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Original Article

Molecular Identification and Genetic Diversity of *Echinococcus granulosus* Sensu Stricto in Livestock from Western and Southwestern Iran

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Abstract

Background: Cystic echinococcosis (CE) is a long-standing, neglected parasitic zoonosis caused by infection with the larval stage of the *Echinococcus granulosus* (*E. granulosus*) tapeworm, affecting both animals and humans with significant health and economic impacts. We investigated the molecular epidemiology of CE in livestock populations in two provinces (Lorestan and Khuzestan) in western and southwestern Iran from 2024 to 2025.

Methods: Overall, 120 CE isolates were collected from infected livestock lung and liver samples at slaughterhouses. After DNA extraction from the germinal membrane of the cysts, PCR-RFLP analysis was performed on the *ITS1* fragment using the restriction enzyme *HpaII* to assess genetic variation. Additionally, twenty *mitochondrial NADH dehydrogenase subunit 1* (*ND1*) amplicons were directly sequenced to identify strains and haplotypes.

Results: One hundred and twenty CE isolates were collected from slaughterhouses in several cities across Lorestan and Khuzestan provinces, including 70 from sheep, 25 from cattle, 10 from buffalo and 15 from goats. RFLP analysis indicated that all isolates belonged to *E. granulosus* sensu stricto (s.s.). Twenty CE DNA samples were randomly selected for *ND1* partial gene amplification. Of these, 14 sequences were characterized as the G1 genotype of *E. granulosus*, while 6 were identified as G3. Low haplotype and nucleotide diversity were observed among the *E. granulosus* s.s. sequences.

***Conclusion*:** This study confirms the presence of *E. granulosus* s.s. (genotypes G1 and G3) among livestock in Lorestan and Khuzestan provinces. To mitigate the associated public health risk, policymakers should prioritize educational programs for livestock keepers and farmers such as preventing dogs from consuming infected organs and restricting livestock access to areas contaminated with parasite eggs as part of integrated health initiatives.

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Introduction

Cystic echinococcosis (CE) is a neglected parasitic zoonosis caused by the larval stage of the *Echinococcus granulosus* sensu lato (s.l.) complex. Adult parasites inhabit the small intestine of dogs, jackals, wolves, and other canids, and their eggs are shed in feces (1). When eggs of *E. granulosus* are ingested by intermediate hosts including various herbivorous and omnivorous animals and, accidentally, humans, the oncosphere is released in the intestine, penetrates the gut wall, and migrates through the circulatory system to different organs, particularly the liver and lungs, where it gradually develops into hydatid cysts. Within the cyst, protoscoleces and daughter cysts form. The definitive host becomes infected by ingesting cyst-containing organs from infected intermediate hosts (2).

CE is widespread in many regions globally, affecting more than 50 per 100,000 people annually (3). Its prevalence in slaughtered livestock ranges from 20% to 95% (4). The WHO has classified CE as a neglected zoonotic disease with significant veterinary and public health implications (5). CE is most common in areas where livestock farming is prevalent and humans and animals live in close proximity, particularly in rural areas such as parts of Africa, the Middle East, Mediterranean Europe, Central Asia, South America, and western China (6).

Iran, the second-largest country in the Middle East by population (92,417,681) and area (1,648,195 km^2) (7), is endemic for CE (8). The disease's prevalence varies depending on climate and the density of definitive hosts (9). In Iran, the overall prevalence of CE in slaughtered livestock is estimated at 15.6%: sheep (5.9%), camels (32.7%), cattle (16.5%), buffalo (5.2%), and goats (6.4%) (10). Furthermore, the overall seroprevalence of human CE in the general population has been reported as 4% (11).

To date, six genotypes/species of *E. granulosus* s.l. have been reported in intermediate and definitive hosts in Iran, including in humans (G1, G3), camels (G1, G3, G5, G6), sheep (G1, G3, G6), buffalo (G1, G3), dogs (G1, G2, G3), goats (G1, G3, G6), and cattle (G1, G3, G5, G6) (12). The variation among genotypes reflects their adaptation to different predator–prey systems across regions.

Mitochondrial DNA (mtDNA) has been widely used as a genetic marker in evolutionary and population genetic studies over the past three decades due to its haploid nature, rapid mutation rate, homoplasmy, and lack of recombination (13,14). We investigated the molecular epidemiology of CE infecting livestock populations in Lorestan and Khuzestan provinces, western Iran.

Materials and Methods

Study area

Lorestan Province (33°34'N, 48°24'E) is located in western Iran, nestled in a scenic valley surrounded by the Zagros Mountains. The province is known for its numerous springs, wetlands, and waterfalls. Lorestan experiences a four-season climate with considerable meteorological diversity. Khuzestan Province (31°20'N, 48°40'E) lies in southwestern Iran, at the head of the Persian Gulf and bordering Iraq. Its climate ranges from semi-desert to warm steppe, with snow-capped mountains in the north extending toward the Persian Gulf in the south (Fig. 1).



Fig. 1: The location of Lorestan and Khuzestan provinces on the map of Iran and Counties where hydatid cyst samples were collected from slaughterhouses

Sample Collection

During routine veterinary inspections, CE samples were obtained from slaughterhouses in various regions of Lorestan and Khuzestan provinces from 2024 to 2025. CE detection was performed through visual inspection and palpation of visceral organs. Cysts were collected aseptically from slaughtered animals and transported under cold-chain conditions to the Parasitology Laboratory of Kermanshah University of Medical Sciences. Protoscoleces or germinal layers were aspirated from cysts and stored in 70% ethanol at −20 °C until further analysis.

The present study was approved by the Ethics Committee of Islamic Azad University, Karaj Branch (Approval code: IR.IAU.K.REC.1403.132).

Genomic DNA Extraction and PCR-RFLP Analysis

Genomic DNA was extracted from protoscoleces and germinal layers using the DNG™-plus Kit (CinnaGen, Iran) according to the manufacturer's protocol. The *ITS1* region was amplified using the forward (5'-GTCGTAACAAGGTTTCCGTAGG-3') and reverse (5'- TAGATG CGTTCGAAGTGTCG -3') primers. PCR amplification was carried out in a 50 µL reaction mixture containing 20 µL of master mix (Amplicon), 4 µL of each primer (5 pmol), 3 µL of genomic DNA, and 20 µL of distilled water. The cycling conditions were as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, and extension at 72 °C for 30 sec; followed by a final extension at 72 °C for 5 min.

PCR products were separated on a 1.5% agarose gel stained with Simply Safe (Eurx, Poland) and visualized under UV light. PCR-RFLP analysis of the *ITS1* fragment was performed using the *HpaII* restriction enzyme, which recognizes the C/CGG sequence, to differentiate *E. granulosus* s.s.

DNA typing and sequence analysis

Twenty samples representing all studied host species and geographic origins were randomly selected for more detailed genetic analysis. PCR was performed to investigate the genetic diversity of *Echinococcus* spp. using primer pairs targeting the *NADH dehydrogenase subunit 1* (*ND1*) gene (MS1, 5'-CGT AGG TAT GTT GGT TTGTTTGGT -3' and MS2, 5'-CCA TAA TCA AAT GGC GTACGAT-3'). PCR amplification was carried out in a 30 µL reaction volume containing 3 µL of genomic DNA, 10 pmol of each primer, and 15 µL of PCR mix comprising 1.25 U of Taq DNA polymerase, 200 µM dNTPs, and 1.5 mM $MgCl_2$ (2× Master Mix RED, Ampliqon, Denmark). Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 45 sec, and extension at 72 °C for 35 sec; followed by a final extension at 72 °C for 10 min.

Phylogenetic and haplotype network analysis

Sequences obtained in this study were trimmed and edited in BioEdit. Previously published *ND1* sequences from Iranian *Echi-*

nococcus specimens were retrieved for comparative analysis. The number of segregating sites, diversity indices (haplotype diversity, Hd; nucleotide diversity, π), and neutrality test (Tajima's D) were calculated using DnaSP ver. 5.10 (15). Haplotype networks of *ND1* regions were constructed in PopART software using the median-joining algorithm (16). For the phylogenetic tree, Maximum Likelihood (ML) analysis was conducted using MEGA 6.0 on the alignment of the *ND1* gene sequences (17). The Hasegawa-Kishino-Yano (HKY) model was identified as the best-fitting model for sequence evolution based on the Bayesian Information Criterion (BIC) and the Modified Akaike Information Criterion (AICc) implemented in MEGA 6.0 software. Phylogenetic analyses were performed with 1000 bootstrap replicates.

Results

Overall, 120 *E. granulosus* isolates were collected from slaughterhouses across various cities in Lorestan and Khuzestan provinces, including 70 from sheep, 25 from cattle, 10 from buffalo and 15 from goats. PCR amplification of the *ITS1* region of rDNA was successfully performed on 118 DNA samples, yielding amplicons of approximately 1 kb. Among the samples used for PCR, 80 were from liver cysts and 40 from lung cysts. RFLP analysis of the *ITS1* rDNA region was conducted using the restriction endonuclease *HpaII*, producing fragment sizes of approximately 300 and 700 bp. The RFLP pattern confirmed that all isolates belonged to *E. granulosus* s.s.

Twenty *E. granulosus* DNA samples (six from sheep, five from cattle, four from buffalo and five from goats) were randomly selected for *ND1* partial gene amplification and sequencing to assess phylogenetic and haplotype diversity. BLAST analysis revealed that 14 sequences were characterized as the G1 genotype of *E. granulosus*, while six sequences were identified as G3. The sequences of *E. granulosus* s.s. isolates (n = 20) were deposited in GenBank (Table 1).

Table 1: Livestock samples of *Echinococcus granulosus* sensu stricto submitted to GenBank

Geographical origin (n)	Isolates name	Host (n)	Cyst location (n)	Number of isolates	Identified genotype	Haplotype number	Accession number
Lorestan (2) and Khuzestan (4)	BO1, KH8, AN3, AN4, AH1, SH10	Sheep (4), Buffalo (1), Cattle (1)	Liver (3), lung (3)	6	G3	1	PX434809, PX434815, PX434821, PX434822 PX434825, PX434826
Lorestan	BO7	Buffalo (1)	Liver	1	G1	2	PX434810
Lorestan	BO10	Sheep (1)	lung	1	G1	3	PX434811
Lorestan (6) and Khuzestan (2)	BO11, KH2, KH7, KH14, KU8, KU15, AN6, AH17	Cattle (4), Buffalo (1), Goat (3)	Liver (4), lung (4)	8	G1	4	PX434812, PX434813, PX434814, PX434816, PX434819, PX434820, PX434823, PX434824
Lorestan and Khuzestan	KU2, SH11	Goat (2)	Liver (2)	2	G1	5	PX434817, PX434827
Lorestan and Khuzestan	KU5, SH18	Sheep (1), Buffalo (1)	Liver (2)	2	G1	6	PX434818, PX434828

Phylogenetic analyses

The phylogenetic tree constructed based on *ND1* gene sequences of *E. granulosus* isolates is shown in Fig. 2. Analysis revealed two main clusters corresponding to genotypes G1 and G3. Although the bootstrap values for the *ND1* phylogenetic tree were relatively low, the two clusters were distinguishable.

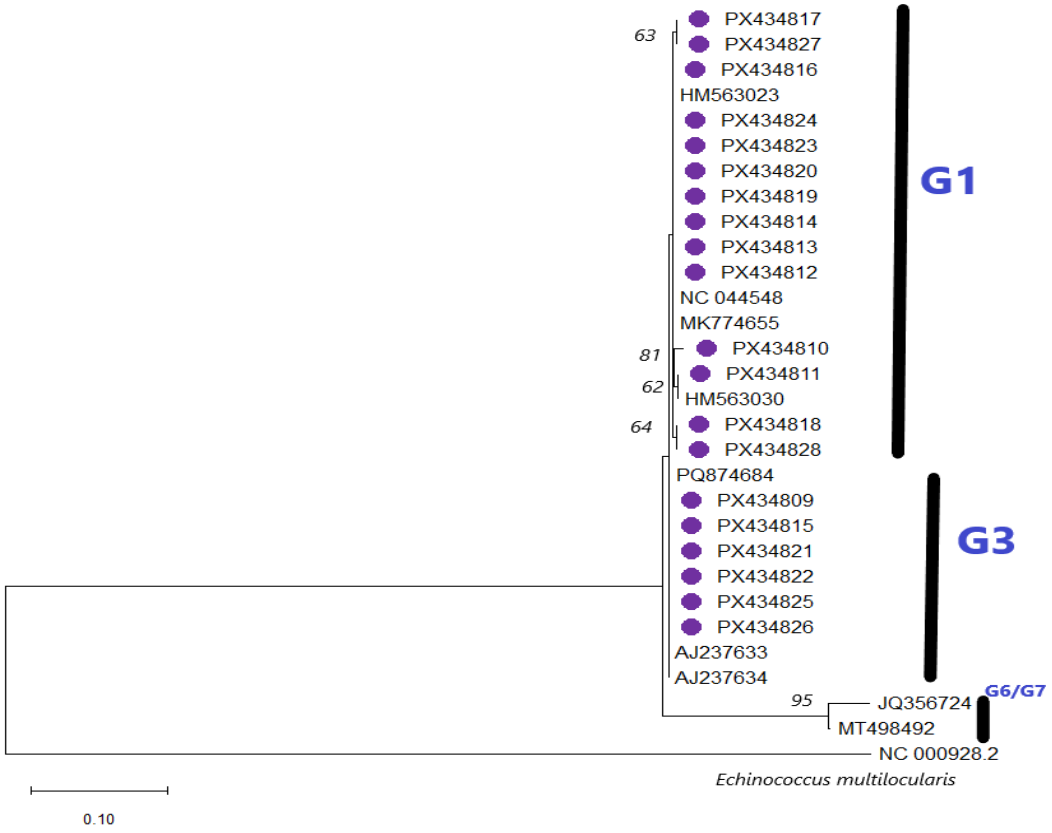


Fig. 2: Phylogenetic tree of *E. granulosus* sensu stricto isolates from Lorestan and Khuzestan provinces (n=20), western Iran based on a 385 bp-long fragment of the *ND1* gene. The tree was designed by using the Maximum-Likelihood test and the Hasegawa-Kishino-Yano (HKY) model. The numbers on the branches are percent bootstrap values from 1000 replicates. Sequences detected in this study are highlighted with the purple circle

Nucleotide diversity analysis

Seven polymorphic sites were identified among the *E. granulosus* s.s. isolates sequenced in this study (Table 2). Both haplotype and nucleotide diversity were low. Tajima's D test showed negative values, indicating a pattern suggestive of recent population expansion, a bottleneck event, or positive directional selection.

Haplotype networks

Overall, 99 *E. granulosus* s.s. sequences from GenBank, representing isolates from various hosts (cattle, buffalo, sheep, and goats) across western and southwestern Iran, were used for haplotype network analysis. The resulting network displayed a relatively diverse topology, including five main star-like clusters with satellite haplotypes. The most frequent haplotype (H4) comprised 56 sequences, predominantly

from southern and southwestern Iran. Most haplotypes were separated by one to three mutational steps (Fig. 3).

Table 2: Diversity and neutrality test for *Echinococcus granulosus* (s.s.) populations from Western and Southwestern Iran

Isolates (no)	Length (bp)	S	H	π±SD	k	Hd ±SD	Tajima's D**
20	385	7	6	0.00317	1.221	0.763	-1.25317

*Abbreviations: S: number of polymorphic sites, H: haplotype, π: nucleotide diversity, SD: Standard deviation, K: average number of pair-wise nucleotide differences, Hd: haplotype diversity.
***P*>0.10

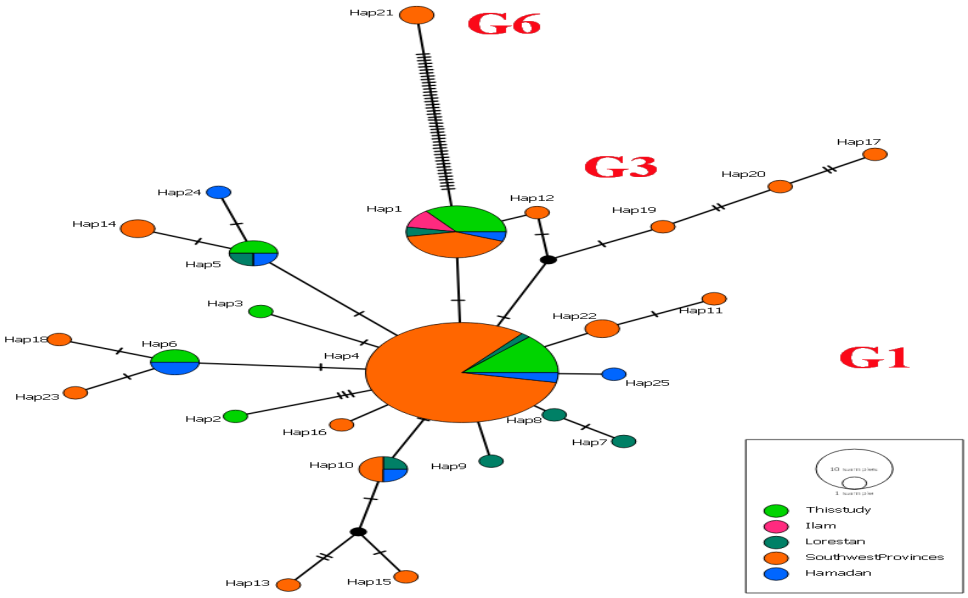


Fig. 3: Haplotype network built with the sequence of the *ND1* gene of *E. granulosus* sensu stricto isolated from sheep, cattle, buffalo and goat in Lorestan and Khuzestan provinces and similar sequences deposited in GenBank isolated from western and southwestern provinces of Iran. The mutational steps are indicated by the short marks crossing the connection lines. Circle size is relative to haplotype frequency

Discussion

Livestock cystic echinococcosis (CE) is widespread across many regions of Iran and poses major challenges for both public health and veterinary sectors. Nevertheless, molecular epidemiological data from several regions remain limited, outdated, or insufficient. This study investigated the molecular epidemiology of CE in livestock populations from Lorestan and Khuzestan provinces, Western and Southwestern Iran.

In the present study, PCR-RFLP analysis targeting the *rDNA-ITS1* region was conducted on livestock CE isolates. PCR-RFLP analysis of the *ITS1* region using the enzyme *HpaII* allows the differentiation of *E. granulosus* s.s. (genotypes G1-G3) from genotype G6 (camel strain) based on specific banding patterns such that genotype G6 produces five distinct bands instead of two (18). Although

this approach is recommended for large-scale epidemiological screening in *E. granulosus* endemic areas due to its low cost and minimal need for laboratory tools, PCR-RFLP cannot distinguish G1-G3 genotypes from each other. Therefore, sequencing was necessary to identify genotypes within *E. granulosus* s.s. (G1-G3). Accurate identification and differentiation between G1 and G3 genotypes are valuable, as these may display varying degrees of infectivity to humans (18).

Our results showed that approximately 70% of the CE isolates sequenced belonged to the G1 genotype. The predominance of the G1 genotype in CE is mainly due to traditional free-range livestock farming, which is also common in our country. These practices facilitate the continuous transmission cycle of the parasite between definitive hosts (dogs) and intermediate hosts (sheep, goats, and cattle) through contaminated environments. Free-range and extensive animal husbandry in open pastures allows stray dogs to easily contaminate grazing pastures and water sources with parasite eggs. Since these animals constantly graze in an open and unprotected environment, they regularly ingest parasite eggs, thereby maintaining the G1 genotype life cycle. Considerable evidence supports the existence of a domestic dog-ruminant cycle in most regions of Iran, where the G1 genotype is dominant (19). Haplotypes belonging to the G1 genotype were the most frequent, and similar haplotypes have been reported in various intermediate hosts across western and southwestern Iran (20, 21) (Fig. 3).

The G3 genotype was first identified in goats, camels, and cattle in Ardabil Province (northwestern Iran) (22) and has since been reported in multiple host species in other parts of the country (23, 24). In this study, genotype G3 represented 30% of CE cases in livestock.

The high prevalence of *E. granulosus* s.s. genotypes G1 (common sheep strain) and G3 (buffalo strain) in the region is of critical importance to public health because they are the primary causes of cystic echinococcosis (CE) in humans worldwide (25). Together, these genotypes, belonging to the *E. granulosus* s.s. complex, are responsible for approximately 88.5% of human CE cases (25). Molecular data indicate that *E. granulosus* s.s. is responsible for 96% of human CE cases in Iran (94% G1 and 2% G3), followed by G6 with 4% of cases (26). Their prevalence is linked directly to the persistence of the parasite in livestock (sheep, cattle) and domesticated dogs, causing significant morbidity in humans and substantial economic losses in the farming industry.

A recent systematic review and meta-analysis of molecular epidemiology data from 2,952 animal hosts (both intermediate and definitive) in Iran revealed that genotypes G1 (92.75%) and G6 (4.53%) are the most prevalent among sheep, cattle, goats, and camels (12). Genotype G3 was also reported in five herbivorous species and dogs, while G7 (0.2%) and G2 (0.06%) were detected in sheep, goats, and dogs, respectively, at much lower frequencies (12). The absence of the G6 genotype in this study could be attributed to the lack of camels (the natural intermediate hosts of the camel strain) in the investigated regions. *E. granulosus* s.s. is the species most commonly adapted to humans; consequently, it accounts for the majority of human CE cases worldwide (25).

A total of six haplotypes were identified in the CE samples studied. The low haplotype diversity could be related to the limited sequence length analyzed, which cannot accurately capture the true, small-scale genetic structure of the populations. Additionally, the low variability may also reflect the sampling of hosts from geographically restricted areas.

One limitation of the current study was that only one cyst was isolated from each host for molecular analysis. Previous studies have demonstrated that individual animals, particularly cattle and camels, may harbor multiple genotypes simultaneously (27), underscoring their critical role in maintaining and transmitting the CE life cycle in different regions. Another challenge was the low sample size for

sequencing due to financial constraints. Also, due to the short length of the sequenced fragments, genetic differences between the isolates studied may have been underestimated, therefore, the results should be interpreted with caution.

Conclusion

This study confirms the presence of *E. granulosus* s.s. (genotypes G1 and G3) among livestock in Lorestan and Khuzestan provinces. To mitigate the associated public health risk, policymakers should prioritize educational interventions for livestock owners and farmers, such as preventing dogs from consuming infected organs and restricting livestock access to contaminated feed or water sources. Regular deworming of dogs also represents a valuable preventive measure within integrated control programs.

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Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Shiee MR, Kia EB, Zahabiun F, et al. *In vitro* effects of tropisetron and granisetron against *Echinococcus granulosus* (s.s.) protoscoleces by involvement of calcineurin and calmodulin. Parasit Vectors. 2021;14(1):197.
2. Badwaik N, Gharde P, Shinde RK, et al. Hydatid Cyst or Echinococcosis: A Comprehensive Review of Transmission, Clinical Manifestations, Diagnosis, and Multidisciplinary Treatment. Cureus. 2024; 16(7):e63713.
3. Hogea MO, Ciomaga BF, Muntean MM, et al. Cystic Echinococcosis in the Early 2020s: A Review. Trop Med Infect Dis. 2024; 9(2):36.
4. Wang Y, Zhang R, Zhang D, et al. Global burden of cystic echinococcosis: a 32-year analysis of 204 countries with 2036 projections. BMC Public Health. 2025 Sep 30;25(1):3213.
5. da Silva AM. Human echinococcosis: a neglected disease. Gastroenterol Res Pract. 2010; 2010:583297.
6. Nocerino M, Pepe P, Ciccone E, et al. Epidemiological update of cystic echinococcosis in livestock and assessment of practices related to its control in the Mediterranean area. Acta Trop. 2024; 255:107240.
7. Fattahi Z, Beheshtian M, Mohseni M, et al. Iranome: A catalog of genomic variations in the Iranian population. Hum Mutat. 2019;40(11):1968-1984.
8. Zeinali M, Mohebali M, Shirzadi MR, et al. Human Cystic Echinococcosis in Different Geographical Zones of Iran: An Observational Study during 1995-2014. Iran J Public Health. 2017;46(12):1623-1631.
9. Ebrahimipour M, Budke CM, Harandi MF. Control of Cystic Echinococcosis in Iran: Where Do We Stand? Trends Parasitol. 2020;36(7):578-581.
10. Khalkhali HR, Foroutan M, Khademvatan S, et al. Prevalence of cystic echinococcosis in Iran: a systematic review and meta-analysis. J Helminthol. 2018;92(3):260-268.
11. Mohemmi N, Khodayari MT, Karamati SA, et al. Update on the Seroepidemiology of Human Cystic Echinococcosis and Associated Risk Factors in Iran: A Systematic Review and Meta-Analysis. Health Sci Rep. 2025;8(4):e70699.
12. Khademvatan S, Majidiani H, Foroutan M, et al. *Echinococcus granulosus* genotypes in Iran: a systematic review. J Helminthol. 2019; 93(2):131-138.
13. Rashidi MR, Mousavi SM, Mohammadi MA, et al. Surface water contamination with *Echinococcus granulosus* eggs in the endemic

regions of cystic echinococcosis in the southeast of Iran: significance and public health implications. J Water Health. 2025;23(9):981-990.
14. Ghanbari F, Ashrafi K, Fasihi Harandi M, et al. Frequency and Sequence Analysis of *Echinococcus granulosus* Sensu Lato Isolated from Buffaloes in Northern Iran. Vet Med Sci. 2025 ;11(3):e70328.
15. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, et al. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Mol Biol Evol. 2017;34(12):3299-3302.
16. Leigh JW, Bryant D. POPART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6(9): 1110–1116.
17. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 2018;35(6):1547-1549.
18. Kinkar L, Laurimäe T, Acosta-Jamett G, et al. Distinguishing *Echinococcus granulosus* sensu stricto genotypes G1 and G3 with confidence: A practical guide. Infect Genet Evol. 2018; 64:178-184.
19. Elyasi H, Hajari AH, Javaheri E. Genetic Identification of *Echinococcus granulosus* in Slaughtered Domestic Animals from Two Northeastern Iranian Cities Using HRM and Sequencing Techniques. Iran J Parasitol. 2025;20(3):346-355.
20. Jamshidi A, Ghatee MA, Haniloo A, et al. Phylogenetic Study of cox1 Gene in *Echinococcus granulosus* Sensu Lato Genotypes in Southwestern Iran. Zoonoses Public Health. 2025;72(5):435-441.
21. Kheirandish F, Badparva E, Mahmmoudv-and H, Beiranvand E, Babaei S, Nasiri B. Genetic Characterization of Hydatid Cysts Isolated from Domestic Animals in Lorestan Province, Western Iran. Iran J Parasitol. 2018;13(1):120-126.
22. Pezeshki A, Akhlaghi L, Sharbatkhori M, et al. Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil Province, northwest Iran. J Helminthol. 2013;87(4):387-91.
23. Nikmanesh B, Mirhendi H, Ghalavand Z, et al. Genotyping of *Echinococcus granulosus* Isolates from Human Clinical Samples Based on Sequencing of Mitochondrial Genes in Iran, Tehran. Iran J Parasitol. 2014;9(1):20-7.
24. Borhani M, Fathi S, Darabi E, et al. Echinococcoses in Iran, Turkey, and Pakistan: Old Diseases in the New Millennium. Clin Microbiol Rev. 2021;34(3):e0029020.
25. Akkaş Ö, Özgökçe M, Aydemir S, Dündar İ, Ekici A. Genotyping of *Echinococcus granulosus* Human Isolates Obtained from the Puncture, Aspiration, Injection, and Re-Aspiration (PAIR) Process in Türkiye. Iran J Parasitol. 2023;18(4):427-434.
26. Rostami S, Shariat Torbaghan S, Dabiri S, 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.
27. M'rad S, Oudni-M'rad M, Bastid V, et al. Microsatellite Investigations of Multiple *Echinococcus granulosus* Sensu Stricto Cysts in Single Hosts Reveal Different Patterns of Infection Events between Livestock and Humans. Pathogens. 2020;9(6):444