632 for G1 strain, and showed significant homology up to 97% to 99% compared to reference genotypes[29]. The phylogenetic results presented here confirmed that the variation between the sequences obtained herein resulted because they belonged to different *E. granulosus* sens lato isolates.

The present study results using PCR –RFLP of ITS1 and genetic sequencing are in line with previous studies on animals and ITS1 gene sequence, and the sheep strain was also confirmed in this case[12]. As an important point, in Iran, genotypes of intermediate hosts are in line with definitive host of *E. granulosus*. For example, in a molecular study, Parsa *et al.* 2012, revealed the presence of G1 genotype (sheep strain) of *E. granulosus* sensu stricto as dominant genotype in dogs[30].

In conclusion, the dominant strain in southwest region of Iran (and also other parts of the country), is G1 or sheep strain, which can invade human liver, lung, and even parotid glands. Thus, the important role of dog-sheep life cycle in the region is recognized. Educating those who are in contact with dogs, gathering and housing stray dogs, and ongoing treatment of pet dogs can be significantly effective in controlling the disease.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors would like to thank Dr Jasem Saki for his help in analysis of the samples.

Authors' contribution

SK, AR and IR conceived and designed the experiments; EY performed the experiments; SK, EY and MF wrote the paper.

References

[1] Rokni M. Echinococcosis/hydatidosis in Iran. *Iran J Parasitol* 2009; **4**: 1-16.

[2] Khalkhali H, Foroutan M, Khademvatan S, Majidiani H, Aryamand S, Khezri P, et al. Prevalence of cystic echinococcosis in Iran: A systematic review and meta-analysis. *J Helminthol* 2018; **92**: 260-268.

[3] Soltani S, Rafiei A, Ramezani Z, Abbaspour MR, Jelowdar A, Kahvaz MS. Evaluation of the hydatid cyst membrane permeability of albendazole and albendazole sulfoxide-loaded solid lipid nanoparticles. *Jundishapur J Nat Pharm Prod* 2017; **12**: e34723.

[4] Shi Y, Wan X, Wang Z, Li J, Jiang Z, Yang Y. First description of *Echinococcus ortleppi* infection in China. *Parasit Vectors* 2019; **12**(1): 1-6.

[5] Ma X, Zhang L, Wang J, Luo Y. Knowledge domain and emerging trends on echinococcosis research: A scientometric analysis. *Int J Environ Res Public Health* 2019; **16**(5): 842.

[6] Khademvatan S, Majidiani H, Foroutan M, Tappeh KH, Aryamand S, Khalkhali H. *Echinococcus granulosus* genotypes in Iran: A systematic review. *J Helminthol* 2018; **93**: 1-8.

[7] Arbabi M, Pirestani M, Delavari M, Hooshyar H, Abdoli A, Sarvi S. Molecular and morphological characterizations of *Echinococcus granulosus* from human and animal isolates in Kashan, Markazi Province, Iran. *Iran J Parasitol* 2017; **12**(2): 177-187.

[8] Han X, Jian Y, Zhang X, Ma L, Zhu W, Cai Q, et al. Genetic characterization of *Echinococcus* isolates from various intermediate hosts in the Qinghai-Tibetan Plateau Area, China. *Parasitology* 2019; **31**: 1-25.

[9] Prevalence and molecular characterization of *Echinococcus granulosus* sensu stricto in northern Xinjiang, China. *Korean J Parasitol* 2019; **57**(2): 153-159.

[10] Schneider R, Gollackner B, Edel B, Schmid K, Wrba F, Tucek G, et al. Development of a new PCR protocol for the detection of species and genotypes (strains) of *Echinococcus* in formalin-fixed, paraffin-embedded tissues. *Int J Parasitol* 2008; **38**: 1065-1071.

[11]Rahimi H, Kia E, Mirhendi S, Talebi A, Harandi MF, Jalali-Zand N, et al. A new primer pair in ITS1 region for molecular studies on *Echinococcus granulosus*. *Iran J Public Health* 2007; **36**: 45-49.

[12]Khademvatan S, Yousefi E, Rafiei A, Rahdar M, Saki J. Molecular characterization of livestock and human isolates of *Echinococcus granulosus* from south-west Iran. *J Helminthol* 2013; **87**: 240-244.

[13]Matini M, Fallah M, Maghsood AH, Saidijam M, Harandi MF. *Echinococcus granulosus* sensu stricto in livestock and human in Hamadan, western Iran. *Iran J Parasitol* 2019; **14**(2): 288-296.

[14]Jenkins DJ, Williams T, Raidal S, Gauci C, Lightowlers MW. The first report of hydatid disease (*Echinococcus granulosus*) in an Australian water buffalo (*Bubalus bubalis*). *Int J Parasitol Parasites Wildl* 2019; **8**: 256-259.

[15]Eckert J, Gemmell M, Meslin FX, Pawlowski Z. *WHO–OIE manual on Echinococcosis in humans and animals: A public health problem of global concern*. Paris: World Organisation For Animal Health Paris; 2001.

[16]Himsworth CG, Jenkins E, Hill JE, Nsungu M, Ndao M, Thompson RA, et al. Emergence of sylvatic *Echinococcus granulosus* as a parasitic zoonosis of public health concern in an indigenous community in Canada. *Am J Trop Med Hyg* 2010; **82**: 643-645.

[17]Shang JY, Zhang GJ, Liao S, Huang Y, Yu WJ, He W, et al. A multiplex PCR for differential detection of *Echinococcus granulosus* sensu stricto, *Echinococcus multilocularis* and *Echinococcus canadensis* in China. *Infect Dis Poverty* 2019; **8**(1): 68.

[18]Ahmadi N, Hamidi M. A retrospective analysis of human cystic echinococcosis in Hamedan province, an endemic region of Iran. *Ann Trop Med Parasitol* 2008; **102**: 603-609.

[19]Hanifian H, Kambiz D, Tappeh KH, Mohammadzadeh H, Mahmoudlou R. Identification of *Echinococcus granulosus* strains in isolated hydatid cyst specimens from animals by PCR-RFLP method in West Azerbaijan–Iran. *Iran J Parasitol* 2013; **8**: 376.

[20]Shir Yazdi S, Mir Shamsi M, Hosseini B, Ebadi M. Cases of the hydatid cyst that were operated upon in Yazd. *J Shahid Sadoughi Univ Med Sci Health Services* 2000; **1**: 25-30.

[21]Kia EB, Rahimi H, Sharbatkhori M, Talebi A, Harandi MF, Mirhendi H. Genotype identification of human cystic echinococcosis in Isfahan, central Iran. *Parasitol Res* 2010; **107**: 757-760.

[22]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**: 588-594.

[23]Parsa F, Haghpanah B, Pestechian N, Salehi M. Molecular epidemiology of *Echinococcus granulosus* strains in domestic herbivores of Lorestan, Iran. *Jundishapur J Microbiol* 2011; **4**: 123-130.

[24]Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006; **55**: S197-S202.

[25]Dousti M, Abdi J, Bakhtiyari S, Mohebali M, Mirhendi S, Rokni M. Genotyping of hydatid cyst isolated from human and domestic animals in Ilam Province, Western Iran using PCR-RFLP. *Iran J Parasitol* 2013; **8**: 47.

[26]Rafiei A, Hemadi A, Maraghi S, Kaikhaei B, Craig P. Human cystic echinococcosis in nomads of south-west Islamic Republic of Iran. *East Mediterr Health J* 2007; **13**: 41-48.

[27]M'rad S, Filisetti D, Oudni M, Mekki M, Belguith M, Nouri A, et al. Molecular evidence of ovine (G1) and camel (G6) strains of *Echinococcus granulosus* in Tunisia and putative role of cattle in human contamination. *Vet Parasitol* 2005; **129**: 267-272.

[28]Sharbatkhori M, Harandi MF, Mirhendi H, Hajialilo E, Kia EB. Sequence analysis of *COX1* and *NAD1* genes in *Echinococcus granulosus* G3 genotype in camels (*Camelus dromedarius*) from central Iran. *Parasitol Res* 2011; **108**: 521-527.

[29]Šnábel 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**: 145.

[30]Parsa F, Fasihi Harandi M, Rostami S, Sharbatkhori M. Genotyping *Echinococcus granulosus* from dogs from Western Iran. *Exp Parasitol* 2012; **132**: 308-312.