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Letter to the Editor

Genetic Identification of *Echinococcus granulosus* Isolates from Domestic Animals in Sabzevar, Northeast Iran

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Dear Editor-in-Chief

Echinococcus granulosus is one of the Cestoda parasitic worms that live in the small intestine of the dog as the final host and the larval stage that causes cystic echinococcosis in the body of intermediate hosts, including many herbivorous animals, such as cattle, goats, camels, horses, as well as humans, cause enormous economic and social damage (1-3).

A wide range of genetic diversity has been described in the mitochondrial genes of *Echinococcus granulosus*. Using mitochondrial DNA sequencing, 10 strains (G1-G10) have been identified as genetically distinct. This includes sheep strain G1, Tasmanian sheep strain G2, buffalo strain G3 (4), *E. equinus* G4, *E. ortleppi* G5 cattle strain, *E. canadenisis* G6- G10, G6 cattle strain, G7 pig strain, and *E. felidis* (lion strain) supposed to occur only in Africa (4-7).

From Dec 2018 to Sep 2019, livestock hydatid cysts including cattle and sheep were collected from Sabzevar Industrial Slaughter, northeast of Iran. Fertile cysts containing clear, colorless liquid were selected for molecular analysis. Hydatid cysts were washed three times with normal sterile saline solution and stored in 70% ethanol at -20 °C until use for the molecular process. DNA Extraction: total genomic DNA (gDNA) was extracted from each cyst as manufacture's instructions (Ge Net Bio-South Korea). The extracted gDNA was evaluated by 2% agarose gel electrophoresis and spectrophotometer (NanoDrop-ND1000) and the DNA stored at -20 °C until molecular analysis.

Ethical approval of this study was obtained from the Ethics Committee at Sabzevar University of Medical Sciences, Sabzevar, Iran.

PCR was performed to amplify a 446bp DNA fragment of the mitochondrial subunit 1 of the *cytochrome c oxidase 1* (*cox1*) gene as previously described (8). The primer sequences utilized were 5´ TTTTTTGGGCATCCTGAGGTTTAT 3´ (For-ward) and 5´ TAAAGAAAGAACATAATGAAAATG 3´ (Reverse).

The PCR reaction carried out in a total volume of 20µl consisted of 2µl PCR buffer (10×), 2 µl dNTPs, 1 µl MgCl2, 1 µl of each primer, 1 unit Taq DNA polymerase, 11µl distilled water and 1 µl DNA. The PCR protocol was as follows: The thermal cycler for *Cox1* primer was set for 95 °C (3 min) for initial denaturation and denaturation at 95 °C (30 sec), annealing at 54 °C (30 sec), extension at 73 °C (30 sec) in 35 cycles, and the final extension 73 °C (5 min). The PCR products were separated with electrophoresis on a 2% agarose gel mixed with safe stain and observed on a UV transilluminator.

PCR products were sequenced by Bioneer Corporation (South Korea). Nucleotide sequence analysis was performed with BLAST (http://www.ncbi.nlm.nih.gov), whereas alignments were undertaken using software packages such as ClustalX and BioEdit. The *Cox1* sequences of the representative isolates were submitted to the GenBank. A phylogenetic tree was obtained by using the Molecular Evolutionary Genetics Analysis (Mega7) software package. The dendrogram was drawn by comparing the sequences obtained in the present study and reference sequences of all described *E. granulosus* genotypes (G1-G10) in the GenBank, and *Taenia saginata* was considered as the outgroup in the model.

The evolutionary history was obtained through the maximum likelihood (ML) approach based on the Kimura 2-parameter model. Primary tree(s) were obtained automatically by applying the neighbor-joining (NJ) method to a matrix of pairwise distances estimated utilizing the maximum composite likelihood method, and then choosing the topology with a higher log-likelihood value. The representative tree was drawn to scale.

The PCR amplification of the Cox1 region of rDNA was successfully performed on 46 animal DNA (13 sheep and 36 cattle) samples. Then we produced amplicons, which were similar to the sheep strain (Fig. 1).

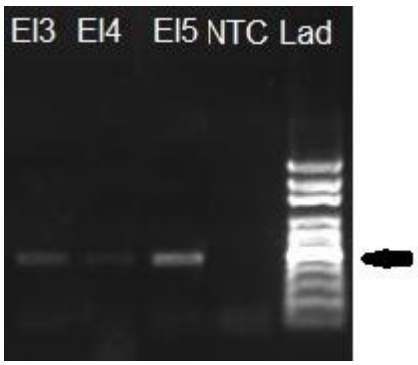

Fig. 1: Agarose gel electrophoresis of cox1-PCR (446 bp) products of *E. granulosus* isolates from livestock Lanes 1, 2, 3 (E13, E14, E15) cox1 gene, Lane 4(NTC): negative control lane 5: ladder 100bp

The samples used for PCR tests were 38 liver and 11 lung hydatid cysts. No amplification was observed in negative control in each PCR run. Twenty-seven representative amplicons of Cox1 DNA, from animal isolates, were subjected to sequencing. Partial consensus sequences of *E. granulosus Cox1* rDNA region of the isolates were achieved and compared with the reference sequence genotype G1 (Accession No. MG 322623.1) (8) and other sequences deposited in GenBank. The alignment of the obtained sequences with the reference sequence revealed 99.9% homology and indicated that the isolates corresponded to *E. granulosus* genotype G1.

Phylogenetic trees were generated by using *Cox1* sequencing (Fig. 2). The alignment was performed using ClustalW and the aligned sequences manually refined in BioEdi software (version 7.2.5) maximum likelihood (ML) trees

were inferred by MEGA 7 software. Nodal support was assessed by bootstrapping with 1000 replicates.

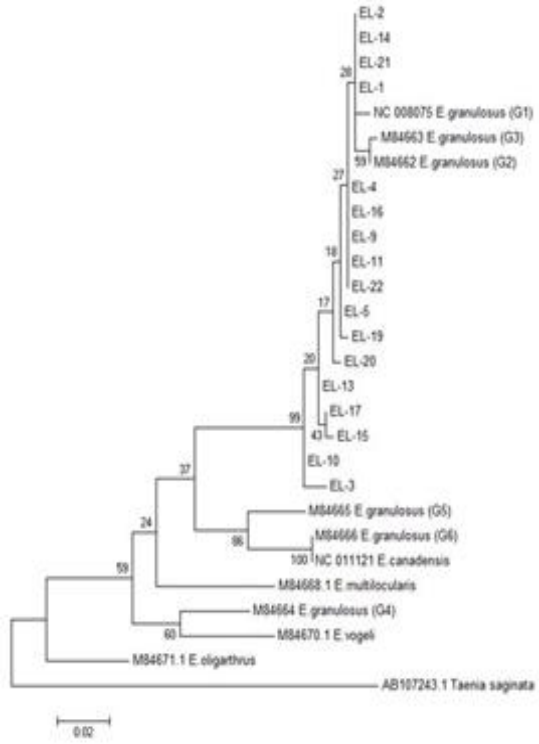

Fig. 2: Molecular Phylogenetic analysis by Maximum Likelihood method

The *Cox1* gene could be used in the molecular diagnosis of hydatid cysts. The predominant genotype in the Sabzevar region is G1 gene. This study could be the basis for further studies to determine the pattern of regional transmission and disease control process.

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Conflict of interest

The authors declare that there is no conflict of interest.

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