Short Communication

Genotyping *Echinococcus granulosus* from Canine Isolates in Ilam Province, West of Iran

*Abdolhossein DALIMI 1, Morteza SHAMSI 1, Afra KHOSRAVI 2, Fatemeh GHAFFAFARIF 1

1. Dept. of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Dept. of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran

**Abstract**

*Background:* Cystic Echinococcosis (CE) is one of the most common parasitic zoonosis caused by *Echinococcus granulosus* worldwide. This study investigated genotype diversity of *Echinococcus granulosus* isolated from stray dogs and golden jackals in Ilam province, West of Iran.

**Methods:** Adult worms were collected from the small intestine of the stray dogs and golden jackals from Ilam Province roads during 2012-2014. DNA was extracted from the adult worms and the partial mitochondrial NADH dehydrogenase subunit1 (*nad1*) was amplified by PCR then the products were digested by using HpaII, RsaI, and AluI restriction enzymes. In order to confirm RFLP results, a number of PCR products were bi-directionally sequenced.

**Results:** Totally, 20 stray dogs out of 75 (26.66%) and two out of 73 (2.74%) golden jackals showed infection with *E. granulosus*. Amplified PCR product for all isolates was a band of approximately 550bp. *AluI* digested the product into two bands of approximately 160bp and 390bp fragments, while the *RsaI* cut the product into 320bp and 230bp fragments and the *HpaII* had no effect on the PCR product for both dog and jackal samples. The isolate sequences of mtDNA*nad1* gene indicated 100% homology with references G1, G2 and G3 sequences in the GenBank database.

**Conclusion:** The genotype of adult *E. granulosus* was similar to larval stage genotypes of parasite (G1-G3 complex).

**Keywords:** *Echinococcus granulosus*, Stray dog, Golden jackal, Genotyping, Iran

**Introduction**

Cystic Echinococcosis (CE) is one of the most common parasitic zoonosis caused by *Echinococcus granulosus* worldwide (1, 2). CE makes many economic problems in animal and human society and it constitutes major public health issues. A sero-positivity rate in different parts of Iran country was within 1.2%-21.4% (3). The infection
rate was reported in dogs from 5% to 49% in
different regions of Iran (3). Within the life
cycle of E. granulosus, canine and wild carn-
ivores can serve as important definitive hosts.

Nowadays, there are 10 distinct genetic types
(Genotypes G1-G10) of different E. granulosus
(4, 5). The most isolate of human E. granulosus
have been indicated to be common with sheep
strain (6, 7). Epidemiological studies along
with genetic characterization have demon-
strated the prevalence of CE and some partic-
ular genotypes of E. granulosus in relation to
type of livestock host. Knowing the genetic
varieties of the parasite has some effects on
epidemiology, pathology, infectivity, and con-
trol of hydatidosis (8). The susceptibility of
human to CE may be correlated with E. granulosus genotype (5). Therefore, it could be
very important to understand the levels of ge-
netic variability and phylogenetic relationships
among and within E. granulosus genotypes and
this by itself has an important role in pro-
grams for infectious disease control, especially
for health's organization (9).

This study investigated the molecular genetic
diversity of adult’s worm of E. granulosus by
using NADH dehydrogenase gene subunit
(nad1) among stray dogs and golden jackals in
Ilam province West of Iran.

Materials and Methods

Parasite

From Feb 2012 to Oct 2014, 75 stray dogs,
73 golden jackals, and 70 red fox carcasses
killed in car accidents were collected from the
road in Ilam Province (Fig.1). After necropsy,
the intestines of canine were evaluated for the
presence of adult worms of E. granulosus. The
separated worms from each infected animal
were placed in a tube, rinsed three times with
phosphate-buffered saline, and preserved in
80% ethanol until further analysis.

Compliance with Ethical Standards:

This study was financially supported by the
Tarbiat Modares University, Tehran, Iran. All
procedures performed in studies involving
animals were in accordance with the ethical
standards of the Tarbiat Modares University
Ethical Committee. All applicable interna-
tional, national, and/or institutional guidelines for
the care and use of animals were followed.

Fig. 1: The collection sites of canine examined from Ilam Province between 2012 and 2014

DNA extraction

The study was done in Parasitology De-
partment of Medical Sciences Faculty of

Tarbiat Modares University, Tehran, Iran in
2012-14. Twenty-two samples (twenty samples
from stray dogs and two from golden jackals)
were examined for DNA extraction. The adult worms were washed three times using sterile distilled water to remove ethanol. Each sample contains 10 *E. granulosus* from each infected animals, randomly selected and provided for repeated freezing and thawing, as well as abrasion and mechanical grinder. Genomic DNA was extracted according to the manufacturer's instruction by using DNA extraction kit (Geneall, Korea). Concentration of DNA was determined by spectrophotometry and the isolates were eluted in 50 μl of ddw at 70 °C and stored at -20 °C until PCR reactions.

**Molecular analysis**

The isolates were analyzed using amplification of mtDNA*nad1* gene. PCRs were carried out in a final volume of 25 μl including (10X buffer 2.5 μl, genomic DNA 4 μl, MgCl2, 1.75 μl, 0.6 μl of each deoxynucleotide triphosphate (dNTPs), 1 μl of each primer, Taq DNA polymerase 0.15 μl and distilled water 14 μl) (10).

The forward (nad1.F) JB11: 5’-AGATTCGTAAGGGGCCTATA-3’and reverse (nad1.R) JB12: 5’-ACCCTAATTCTACTTCTC-3’primers were employed for PCR amplification. The following temperature profile was used for DNA amplification. Initial denaturation 5 min for 94 °C, denaturation 1min for 94C; annealing 1min for 50C; extension 1min for 72 °C, number of cycles 35 and final extension 10 min for 72 °C.

PCR products were loaded on 1.2% (w/v) agarose gel (Sinnagen, Iran) in SB buffer (10 mM Sodium hydroxide, pH adjusted to 8.5 with Boric acid) (11) and stained with 0.5 μg/ml ethidium bromide. Electrophoresis was carried out for 50 min at 80 V. The bands were visualized in UV transilluminator and digitally were photographed (10, 12).

**PCR-RFLP and DNA Sequencing**

This method was done on PCR products mtDNA*nad1* regions of isolates from animals with 3-base cutting restriction endonucleases, *Alu*I, *Rsa*I and *Hpa*II. Electrophoresed products were stained with 0.5μg/ml ethidium bromide and then the created bands were photographed and observed by the documentation gel system. In order to confirm RFLP results, a number of PCR Product were randomly bi-directionally sequenced using PCR primers by the Seqtech Company in the USA. Results were analyzed by Mega ver. 6 software package and compared with the recorded results in GenBank.

**Results**

Totally, twenty stray dogs out of 75 (26.66%) and two out of 73 (2.74%) golden jackal showed infection with *E. granulosus*. No foxes revealed to be infected. *Nad1* gene was successfully amplified for all isolates. Amplified fragment size of nad1 gene of dogs and jackals isolates both showed approximately 550bp amplicons in PCR reaction (Fig.2).

![Fig. 2: PCR products of mtDNA-nad1 of adult *E. granulosus* on agar gel: M: marker with 50bp molecular weight, Co.n: control negative, 1: dog, 2: jackal samples](http://ijpa.tums.ac.ir)

The *Alu*I digested the product into two bands for both dog and jackal isolates of approximately 160bp and 390bp fragments (Fig.3) while the *Rsa*I cut the product into 320bp and 230bp fragments for both dog and jackal samples (Fig.4). The *Hpa*II restriction enzyme
had no effect on the PCR product for \textit{nad1} and after digestion intact 550bp fragment was seen (Fig.5). Sequence data of amplified fragment (471bp) of ten isolates from dogs and two isolates from jackal showed 100% homology with GenBank reference sequence for G1 (AJ237632), G2 (AJ237633) and G3 (AJ237634) genotypes (Fig.6). Phylogenetic tree was drawn and the similarity of jackal samples with G1 genotype (sheep strain), dog samples with G2 genotype (Tasmania sheep strain) and G3 genotype (Buffalo strain) (Fig.6). Therefore, the presence of G1- G3 complex of \textit{E. granulosus} was confirmed in Ilam Province. The sequence data were registered into GenBank reference sequences with Accession Nos. (KT338943 for dog isolates and KT338944 for jackal samples).
Fig. 6: Phylogenetic relationships among *E. granulosus* isolated from dogs and jackals based on mtDNA nad1 gene sequence. The evolutionary history was inferred using the neighbor-joining method, supported by 1000 bootstrap replicates.

**Discussion**

Iran has geographically been located in high prevalence region of echinococcosis. The infection rate of hydatidosis in cattle, sheep, and goat based on abattoir data in Western Iran (Ilam, Lorestan, Kermanshah and West Azerbaijan provinces), were 11.1%, 16.4%, 12.4% and 6.3%, respectively (13). The rate of infection in stray dogs was reported 22.3% in the north of Iran, 17.6% in Kerman, 22% in Mashhad, 12.5% in the west Azerbaijan, 11.4% in Kordestan, 16.7% in Kermanshah, 30.9% in Lorestan and 9% in Ilam (14). However, spillover events from domestic to sylvatic cycles are suspected to be frequent in areas where infected viscera cheered livestock are known to be accessible to jackals and foxes (13).

Up to date, some studies have been performed on the molecular characterization of *E. granulosus* isolates from humans, sheep, goats, cattle, and camels in different parts of Iran (15-40). The result of this type of studies indicated the presence of G1, G2, G3 and G6 genes in our country (21-40). In a study that conducted in Ilam Province, three genotypes were detected as G1, G2, and G3 (17). In this study, on mtDNA of nad1 gene of adult worms, the results were similar to that of others. G1 genotype has been introduced as a dominant variant in Iran by some studies (18, 19, 21, 22, 24, 27). In addition, the presence of camel (G6 genotype) strains has been reported in Iran (16, 18, 20, 30, 31). Sequencing of the nad1 and *cox1* genes and ITS1-PCR coupled to RFLP confirmed these observations. The same genotype for sheep and human isolates, while different ones for camel (18). The genotype diversity of three mitochondrial genes *cox1*, *nad1* and *atp6* was investigated and also partial sequences of 12S rRNA gene in their isolates and confirmed the presence of G1 and G6 genotypes in different intermediate hosts, including cattle, camels, sheep, buffalo and goats in different geographic areas in Iran (21). Among 55 samples from domestic animals and humans in Ardabil, the G1 was the dominant strain in Iran and G3 genotype was reported in two human isolates (22).
The present study indicated the presence of (G1-G3 complex) of *E. granulosus* among dogs and jackals in Ilam Province. In a molecular study, all human and sheep were reported to be of (G1-G3 complex) genotypes (17). By analyzing *cox1* and *nad1* gene sequences of the adult worms isolated from dogs in Lorestan near to Ilam, about 75% of the cases specified as G1, 10% as G2 and only 15% as G3 genotypes (23). These findings are, more or less, in accordance with our research results. Our findings also were confirmed by two studies that all human isolates was reported as G1 genotype (24, 25). G1, G2 and G3 are the most common genotypes of the parasite in intermediate hosts such as sheep, camel, buffalo and occasionally human throughout the world (1, 6, 7, 21, 22). In addition to stray dogs and golden jackals were found to be infected with G1, G2, and G3 genotypes. By this study, G1 genotype in golden jackal has been reported for the first time in the world.

**Conclusion**

The infected definitive hosts (dogs and jackal) were harbored the G1-G3 genotypes are predominant in the transmission cycle of *E. granulosus* in West of Iran.

**Acknowledgements**

This work is part of Ph.D. thesis supported financially by Tarbiat Modares University. The authors would like to thank the colleagues that helped us in this study, particularly the staff of Parasitology Department of Tarbiat Modares University. We also thank Ilam University and Ilam University of Medical Sciences for their cooperation.

**Conflict of Interest**

All authors declare that they have no conflict of interest.

**References**

1. McManus DP, Thompson RC. Molecular epidemiology of cystic Echinococcosis. Parasitology. 2003;127 Suppl:S37-51.
2. Heidari Z, Mohebali M, Zarei Z et al. Seroepidemiological study of human hydatidosis in Meshkinshahr district, ardabil province, Iran. Iran J Parasitol. 2011;6(3):19-25.
3. Rokni MB. Echinococcosis /hydatidosis Iran. Iran J Parasitol.2009;4(1):1-16.
4. Thompson RC. The taxonomy, phylogeny and transmission of *Echinococcus*. Exp Parasitol. 2008;119(4):439-46.
5. Nakao M, McManus DP, Schantz PM, Craig PS, Ito A. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitology. 2007;134(Pt 5):713-22.
6. Moro PL, Nakao M, Ito A, Schantz PM, Cavero C, Cabrera L. Molecular identification of *Echinococcus* isolates from Peru. Parasitol Int. 2009;58(2):184-6.
7. Utuk AE, Simsek S, Koroglu E, McManus DP. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. Acta Trop. 2008;107(2):19-34.
8. Eckert J, Thompson RC. Intra specific variation of *Echinococcus granulosus* and related species with emphasis on their infectivity to humans. Acta Trop. 1997;64(1-2):19-34.
9. Rosenzvit MC, Zhang LH, Kamnetzky I, Canova SG, Guarnera EA, McManus DP. Genetic variation and epidemiology of *Echinococcus granulosus* in Argentina. Parasitology. 1999;118 (Pt 5):523-30.
10. Bowles J, McManus DP. Rapid discrimination of *Echinococcus* species and strains using a polymerase chain reaction based RFLP method. Mol Biochem Parasitol.1993;57(2):231-9.
11. Brody JR, Kern SE. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques. 2004;36(2):214-6.
12. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol. 1992;54(2):165-73.
13. Dalimi A, Sattari A, Motamedi G. A study on intestinal helminthes of dogs, foxes and jackals

Available at: [http://ijpa.tums.ac.ir](http://ijpa.tums.ac.ir)
in the western part of Iran. Vet Parasitol. 2006;142(1-2):129-33.

14. Abdi J, Panahi J, Havasian M, Sayehmiri K. The prevalence of *Echinococcus granulosus* infection in various domestic herbage animals from southwest Iran. J Helminthol. 2013;37(2):240-4.

15. Eslami A, Hosseini SH. *Echinococcus granulosus* infection of farm dogs of Iran. Parasitol Res. 1998;84(3):205-7.

16. Harandi MF, Hobbs RP, Adams PJ, Mobedi I, Morgan-Ryan UM, Thompson RC. Molecular and morphological characterization of *Echinococcus granulosus* human and animal origin in Iran. Parasitology. 2002;125(Pt 4):367-73.

17. Dousti M, Abdi J, Bakhityari S, Mohebali M, Mirhendi Sh, Rokni M. Genotyping of hydatid cyst isolated from human and domestic animals in Isfahan Province, Western Iran. Iran J Parasitol. 2013;8(1):47-52.

18. Ahmadi N, Dalimi A. Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. Infect Genet Evol. 2006;6(2):85-90.

19. Parsa F, Haghighan B, Pestechian N, Salehi M. Molecular epidemiology of *Echinococcus granulosus* strains in domestic herbivores of Lorestan, Iran. Jundishapur J Microbiol. 2011;4(2):123-30.

20. Zhang L, Eslami A, Hosseini SH, McManus DP. Indication of the presence of two distinct strains of *Echinococcus granulosus* in Iran by mitochondrial DNA markers. Am J Trop Med Hyg. 1998;59(1):171-4.

21. Rostami Nejad M, Taghibour N, Nochi Z et al. Molecular identification of animal isolates of *Echinococcus granulosus* from Iran using four mitochondrial genes. J Helminthol. 2012;86(4):485-92.

22. Pezeshki A, Akhlaghi I, Sharbatkhori M, Razmjou E, Oormazdi H, Mohebali M, Meamar AR. Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil Province, northwest Iran. J Helminthol. 2013;87(4):387-91.

23. Parsa F, Fasih Harandi M, Rostami S, Sharbatkhori M. Genotyping *Echinococcus granulosus* from dogs from Western Iran. Exp Parasitol. 2012;132(2):308-12.

24. Khademvatan S, Yousefi E, Rafiei A, Rahdar M, Saki J. Molecular characterization of livestock and human isolates of *Echinococcus granulosus* sensu lato in stray dogs in a hyperendemic Middle East focus, northwestern Iran. Parasit Vectors. 2015;8:409.

Available at: [http://ijpa.tums.ac.ir](http://ijpa.tums.ac.ir)
34. Gholami Sh, Sosari M, Fakhar M, Sharif M, Daryani A, Hashemi M, Vahadi M. Molecular characterization of Echinococcus granulosus from hydatid cysts isolated from human and animals in Golestan Province, North of Iran. Iran J Parasitol. 2012;7(4):8-16.

35. Farhadi M, Fazaeli A, Haniloo A. Genetic characterization of livestock and human hydatid cyst isolates from northwest Iran, using the mitochondrial cox1 gene sequence. Parasitol Res. 2015;114(12):4363-70.

36. Shahnazi M, Hejazi H, Salehi M, Andalib AR. Molecular characterization of human and animal Echinococcus granulosus isolates in Isfahan, Iran. Acta Trop. 2011;117(1):47-50.

37. Rostami Nejad M, Nazemalhosseini Mojarad E, Nochi Z et al. Echinococcus granulosus strain differentiation in Iran based on sequence heterogeneity in the mitochondrial 12S rRNA gene. J Helminthol. 2008 82(4):343-7.

38. Pour AA, Hosseini SH, Shayan P. Comparative genotyping of Echinococcus granulosus infecting buffalo in Iran using cox1 gene. Parasitol Res. 2011;108(5):1229-34.

39. 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.

40. Rostami S, Shariat Torbaghan S, Dabiri S, Babaei Z, Ali Mohammadi M, Sharbatkhori M, Fasihi Harandi M. Genetic characterization of Echinococcus granulosus from a large number of formalin-fixed, paraffin-embedded tissue. Am J Trop Med Hyg. 2015;92(3):588-94.