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ORIGINAL ARTICLE

Genotyping of *Echinococcus granulosus* isolates from livestock based on mitochondrial cox1 gene, in the Markazi province, Iran

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Abstract Hydatidosisis a parasitic disease caused by the larval stage of *Echinococcus granulosus* with different genotypes, and major complications in vital organs such as liver, lungs and, brain. Also, this parasite can infect animals and cause economic damages. Recently, some investigations indicated that the genetic variation of the parasite affects the antigenic, immunogenic and pathogenic features. Therefore, present study conducted to genotyping of the *E. granulosus* larva based on mitochondrial cox1 gene in livestock in the endemic areas of Markazi province, Iran. In this study, 49 hydatid cysts samples collected from 36 sheep, 11 goats and 2 cattle from different slaughterhouses of Markazi province in central part of Iran, 2017. The mitochondrial cox1 gene was amplified and genotyping were accomplished using sequence analysis. The sequencing analysis indicated that the main genotype G1 (61%) and G3 (37%) were identified. Also, one of the samples shows similarity with the G2 (2%) genotype. The results showed the statistically significant differences between the genotypes in different livestock ($P < 0.05$). This study indicated that the main genotypes of *E. granulosus* in Markazi province are G1 and G3 which are related to dog/sheep strain. Therefore, parasite control in dogs and sheep can reduce the risk of transmission of infection to humans.

Keywords *Echinococcus granulosus* · cox1 · Livestock · Iran

Introduction

Hydatidosis is a parasitic disease caused by the larval stage of *Echinococcus granulosus* (Fadakar et al. 2015). The Hydatidosis prevalence has been raised in recent decades. The geographical distribution of the disease is depended on its host, for instance sheep as interface Host. The *E. granulosus* prevalence is higher in Moderate climate such as, Mediterranean, Asia, China, Russia and the north of the Africa (Grosso et al. 2012). This disease can cause the major complications in vital organs such as, liver, lungs and, brain. Also, this parasite can infect animals and cause economic damages (Rokni 2009). There are different investigations conducted in Iran for assessment of phonotypical and genotypic features of *E. granulosus* by using mitochondrial cox1 and Nad1 genes (Dousti et al. 2013; Mitra Sharbatkhori et al. 2011). The investigations introduce ten genotypes for this parasite which they called as G1 to G10 (Sharbatkhori et al. 2011; Nejad et al. 2010). The inducted studies showed that the major genotype in Iran and also the worldwide is the G1 (Harandi et al. 2002; Kinkar et al. 2018).

Recent investigations indicated that the genetic variation of the parasite affects the antigenic, immunogenic and pathogenic features (Kinkar et al. 2018; Eckert et al. 2001; Gholami et al. 2011; Romig et al. 2015). The parasite dominate genotype is different in the geographical regions which it indicate the importance of the genotyping the

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parasite in different geographical regions (Eckert et al. 2001; Gholami et al. 2011; Romig et al. 2015; Kinkar et al. 2018). The genotyping of the parasite can be important in prevention of transmission and the treatment in human populations. In a previous study, the high prevalence of the Echinococcosis was reported in livestock in Markazi province in the central part of Iran (Ghasemikhah et al. 2015).

Therefore, present study conducted to genotyping of the *E. granulosus* larval stage based on mitochondrial cox1 gene in livestock in the endemic areas of Markazi province, Iran.

Materials and methods

Sampling and sample preparation

Totally, 100 hydatid cyst specimens were collected from the slaughter houses in Markazi province, located in central part of Iran in 2017. The specimen enrolled into the current cross-sectional study was 49 hydatid cysts from 36 sheep, 11 goat and 2 cattle isolates from different slaughterhouses which confirmed macroscopically and microscopically. Also, this project supported by Ethical Committee of Hamadan University of Medical Sciences (Code no: P.16.35.6.399).

Nucleic acids extraction

DNA was extracted from the samples by using the commercial DNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. Evaluation of purified nucleic acids was performed by using Nano Drop ND-1000® spectrophotometry in 260 nm (Thermo Fisher Scientific Inc., Waltham, MA, USA). Extraction product DNA was kept at − 20 °C.

Mitochondrial PCR amplification

A conventional-PCR (cPCR) performed for detection of *E. granulosus* by mitochondrial cox1gene. The forward primer (JB3) (TTT TTT GGG CAT CCT GAG GTT TAT) and reverse (JB4.5) (TAA AGA AAG AAC ATA ATG AAA ATG) were used by 450 bp amplicon sizes (Bowles et al. 1992). The reaction mix contains 0.5 µM of template DNA or controls, 0.5 mM of dNTP mix, 1 µM of each primer, 1.5 µM $MgCl_2$, and 0.5 units/µl of Taq DNA polymerase (Fermentas GmbH, Germany), 2 µl PCR buffer and sterilized DW added to round out the total volume to 15 µl for each reaction. A Bio-Rad thermo cycler (T100™ Thermal Cycler) was used for heating programs. Thermo cycler heating protocol was included 5 min at 95 °C, 40 cycles of the 60 s at 90 °C, 30 s at 60 °C, 120 s at 72 °C and one final extension step at 72 °C for 10 min. Also, PCR products gel electrophoresis was performed by Tris-Boric Acid-EDTA 1 × buffer system and the 1.5% agarose gel stained by Ethidium bromide for visualization the UV.

Sequencing

The cox1-PCR products were sequenced employing the same primers conducted in the primary PCR. The obtained data were aligned by the reference genotypes of *E. granulosus* in Gene bank to determine the genotypes using CLC Main Workbench 5 software

Statistical analysis

Statistical analyses and the basic descriptive and frequency variables performed by SPSS version 16 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered with P values < 0.05.

Results

The PCR amplification of 49 hydatid cyst samples have shown 450 bp band for cox1 gen and genotypes were determined based on sequencing (Fig. 1). The result indicated that the main genotype in of *E. granulosus* in this study was G1. The G1 isolates prevalence in this study was 61% and follows by G3 37% in samples. The additional data of genotypes are shown in Table 1.

Also, in this study the prevalence of G1 genotype of *E. granulosus*have shown 57.5% in sheep, 81.8% in goats. Significant difference was found in the different livestock and genotypes ($P < 0.05$), but no significant differences with the involved organ (Table 1). The majority of the hydatid cyst samples isolates in this study obtained from liver (40 samples) ($P > 0.05$).

Discussion

This study is conducted to determined genotypes by mitochondrial cox1 gene of *E. granulosus* larval stage isolates from sheep, goats and cattle in the Markazi province, central part of Iran. The main reason for using mitochondrial genes is this genes conservation (Kinkar et al. 2017). The result of the current study indicated that the main genotype in of *E. granulosus* larval by cox1 gene is G1. The G1 isolates prevalence in this study was 61% and follows by G3 37% in our samples. In the similar study conducted by Shahnazi et al., in 2014 indicated that the major genotype of the *E. granulosus* in Esfahan, Iran is the G1 genotype (Shahnazi et al. 2011). Also, study in Isfahan

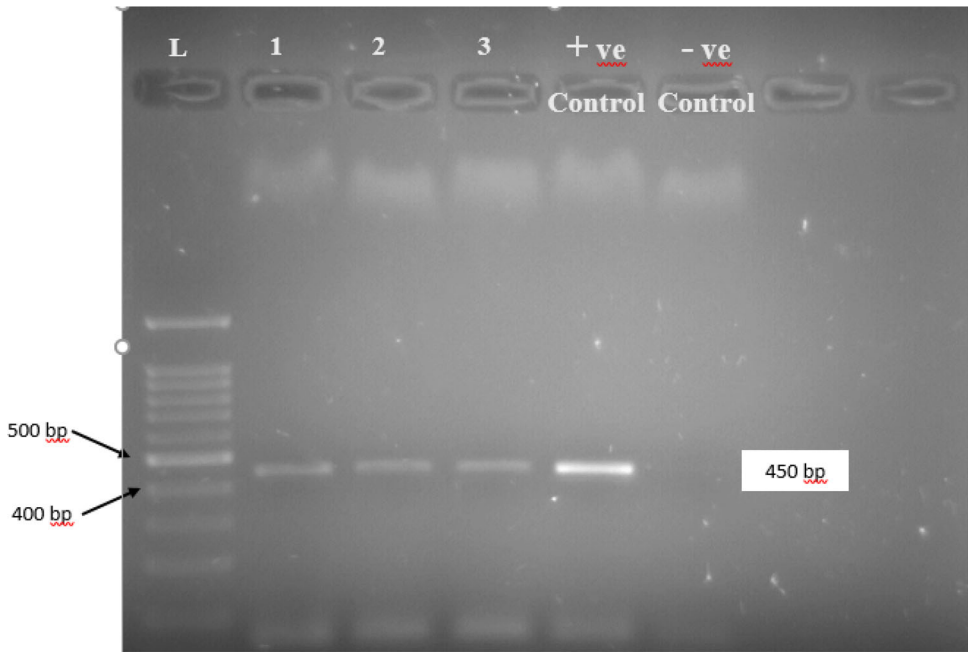

Fig. 1 *E. granulosus* DNA amplification by PCR with COX1 gene on gel electrophoresis 1.7% agaros. All three samples shows sheep isolates in this figure, C+: positive control; C−: negative control, DNA marker 100 bp

Table 1 Genotypes distribution of *Echinococcus granulosus* in different livestock in the Markazi province, Iran

Genotype		Livestock				Organ		
Name	Count (%)	Sheep (%)	Goat (%)	Cattle (%)	*P* value	Liver (%)	Lung (%)	*P* value
G1	30 (61)	21 (57.5)	9 (81.8)	0	0.037	23 (57.5)	7 (77.8)	0.227
G2	1 (2)	0	0	1 (50)		1 (2.5)	0	
G3	18 (37)	15 (41.5)	2 (18.2)	1 (50)		16 (40)	2 (22.2)	

confirmed the G1 genotype of the *E. granulosus* in 74.2% of samples and induce this genotype as major genotype in this province. In the other study by Dousti et al., in 2014 G1 reported as the main genotype in Ilam, Iran (Dousti et al. 2013). The results in our study confirm these data and indicate the importance of the G1 genotype in Iran. Also, the differences in the reported prevalence are due to the difference in the samples in these studies.

In our study, the major genotype of *E. granulosus* was G1 in sheep, goat isolates and cattle isolates G2 and G3 genotype. Also, in compare to study in the north of Iran it is been showed the 100% of sheep isolates were G1 (Fadakar et al. 2015). The conducted results from these study confirmers our result in the association of the livestock and genotype. Furthermore, in the study conducted in 2016 by Hizem et al. has shown that there is no association between the infected organ and the *E. granulosus* infected genotype. Also, there was no difference between the infected organ and parasite genotype. The result from present study also confirmers this finding (Hizem et al. 2016).

Some of conducted studies and their result about the genotypes of *E. granulosus* in Iran is summarized in Table 2. Khademvatan et al. (2018) in a review study on 73 documents from Iran different regions, indicated that the major genotypes are G1, G6 and, G3 which they have been reported in 320, 13 and 7 cases respectively (Khademvatan et al. 2018). Also, Sharafi et al., in 2014 investigated the genotypes prevalence of *E. granulosus* in Iran. Their result shows that G1, G2, G3 and G6 are the most common genotypes in humans in Iran (Sharafi et al. 2014). The differences in these studies and the present study could be justified by differences in samples and sample size. Our present study indicated that the main genotypes in Markazi province are G1 and G3 which are related to sheep strain. In the life cycle of these genotypes, the dog is the definitive host and livestock are known as intermediate host. Therefore, parasite control in dogs and sheep can reduce the risk of transmission of infection to humans. Investigations conducted in this field show the needs for the further studies about the association of the genotypes and different

Table 2 The genotypes of *E. granulosus* reported from different provinces of Iran

Province	Host (genotype)	References
Isfahan	Human (G1, G6), goat (G1), cattle (G1, G6), camel (G1, G6)	Shahnazi et al. (2011)
Ilam	Human (G1, G3), sheep (G1, G3)	Shamsi et al. (2015)
Tehran	Human (G6)	Sadjjadi et al. (2013)
Kerman	Human (G6), sheep (G1, G3), goat (G1), cattle (G1, G3), camel (G1, G3)	Hajialilo et al. (2012)
Golestan	Human (G1, G6), sheep (G1), cattle (G1), camel (G1, G6)	Gholami et al. (2012)
Zanjan	Human (G1, G3), sheep (G1, G3), cattle (G1, G3)	Haniloo et al. (2013)
Tehran	Human (G1, G3, G6)	Nikmanesh et al. (2014)
Lorestan	Dog (G1, G2, G3)	Parsa et al. (2012)
Khuzestan	Human (G1), sheep (G1), goat (G1), cattle (G1)	Khademvatan et al. (2013)
Kohgiluyeh and Boyer-Ahmad	Human (G1), sheep (G1), cattle (G1)	Sadri et al. (2012)
Ardabil	Human (G1, G3), sheep (G1, G3), goats (G1), cattle (G1, G3)	Pezeshki et al. (2013)
Khorasan	Human (G1, G6)	Gezehegn et al. (2017)
Alborz, Tehran, Kerman	Human (G1, G2, G3, G6)	Rostami et al. (2015)
Golestan	Human (G1), sheep (G1), goat (G1), cattle (G1, G2, G3), camels (G1, G3, G6), buffalo (G1)	Sharbatkhori et al. (2016)

types of livestock. The major limitation of this study could be the sample size.

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Author's contributions This study was conducted by BA, AHM, BK, MF, MM, SG, ASP, and RG. Also, RG designed the study. Laboratory evaluation was performed by BA, AHM and BK. Manuscript preparation was conducted by MF, MM, SG, ASP and RG. BA and AHM participated in the data analysis. BA and RG performed the statistical analysis. All authors includes BA, AHM, BK, MF, MM, SG, ASP and RG read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest There was not any conflict of interests by all authors.

Ethical standard All experimental procedures were approved by the Ethics Committee of Arak University of Medical Sciences, Iran.

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