



Genotyping of *Echinococcus granulosus* isolated from canine in Northwest Iran

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Abstract

Echinococcus granulosus is a parasitic tapeworm that causes cystic echinococcosis, a potentially life-threatening zoonotic infection affecting humans and animals across the globe. In Iran, the prevalence of this parasite remains a significant public health concern, particularly in the northwest region. This study aimed to investigate the genotypes of *E. granulosus* isolated from canines in the northwest of Iran. A total of 87 samples were collected from the Mughan plain area in Ardabil province, including 47 stray dogs (*Canis familiaris*), 25 golden jackals (*Canis aureus*), and 15 red foxes (*Vulpes vulpes*), and molecular analysis was performed for partial cytochrome c oxidase subunit 1 and nad1 genes. Phylogenetic analyses were carried out on the obtained sequence. The findings revealed that 9 out of 87 (10.3%) samples were infected with *Echinococcus* parasites, with a frequency of 1 (4%) and 8 (17%) among golden jackals and stray dogs, respectively. Overall, all (100%) *E. granulosus* adult samples were related to the G1 genotypes. This study provides comprehensive data regarding the epidemiological and molecular characteristics of echinococcosis in canines in northwest Iran.

Keywords Echinococcosis · Genotype · Canine · Iran

Introduction

Echinococcosis is a zoonotic disease caused by helminths, which includes alveolar echinococcosis (AE) and cystic echinococcosis (CE) (Khalkhali et al. 2018). This disease has been studied in various countries as it is a major zoonotic infection and one of the most widespread parasitic diseases (Ebrahimipour et al. 2017). Dogs and wild canines such as jackals, wolves, and foxes are the definite hosts of *Echinococcus*, while ruminants, horses, camels, and even humans are considered intermediate hosts (Mirahmadi et al.

2021). The eggs of adult worms are scattered in the environment through the feces of canids, which can be eaten by humans and ruminants. In Iran, stray dogs and wild canines are found in large populations near human societies and agricultural farms (Heidari et al. 2019). *E. granulosus* sensu lato and *E. multilocularis* are the two species that are relevant to echinococcosis. They are responsible for virtually all the human and animal burden of the disease, causing human cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively (Casulli et al. 2022). Identification of strains of *E. granulosus* in dogs is crucial in parasite control and eradication. According to molecular studies of NADH dehydrogenase 1 and cytochrome c oxidase subunit 1 (CO1), ten distinct genotypes (G1–G10) of *E. granulosus* have been reported in intermediate and definitive hosts worldwide (Khademvatan et al. 2019). Prevalence and genotyping studies of echinococcosis are of great importance for epidemiological understanding and hydatid disease control. Due to the high importance of wild carnivores in the transmission of CE, epidemiological and molecular studies in this type of host are necessary in endemic regions (Grech-Angelini et al. 2019). High prevalence rates of echinococcosis have been reported in Eurasia, Africa, Australia, and South America. CE is considered a global public health concern and is

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endemic in many parts of the world, including the Middle East (Galeh et al. 2018). In different parts of Iran, the mean prevalence of echinococcosis is 8.8% with a range from 8.1 to 11.9% (Khalkhali et al. 2018). However, more studies are required to identify different genotypes of *E. granulosus* involved in canine echinococcosis. This study aimed to investigate the prevalence and molecular identification of *E. granulosus* in canines in northwest Iran.

Materials and methods

The terminology recommended by the World Association of Echinococcosis (Vuitton et al. 2020).

Study area and participants

Between January 2018 and December 2021, a total of 87 samples were collected from canines in the Mughan Plain area of the Ardabil Province in northwest Iran, which included 47 stray dogs (*Canis familiaris*), 25 golden jackals (*Canis aureus*), and 15 red foxes (*Vulpes vulpes*). The specimens were collected from road accidents in the area during these years.

Parasitological procedure

The small intestine of the animals was removed and the alimentary canal was examined for parasites. The contents of the colon and epithelial scraping were washed and examined under a stereomicroscope for parasite presence. Isolated specimens were preserved in 96% alcohol for DNA analysis.

DNA extraction and multiplex PCR

Genomic DNA extraction was performed on four randomly selected worms from each infected animal using the DNA extraction kit (Takapouzist, Iran). The mitochondrial genes, Cox1 and Nad1, were amplified using specific primers. Polymerase chain reaction (PCR) products were analyzed by gel documentation system. Bidirectional sequencing of the Cox1 and Nad1 genes from the selected PCR amplicons was performed by Pishgam Company for haplotype identification (Tehran, Iran).

Sequencing and phylogenetic analysis

The sequences were compared using the BioEdit software ver: 7.2 (<https://bioedit.software.informer.com/7.2/>) and the alignment was performed using the ClustalW module. A phylogenetic analysis was performed using the maximum likelihood method with 1000 bootstrap replicates (MEGA7.0).

Table 1 The frequency of collected samples based of canine's gender

| Canine | No. | Gender | Number | Percentage | Infected with <i>Echinococcus</i> (%) |
|----------------|-----|--------|--------|------------|---------------------------------------|
| Stray dogs | 47 | Male | 37 | 78.7 | 6 (16.2%) |
| | | Female | 10 | 21.3 | 2 (20%) |
| Golden Jackals | 25 | Male | 10 | 40 | 1 (10%) |
| | | Female | 15 | 60 | 0 |
| Red foxes | 15 | Male | 8 | 53.33 | 0 |
| | | Female | 7 | 46.7 | 0 |
| Total | 87 | Male | 55 | 63.2 | 7 (12.7%) |
| | | Female | 32 | 36.8 | 2 (6.2%) |

Table 2 The relationship between age and parasitic infection in the collected domestic and wild canines

| Animal | Life stage | Number | Infection | P-value |
|------------|------------|--------|-----------|---------|
| Total (87) | Young | 69 | 47 | 0.363 |
| | Aged | 18 | 13 | |

Results

Epidemiological survey

According to our study, out of 87 samples taken from canines, 55 (63.2%) were male and 32 (36.7%) were female. We found no significant difference between the prevalence of *Echinococcus* infection in females (6.2%) and males (12.7%) (P-value > 0.05) (Refer to Table 1). A total of 9 out of 87 (10.3%) canine samples were found to be infected with the *Echinococcus* parasite. The results further indicated that while the frequency of *Echinococcus* sp. parasites was 1 (4%) in golden jackals, it was significantly higher (8 or 17%) in stray dogs. The results showed that, there is no significant relationship between age (0.363), and gender of infected animals (0.085), with parasitic infection (Tables 2 and 3).

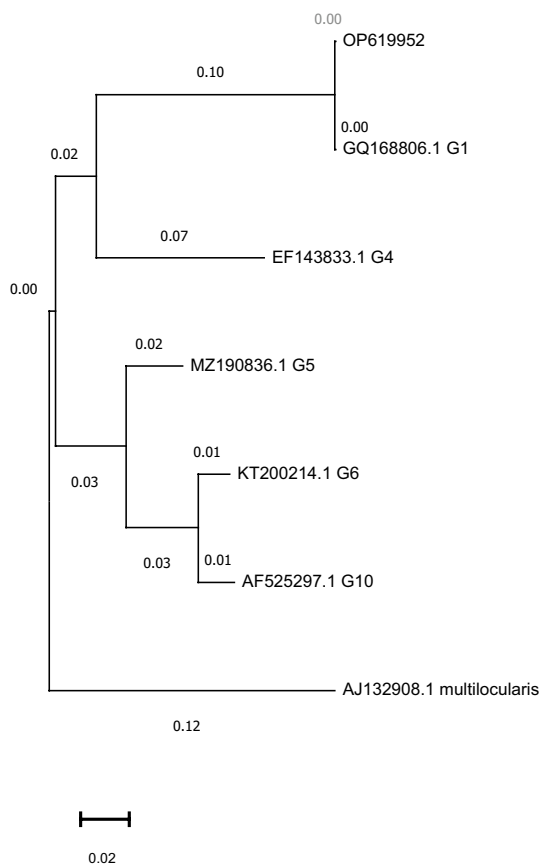
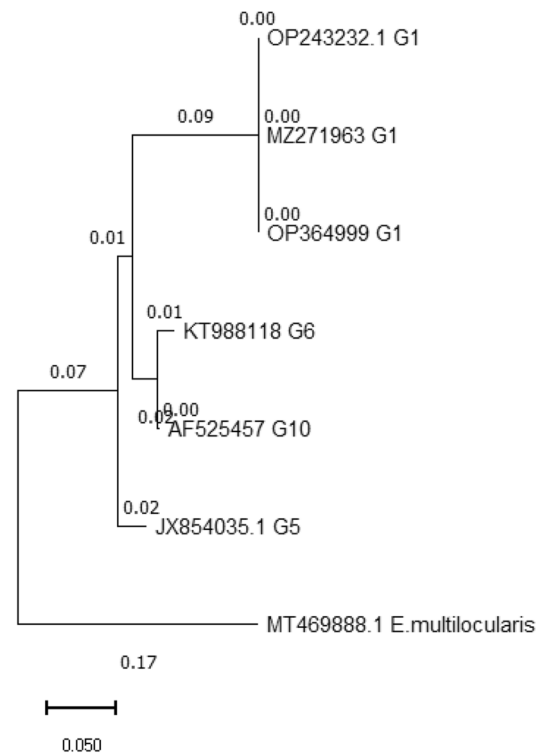
Molecular study

Our study successfully performed molecular amplification of the cox1 and nad1 genes, resulting in 375 and 471 bp amplicons, respectively, for all isolated samples. Furthermore, all amplified samples were successfully sequenced for both genes. The cox1 (accession No.: OP364999-OP365007) and nad1 (accession No.: OP619952-OP619960) sequences of isolates obtained in the present study were registered in NCBI GenBank. Our

Table 3 The relationship between gender of animals and parasitic infection in the collected domestic and wild canines

| Canine | No. | Gender | Number | Percentage |
|----------------|-----|--------|--------|------------|
| Stray dogs | 47 | Male | 37 | 78.7 |
| | | Female | 10 | 21.3 |
| Golden Jackals | 25 | Male | 10 | 40 |
| | | Female | 15 | 60 |
| Red foxes | 15 | Male | 8 | 53.33 |
| | | Female | 7 | 46.7 |
| Total | 87 | Male | 55 | 63.2 |
| | | Female | 32 | 36.8 |

results indicate that all nine positive isolates were related to the G1 genotype with no intra-genotype polymorphism observed in any of the *cox1* and *nad1* gene sequences (Figs. 1 and 2). Additionally, no deletions or insertions were observed in the acquired data from the G1 genotype of the *cox1* gene.

**Fig. 1** Phylogenetic analysis of *Echinococcus granulosus* isolates using *nad1* genes. The phylogenetic tree is drawn to scale, with lengths of the branch measured in the number of substitutions per site. *Echinococcus multilocularis* (aj132908) was used as the out-group**Fig. 2** Phylogenetic analysis of *Echinococcus granulosus* isolates using *cox1* genes. The phylogenetic tree is drawn to scale, with lengths of the branch measured in the number of substitutions per site. *Echinococcus multilocularis* (MT469888) was used as the out-group

Discussion

Echinococcosis prevalence in definitive hosts remains unclear in different parts of Iran (Khalkhali et al. 2018). Previous investigations on the epidemiology of echinococcosis in definitive hosts in Iran, such as golden jackals and stray dogs (Siyadatpanah et al. 2019, Siyadatpanah et al. 2020), have highlighted the need for further research in this area. To our knowledge, this study is the first molecular characterization of *E. granulosus* isolated from canines in the Ardabil province. We found that pronounced *E. granulosus* s.s. infections are present in golden jackal and stray dog populations in the northwestern part of Iran. Furthermore, the prevalence of *E. granulosus* in stray dogs (17%) suggests that these animals are susceptible species with the potential to spread the worm. However, the prevalence of *E. granulosus* s.l. (1.7%) suggests a mild contribution of jackals to the maintenance of this parasite in the studied areas.

Previous studies have shown that golden jackals can contribute to the transmission of both *E. multilocularis* and *E. granulosus* s.l. However, in our study, no infections among red foxes were detected contrary to previous studies in the same region of Iran (Mirahmadi et al. 2021). In Iran, several

studies have been conducted on the epidemiology of echinococcosis in dogs, and the mean prevalence of cystic echinococcosis (CE) has been shown to range from 8.1 to 11.9%, with a mean of 8.8% (Khalkhali et al. 2018). We found that male dogs were more infected with echinococcosis than females, although this difference was not statistically significant ($p > 0.05$).

The infected animals in our study lived in urban and peri-urban areas, and the high frequency of parasites in these animals is a risk factor for human echinococcosis in Iran. Molecular analysis was performed on all isolated *Echinococcus* samples in our study, and all were characterized as the g1 genotype of *E. granulosus*. Determining different genotypes of *E. granulosus* from isolated samples is important for the prevention of echinococcosis in endemic regions (Spotin et al. 2018). The various genotypes of *E. granulosus* exhibit different prevalence, longevity, morphology, period of egg production, host specificity, geographical distribution, and pathogenicity (Berenji et al. 2019). Among the various genotypes, the G1 genotype of *E. granulosus* has garnered significant attention due to its association with high infectivity and pathogenicity to humans (Laurimaa et al. 2015). Previous studies on the molecular identification of echinococcosis in Iran have identified G1–G3 and g6 genotypes from definitive hosts (Heidari et al. 2019). Similarly, a study from another part of the world reported G1 as the main genotype in naturally infected dogs (Kim et al. 2020). One of the major limitations of this study was the relatively small sample size of *E. granulosus* isolates analyzed. As a result, the findings may not be fully representative of the entire canine population in northwest Iran, potentially limiting the generalizability of the results.

Conclusion

The findings of the current study revealed valuable data about the epidemiological and molecular characteristics of echinococcosis in canine as a definitive host in Ardabil, Northwest Iran. Its suggest to utilize advanced molecular techniques such as next-generation sequencing or microsatellite analysis to obtain more detailed genetic information about *E. granulosus* isolates. This could help determine the specific genotypes and haplotypes prevalent in different areas of northwest Iran. The increasing public health concern of echinococcosis requires surveillance and early diagnosis of the infection in at-risk populations in the country.

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Authors' contribution ZH, BA and BMG: Corporate in the design of the study, performed the experiment, and drafting the manuscript; ZZ, MM and ZH: Design of the study, analysis of the result and drafting the manuscript; ZH: critical analysis of the data and drafting the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflict of interests.

Ethical approval Ethical approval was obtained from the Ardabil University of Medical Sciences (IR.ARUMS.REC.1396.159).

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