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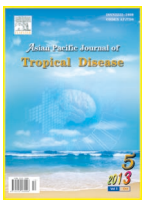

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Genotypic characterization of *Echinococcus granulosus* in Iranian goats

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors evaluated the genotypic characterization of *E. granulosus* in Iranian goats that were found to be different between isolates. The results are interesting and suggested that G1 in goats plays as an important reservoir host where sheep lives.
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ABSTRACT

Objective: To isolate and characterize the genotype of *Echinococcus granulosus* (*E. granulosus*) from goats in Mazandaran Province, Northern Iran.
Methods: A total of 120 goats were screened from abattoirs of Mazandaran Province, Northern Iran. Forty out of 120 samples were infected with cystic echinococcosis and 29 out of 40 infected samples were fertile hydatid cysts (containing protoscolices) which were collected from the livers and lungs of infected goats. DNA samples were extracted from the protoscolices and characterized by mitochondrial DNA sequencing of part of the mitochondrial cytochrome C oxidase subunit 1 gene.
Results: Sequences analysis of nine fertile hydatid cysts indicated that all isolated samples were infected with the G1 sheep strain and two sequences were belonged to G1[4] and G1c microvarients of the G1 genotype.
Conclusions: The results showed that goats act as alternative intermediate hosts for sheep strain. G1 genotype seems to be the main route of transmission and it should be considered in further studies.

KEYWORDS
Echinococcus granulosus, Genotype, Microvarients, CO1, Goat

1. Introduction

Echinococcus granulosus (*E. granulosus*) in carnivores and its larval stage (metacestode) in herbivores has been recognized as the most important helminthic disease. The WHO reported that cystic echinococcosis (CE) is one of the seven important neglected diseases worldwide[1]. Metacestodes are found in sheep, goats, buffaloes, camels, horses, cattle and pigs, especially in their livers and lungs[2]. In Iranian goats, prevalence rate of CE in production animals reported in different provinces is 1.7%-29.4%. Also, prevalence rates of CE in other Iranian ruminants, such as sheep, cattle, buffaloes

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and camels have been reported between 1.3%-74.4%, 1.3%-38.3%, 9%-25.7% and 11%-35.2%, respectively[3-8]. The Iranian Veterinary Organization reported that the number of goats is approximately 25 800 000. The genotyping of *Echinococcus* strains is important for their biology. For several past decades taxonomic of the *Echinococcus* strains was done in many revisions[2,9,10]. At present 16 species and 13 subspecies were determined in the genus *Echinococcus*[11]. To date, ten genotypes (G1-G10) have been characterized within *E. granulosus* species complex by using mitochondrial data. This complex was divided into four species: *E. granulosus sensu stricto* (G1-G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6-G10). These genotypes are including sheep strain (G1), Tasmanian sheep strain (G2), buffalo strain (G3), horse strain (G4), cattle strain (G5), camel strain (G6), pig strains (G7 and G9) and Cervid strains (G8 and G10)[11]. G5 is genetically very distinct from the other genotypes[11]. So far, 3 genotypes (G1-G3) of *E. granulosus* and 5 genotypes (G6-G10) of *E. canadensis* were found in Iran[11-17]. Several intermediate hosts infected with hydatid cyst were reported in Iran[6,12,15-23]. Therefore, it is important to characterize the genotype variations of *E. granulosus* in endemic and hyper endemic regions of Iran. The epidemiological study demonstrates that Iran country has been divided into hyperendemic (northern part) and endemic areas (southern part)[24]. However, there is less informative about the genotypes of *E. granulosus* found in goats in northern Iran[22]. The present study aimed to isolate *E. granulosus* from goats by using genetic characteristics in Mazandaran Province, Northern Iran.

2. Materials and methods

2.1. Samples collection

From the spring to the winter of 2010, 120 goats were screened from abattoirs of Mazandaran Province, Northern Iran. Forty out of 120 samples were infected with CE and among them twenty nine (29/40) fertile hydatid cysts were collected from the livers and lungs of infected goats. All hydatid cysts were obtained under aseptic conditions. Cyst contents were aspirated and examined under a light microscope to confirm the presences of protoscolices. The protoscolices were washed three times with phosphate buffered saline (pH 7.2). Viability of protoscolices was checked by using 0.1% aqueous eosin stain and by observing flame cell activity[25]. Dead protoscolices absorbed eosin and colored red, but alive protoscolices remained colorless. In order to make molecular analysis, protoscolices were stored in 70% ethanol at 4 °C.

2.2. DNA extraction and polymerase chain reaction (PCR)

The genomic DNA was extracted from 50 μL of protoscolices in each 29 fertile hydatid cysts using a DNA purification kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. DNA was stored at -20 °C till molecular analysis. The fragment of 440 bp of mitochondrial cytochrome C oxidase subunit 1 (CO1) gene was amplified using the forward primer (J3: 5′-TTTTTTGGCCATCCTGAGGTTTAT-3′) and reverse primer (J4.5: 5′-TAACGACATAACATAATGAAAATG-3′) which were designed based on the mitochondrial G1 genome sequence[26]. The PCR program was performed by one cycle primary denaturation (5 min at 95 °C), followed by 35 cycles of denaturation step (45 s at 94 °C), annealing step (45 s at 56 °C) and final extension (72 °C for 10 min). Double distilled water instead of DNA was included in each set of PCR reaction as the negative control.

2.3. Sequence analysis

The PCR products were purified by using PCR product purification kit (Fermentas) according to the manufacturer's instructions. DNA sequencing was performed for nine of the samples in two directions (forward and reverse primers) by Sanger's method (Kowsar Biotech Company) in Iran. The sequencing chromatograms were analyzed by the Chromas software (version 3.1) and were compared by BLAST in GenBank to other registered sequences. The sequence of nucleotides was aligned using the multiple alignments clustal W method in Lasergene's MegAlign Program (DNA Star).

2.4. Phylogenetic analysis

The phylogenetic analysis was done based on alignments obtained from clustal W of a 440 bp sequence. The phylogenetic tree was constructed by applying MegAlign (DNA Star) Program. A correspondent nucleotide sequence of *Taenia crassiceps* (GenBank accession number: AB033411) was used as an out group. Evolutionary distance (nucleotide substitutions: ×100) was obtained using the general time reversible evolutionary model, arranging for a γ-shaped variation in mutation rates between codons. The extracted DNA was separately used to amplify the CO1 gene.

3. Results

The PCR product demonstrated an expected fragment of 440 bp in length. Nine PCR products from hydatid cysts of goats were sequenced. The BLAST analysis of the sequences indicated that all nine isolates were of the common sheep strain (G1). The phylogenetic tree relationship between the nine isolated sequences, various genotypes of *E. granulosus* and microvariants of G1 genotype was shown in Figure 1. The sequencing results of nine isolates were documented as G1 that registered in the GenBank under the following accession numbers: JN792923, JN792924, JN792925, JN792926, JN792927, JN792928, JN792929, JN792930, and JN792931. Sequences of the different genotypes of *E. granulosus* (G1, G2, G3, G4, G5, G6 and

G7) and the microvariants of the G1 genotypes (G1A, G1B, G1C, G1D, G1E and G1[1], G1[2], G1[3], G1[4]) deposited in the GenBank as references (accession numbers: M84661/62/63/64/65/66/67, AF458871/72/73/74/75 and EF393619, EF595654, EU178103, EU178104, respectively)[27-29]. Based on molecular reports[12-20], 8 genotypes of *E. granulosus* were found in different provinces of Iran (Figure 2).

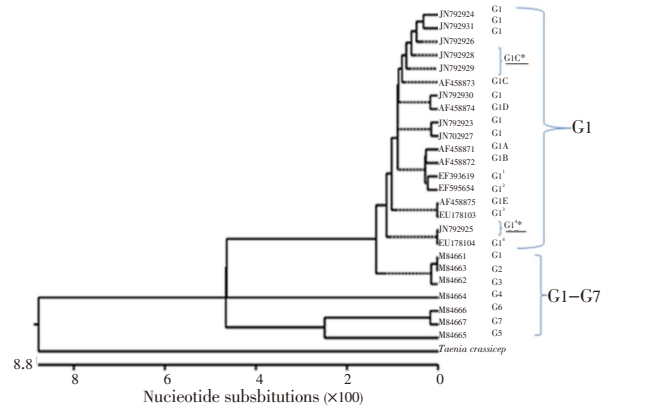

Figure 1. The phylogenetic relationships between genotypes G1-G7 as reference sequences and *Echinococcus* isolates from goats obtained from Mazandaran, Iran. *Taenia crassiceps* was used as the outgroup. Microvariants of G1 genotype originated from goat isolates were characterized by asterisk/underline.

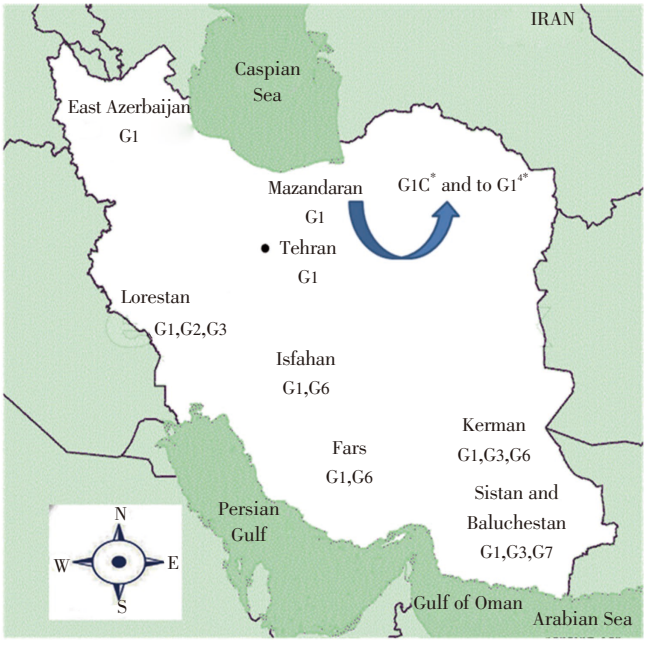

Figure 2. Geographical areas of *E. granulosus* genotypes and microvariants in map of Iran based on present study (asterisk) and previous studies. Strains of G6-G10 have been reported in different geographical locations without exact determination of geographical regions in Iran map.

4. Discussion

Until now, few studies have been done on the genotypes of *E. granulosus* originating from goats in Iran. Albeit, 3 genotypes of *E. granulosus* (G1-G3) and 5 genotypes of *E. canadensis* (G6-G10) have been reported in production animals in different provinces of Iran[11-17]. The present study is the secondary strain characterization of goats in Mazandaran, Northern Iran[22]. The prevalence rate of CE in Iranian goats, cattle, buffaloes and camels has been reported between 1.7%-29.4%, 1.3%-74.4%, 1.3%-38.3%, 9.0%-25.7% and 11.0%-35.2%, respectively. These statistics shows that CE should be considered as a hyperendemic disease in the province of Mazandaran[24]. Incidence rates of human hydatidosis are up to 1.18/100 000 in Mazandaran where sheep and goat for heterogeneous and symbiosis are played as important intermediate hosts[30]. In this current study CO1 gene was used as one of the applicable targets for identification of strains, genotypes and microvariants of *Echinococcus*[14,31]. The CE prevalence in studied goats was 33.3% (40/120). Of 120 samples from goats, 29 isolates (24.1%) were fertile hydatid cysts and 9 isolates (31%) from 29 isolates were genetically studied and were characterized as G1 (sheep strain) genotype. In the present study, absence of G6 genotype in Mazandaran's samples could be due to the low samples analyzed since in previously reports only 15% of the samples were from G6 genotype[22]. The G1 genotype of *E. granulosus* is the dominant strain both in animals and human in Iran and around the world[32]. This study demonstrated that the sheep strain was found in all of the isolated sequences based on the sequences of CO1 gene so it seems that goats are a good intermediate host for the sheep strain. Also, G1 microvariants were characterized based on cox1 gene. Generally the microvariants differ from other groups of the same species in gene frequencies. This variability may be described on the basis of differences in nucleic acid sequences[2]. The results of microvariant showed that two isolated sequences (accession numbers: JN792928, JN792929) belonged to G1c microvariant (accession number: AF458873) and one isolated sequence (JN792925) belonged to G1[4] microvariant (accession number: EU178104). At present several studies have been performed on the genotypes/strains of *E. granulosus* originating from goats in Iran[12,16,17,20,22,23]. G1 strain has been reported in goat's isolates from Spain and Turkey[33-35]. Although, a study has reported goats acted as reservoir host for *E. canadensis* (G6) in Neuquen, Argentina[36]. Another study in India showed goats acted as a new host for the G3 buffalo strain of *E. granulosus*[37]. The results of the present study are in agreement with studies demonstrating that sheep strain of *E. granulosus*, is present in livestock in Iran[6,12,15-23,38]. The study of genotyping hydatid cyst in important intermediate hosts in Iran endemic areas is essential to characterize the life cycle, host specificity and classification of *E. granulosus* strains. Although more numbers of goats are required to be surveyed in different parts of Iran. It would be shown that goats in Northern Iran may play a key role in the epidemiology of CE in Iran. All together, our results from analysis of goat isolates confirmed the existence of the G1 in province of Mazandaran, however G1 is considered as the predominant genotype in sheep, cattle and goats. This paper presents the G1[4] and G1c microvariants of the G1 genotype from goats in Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

E. granulosus in carnivores and its larval stage (metacestode) in herbivores has been recognized as the most important helminthic disease. The WHO reported CE is one of the seven important neglected diseases worldwide. Metacestode is found in sheep, goats, buffaloes, camels, horses, cattle and pigs, especially in their livers and lungs. In Iranian goats, prevalence of CE were 1.7%-29.4%. The Iranian Veterinary Organization reported the numbers of goats are approximately 25 800 000. Sixteen species and 13 subspecies were determined in the genus *Echinococcus*. To date, ten genotypes (G1-G10) have been characterized within *E. granulosus* species complex by using mitochondrial data.

Research frontiers

It is important to characterize the genotype variation of *E. granulosus* in endemic (southern) and hyper endemic (northern) regions of Iran. There is less information about the genotypes of *E. granulosus* found in goats in northern Iran. The study isolated *E. granulosus* from goats by using genetic characteristics in Mazandaran province, Northern Iran.

Related reports

Viability of protoscoleces was checked by using the eosin test and by observing flame cell activity.

The fragment of 440 bp of mitochondrial CO1 gene was amplified using the forward primer (J3: 5′-TTTTTTGGCCATCCTGAGGTTTAT-3′) and reverse primer (J4.5: 5′-TAACGACATAACATAATGAAAATG-3′) which based on the mitochondrial G1 genome sequence.

The sequencing results of nine isolates were documented as G1 that registered in the GenBank under the following accession numbers: JN792923, JN792924, JN792925, JN792926, JN792927, JN792928, JN792929, JN792930, and JN792931. Sequences of the different genotypes of *E. granulosus* (G1, G2, G3, G4, G5, G6 and G7) and the microvariants of the G1 genotypes (G1A, G1B, G1C, G1D, G1E and G11, G12, G13, G14) were deposited in the GenBank as references (accession numbers: M84661/62/63/64/65/66/67, AF458871/72/73/74/75 and EF393619, EF595654, EU178103, EU178104, respectively).

Innovations & breakthroughs

Sequences analysis of nine fertile hydatid cysts found in Iranian goats indicated that all isolated samples were of the G1 strain and two sequences belong to G1[4] and G1c microvarients of the G1 genotype.

Applications

There are 10 genotypes (G1-G10) in *E. granulosus*. Three genotypes of *E. granulosus* (G1, G2 and G3) and one genotype of *E. canadensis* (G6) were found in Iran. Goat is an alternative intermediate host for sheep strain where sheep and goats have symbiosis living together. G1 genotype seems to be the main route of transmission.

Peer review

This is a good study in which the authors evaluated the genotypic characterization of *E. granulosus* in Iranian goats that were found to be different between isolates. The results are interesting and suggested that G1 in goats plays as an important reservoir host where sheep lives.

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