Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Short Communication

The Molecular Epidemiology of Cystic and Alveolar Echinococcosis in Southeast Turkey

***Fadime Eroglu [1,2], Mehmet Dokur [3], Yüksel Ulu [4,5]**

1. *Department of Parasitology, Faculty of Medicine, Aksaray University, Aksaray, Turkey*
2. *FaBiyosit Microbiology-Biotechnology R&D Co., Adana, Turkey*
3. *Department of Emergency, Biruni University, Istanbul, Turkey*
4. *Department of Medical Pathology, Kilis State Hospital, Kilis, Turkey*
5. *Department of Medical Pathology, Istanbul Basaksehir Cam and Sakura City Hospital, Istabul, Turkey*

Received 12 Aug 2020
Accepted 25 Nov 2020

Keywords:
Cystic echinococcosis;
Alveolar echinococcosis;
Molecular methods;
Immigration

***Correspondence Email:**
eroglufadime@hotmail.com

Abstract

Background: The migration of humans and climatic and environmental changes cause the emergence of infectious diseases. This study aimed to investigate the changes in the molecular epidemiology of the echinococcosis in the southeast region of Turkey after migrations.

Methods: Overall, 159 tissues samples were taken from suspected cases of echinococcosis at the Kilis State Hospital in the southeast region of Turkey. All of the tissues samples were analyzed using histopathology methods, PCR, Real-time PCR methods, DNA sequencing, and phylogenetic analyses in laboratories.

Results: The positivity values of the histopathology, the polymerase chain reaction, and the Real-time PCR methods were found to be 14.5% (23/159), 15.7% (25/159), and 16.9% (27/159), respectively. 32.0 % (8/25) *E. multilocularis* of *Echinococcus* isolates and 68% (17/25) *E. granulosus* of *Echinococcus* isolates were identified using PCR methods. 58.8% (10/17) of the *E. granulosus* isolates were found to be Genotype 1% and 41.2% (7/17) *E. granulosus* isolates were found to be Genotype 3.

Conclusion: Molecular methods play an important role in the epidemiology, treatment, and diagnosis of diseases. Increasing immigration in a geographical area may create social, economic, and health problems in that area. For this reason, epidemiological studies of infectious diseases should be updated in areas with immigration.

Introduction

Echinococcosis disease is an infectious disease caused by tapeworms belonging to the species *Echinococcus*. This parasite has a worldwide distribution and is considered a neglected tropical disease by the World Health Organization (WHO) (1). The echinococcosis is divided two main types: alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* and cystic Echinococcosis (CE) caused by *E. granulosus* (2, 3). AE is also difficult to diagnosis at an early stage, and the lesions are typically large, multiple, and even infringing on adjacent structures at the first diagnosis (3). CE disease can be seen in a variety organs including the liver, lung, kidney, spleen, brain, and heart as well as the skeleton (4). The clinical progression of CE relies on the organ involved, the size of the cysts and their position within the organ, the mass effect inside the organ and upon contiguous structures and the complication incidence rate of cyst rupture (5).

AE and CE diseases that cause morbidity and mortality in humans and significant economic losses in livestock (6,7). Thus, the early diagnosis of these diseases is important for public health and the economies of countries all over the world. The diagnosis of AE and CE requires analysis with a detailed history, a physical examination, radiological imaging, and serological and molecular laboratory methods (8-10). Molecular methods such as polymerase chain reaction (PCR), Real-Time PCR, and DNA sequencing can have used to distinguish the parasite species in echinococcosis. It is important to determine the etiological agent in AE and CE patients for treatment of the progression of these diseases.

The aim of epidemiology was to study the distribution, determinants, and causes of health-related problems in order to prevent and control them effectively (11). Despite the beneficial effects of traditional epidemiological studies on the prevention and control of many diseases, important questions still remain about the biological mechanisms underlying infectious diseases (11). Therefore, in recent years, molecular epidemiology studies have been developed that can provide valuable information about the transmission probability of infectious diseases.

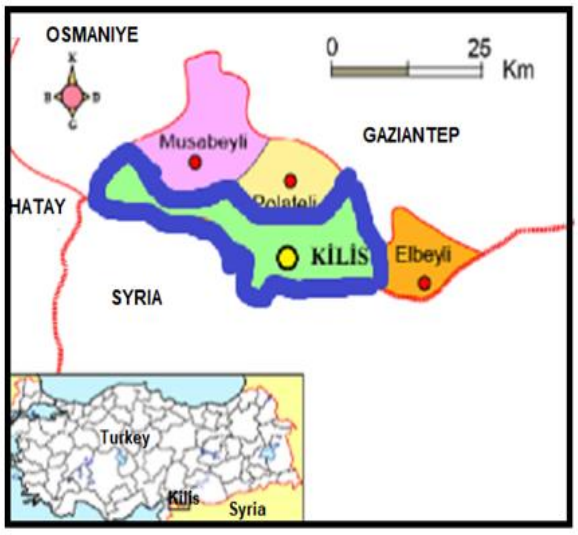

Fig. 1: The Kilis Province's place in Turkey and neighboring of this province

Because of its geographic location and events in neighboring countries like war and unemployment, Turkey has received many immigrants, particularly from Syria, Iran and Azerbaijan (11). In Kilis, a Turkish Province located directly on the border with Syria, the number of local residents has been eclipsed by that of Syrians (12) (Fig.1). The sociological, economic, and health problems in the province of Kilis have changed due to Syrian refugees. The new situation in the province of Kilis brings new health problems that need to be investigated and solved. Thus, this study aimed to determine the *Echinococcus* species, an etiological agent of echinococcosis patients, using molecular methods and to investigate changes in the molecular epidemiology of the echinococcosis arising from the migration in the province of Kilis in southeast Turkey.

Materials and Methods

Collection of Samples

Overall, 159 tissue samples were obtained from suspected echinococcosis patients in the Kilis State Hospital Pathology Laboratory in the southeast region of Turkey.

Permission was obtained from the Kilis Public Hospitals Association (Decision No: 36809483/5848/Kilis) and an ethics committee document was obtained from the ethical committee of Cukurova University (Decision No: 80/31Aug.2018).

All samples were analyzed using histopathology, PCR, Real-time PCR, DNA sequencing, and phylogenetic methods.

PCR Analysis

DNA was extracted from the tissue samples by using the QIAmp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) for the PCR assay. The NADH dehydrogenase subunit 2 (nad2) gene was amplified by the PCR assay according to the protocols for the detection of *E. multilocularis* (13). The PCR assay was carried out in a 25 µl reaction mixture containing 5 µl DNA, 12.5 µl 2X DreamTaq DNA Polymerases (ThermoFisher Scientific, California), 1 µl of each primer (nad2F: gcgttgattcattgatacattgt and nad2R: tagtaaagctcaaaccgagttct), and 4.5 µl sterile water. Thermal reactions were performed for 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 45 sec, and extension at 72 °C for 60 sec in this study.

The identification of *E. granulosus* was based on the amplification of the cytochrome c oxidase subunit gene (CO1) with the use of the primer set JB3 (ttttttgggcatcctgaggtttat) and JB4.5 (taaagaaagaacataatgaaaatg) (14). The PCR assay was carried out in a 25 µl reaction mixture containing 5 µl DNA, 12.5 µl 2X High Fidelity DNA Polymerases (Thermo Fisher Scientific, California), 0.75 µl of each primer, and 6 µl of sterile water. The PCR amplification was performed for 35 cycles of 45 sec at 95 °C, 45 sec at 50 °C, and 60 sec at 72 °C followed by a final extension step of 10 min at 72 °C. The 10 µl of all of PCR products were electrophoresed on 1.5% agarose gels in ethidium bromide as described previously for the detection.

Real-Time PCR Analysis

All of the DNA samples were analyzed with both genus-specific and species-specific primers by the Real-time PCR method. The forward 12S F-gttaagctaagtctatgtgctgc and reverse primers 12S R-ctctcttcacatcaacaaactcatttaa were used to amplify a 126 bp portion of the 12S mtDNA gene for identification of the *Echinococcus* genus-species. The Real-time PCR mixture contained 5 µl of extracted DNA, 1 µl of each primer, 12.5 µl of the SYBR Green master mix, and 5.5 µl of sterile water. The amplification of the PCR conditions were the activation of the polymerase enzyme (95 °C for 3 min), and 35 cycles of amplification were performed, each one including 94 °C for 30 sec, 62 °C for 45 sec, and 72 °C for 45 sec, followed by a final extension at 72 °C for 10 minutes. Fluorescence signals were measured

once in each cycle at the end the of extension step (15).

The DNA of *E. multilocularis* was detected by Real-time PCR targeting a part of the 12S rRNA gene as previously reported for Real-time PCR analysis (16). The Real-time PCR mix was comprised of 10 µL of TaqMan 2X universal master mix (Qiagen, Hilden, Germany), 1 µL Em-R primer (Em-R:ggcttacgccggtcttaactc), 1 µL Em-F (Em-F:ctgtgatcttggtgtagtagttgagattt), 0.5 µL probe Em-Probe: fam-tggtctgttcgacctttttagcctccat-tamra), and 5 µL of genomic DNA samples. The Real-time PCR mixtures were preincubated at 50 °C for two min followed by denaturation at 95 °C for 10 min, 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min using a RotorGene thermocycler (Qiagen, Hilden, Germany).

Genomic DNA was amplified using specific primers for the cox1 gene, the forward JB3 primer (ttttttgggcatcctgaggtttat), and the reverse JB4.5 primer (taaagaaagaacataatgaaaatg) for the identification of *E. granulosus*. The Real-time PCR reaction contained 10 µL master mixes (Type-it HRM PCR Kit, Qiagen, Hilden, Germany), 9 µL of distilled water, 1 µL of each primer, and 4 µL of genomic DNA (17). The Real-time PCR was performed under the following conditions: an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 sec, 55 °C for 30 sec, 72 °C for 27 sec, and a final extension step at 72 °C for 5 minutes. The fluorescence signal was measured at each cycle after the extension step. The melting experiment was performed from 55 °C to 95 °C at 1 °C/sec with continuous fluorescence monitoring. The melting peaks were visualized by plotting the first derivate against the melting temperature, and the T_m was defined as the peak of the curve. Quantitative detection and T_m were obtained using the Rotor-gene 6,000 series software version 1.7 (Corbett, Hilden, Germany).

DNA Sequencing and Phylogenetic analyses

The cycle sequencing kits and a BigDye terminator (Applied Biosytems) were used for the DNA sequencing of the PCR products. The resultant sequence ladders were read with an ABI PRISM 377 genetic analyzer (Applied Biosytems, U.S). The National Center for Biotechnology Information BLAST search primarily confirmed the identity of the sequences with deposited *E. multilocularis* and *E. granulosus*. The results of the DNA sequences both of species were incorporated in the construction of a phylogenetic tree using MEGA ver. 7 software and the maximum likelihood method. The level of confidence was performed by bootsrapping (500 replicates), and the genetic distances calculated between the *E. multilocularis* isolates were described by the same software program.

Results

Out of 159 suspected patients, 56.0% (89/159) males and 44.0% (70/159) females were included in the study. According to the results of the histopathology examination, 5% (23/159) of the patients were positive. All of the DNA samples were screened for the presence of *E. multilocularis* and *E. granulosus* with specific primers using PCR methods. There was a rate of 15.7% (25/159) positive and 84.3% (134/159) negative in all tissue samples according to the PCR results. 32.0% (8/25) *E. multilocularis* of *Echinococcus* isolates and 68% (17/25) *E. granulosus* of *Echinococcus* isolates were identified using PCR methods (Table 1). 58.8% (10/17) of the *E. granulosus* isolates were found to be Genotype 1 and 41.2% (7/17) *E. granulosus* isolates were Genotype 3 in this study (Table 2).

Table 1: The sensitivity / specificity and predictive value of results in histopathology, PCR and Real-Time PCR methods in this study.

Methods	*Positive (%)*	*Negative (%)*	*Sensitivity (%)*	*Specificity (%)*
Histopathology	14.5	85.5	92.8	100
PCR	15.7	84.3	100	97.8
Real-Time PCR	16.9	83.1	100	95.6

Table 2: The identification of *Echinococcus* species caused agents of echinococcosis in southeast of Turkey.

Species	*Positive (%)*	*Negative (%)*	*Genotype 1*	*Genotype 3*
Echinococcus multilocularis	33.3	66.7	0	0
Echinococcus granulosus	66.7	33.3	58.8	41.2

All DNA samples were analyzed using species-specific primers and were probed to identify the *E. multilocularis* and *E. granulosus* species by Real-time PCR methods. The sample was found to be 16.9% (27/159) *Echinococcus* positive and 83.1% (132/159) *Echinococcus* negative according to results of the Real-time PCR method. Cycle threshold values were found to be between 15.1 and 29.3 with a median threshold of 22.2 cycles in Real-time PCR analysis. It was found that PCR is 100% sensitivity and 97.8% specific for the diagnosis of *Echinococcosis*. In addition, Real-time PCR's sensitivity was 100% and 95.6% specific in this study. The sensitivity of the histopathology examination was found to be 92.8%, and the sensitivity of the molecular methods was higher than that of the histopathology methods (Table 1). 33.3% (9/27) of the *Echinococcus* isolates were detected as *E. multilocularis,* and 66.7% (18/27) of *Echinococcus* isolates were identified as *E. granulosus* by the Real-time PCR method.

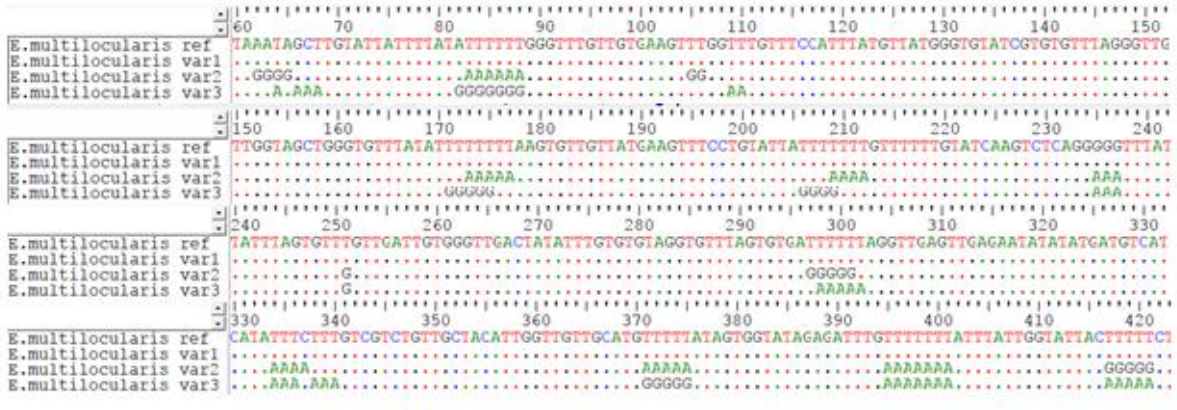

Fig. 2: The results of DNA sequencing in nad2 gene of *Echinococcus multilocularis* in this study. There are 0.076 genetic distance between *Echinococcus multilocularis* isolates and there is no polymophorphism between *Echinococcus granulosus* isolates in this study

The results of DNA sequence confirmed that the *Echinococcus* genotyping was correct in this study. The polymorphism was detected in two of the *E. multilocularis* isolates while no

polymorphism was detected in one of the *E. multilocularis* isolates with DNA sequencing (Fig. 2). The overall average of genetic distance was found to be 0.08 among the *E. multilocularis* isolates, and the phylogeny tree is shown in Fig. 3. No polymorphism was detected between the *E. granulosus* isolates in our study.

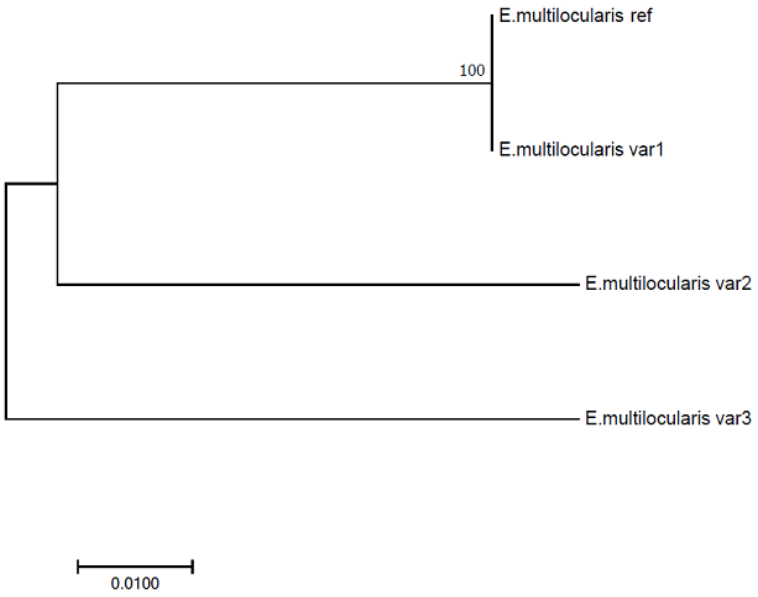

Fig. 3: Phylogeny tree indicate *Echinococcus multicularis* variety. Neighborjoining tree displaying the relationships amongst *Echinococcus multicularis* isolates and bootstrap value is 1000 in this figure

Discussion

Echinococcosis is a zoonotic disease caused by tapeworms of the genus *Echinococcus*. Echinococcosis is divided into AE and CE, both of them generally severe. AE disease is caused by infection with the larval stage of *E. multilocularis*, an approximate 1-4 millimeter long tapeworm found in foxes, coyotes, and dogs. CE, also known as hydatid disease, is caused by infection with the larval stage of *E. granulosus*, an approximate 2-7 millimeter long tapeworm found in dogs, sheep, cattle, goats, and pigs. The WHO reported that echinococcosis was the cause of 19.300 deaths with 90% mortality in untreated patients (18, 19).

AE and CE disease first begins in the liver, but it can also spread to other organs such as the lungs and the brain via the hematogenous route. Multiorgan involvement and the aggressive appearance of lesions cause confusion between AE and CE disease with a metastatic malignancy. Thus, histopathological confirmation is essential for a definite diagnosis of echinococcosis (20).

AE and CE can be easily confused with primary hepatic malignancies and metastases and is misdiagnosed more often compared to other helminth diseases (21,22). While imaging findings in AE and CE are generally strongly suggestive of aggressive malignancy, the general condition of patients is uncharacteristically good. Therefore, clinical findings of the disease should be supported by laboratory techniques. In recent years, PCR and Real-time PCR methods were developed in order to permit a sensitive and specific identification of *E. multilocularis* and *E. granulosus* from clinical samples (23,24). In many studies, it is important to distinguish the *Echinococcus* species, and it can be done with a high melting curve Real-Time PCR analysis. The results of our

study also showed that *E. multilocularis* and *E. granulosus* could be distinguished with a high melting curve Real-time PCR analysis.

Molecular methods such as PCR and Real-time PCR have been observed in the last decade to diagnose of infectious diseases with high sensitivity (25). There are ten different genotype strains (G1-G10) with closely related genotypes that constitute the species *E. granulosus* (25). Determining the geographical distribution and uniformity of host-adapted genotypes of *E. granulosus* are important in confirming the taxonomic designation of *E. granulosus* strain (26). Molecular diagnostic methods are important for epidemiology, therapy, and control of echinococcosis in our country.

In recent years, health problems have increased due to the presence of asylum seekers and refugees in Turkey (27). The geographical and political situation of the country has caused the annual immigration numbers of the country to change. There are some studies regarding *Echinococcus* species in Kilis and its neighbor province Gaziantep. These studies have reported Genotype 1 of *E. granulosus* but not Genotype 3 of *E. granulosus* and *E. multilocularis* in these regions (28, 29). The results of our study showed Genotype 3 of *E. granulosus* in Kilis. There is no genotype in the *E. multilocularis* isolates. These new results of our study may be related to immigration variability. The rate of net migration in the province of Kilis is (-13.9%) according to the Turkish Statistical Institute (27). The many citizens who live in the Kilis Province migrate to neighboring places such as Gaziantep Province due to social, economic, and political reasons. This situation may have caused the molecular epidemiology of echinococcosis in our country to be changed and for Genotype 3 of *E. granulosus* and *E. multilocularis* to spread to the other province. Polymorphism is a principle in biology in which a species can have many different forms, and polymorphism may arise due to various geographical reasons. We detected polymorphism in the *E. multilocularis* isolates in the Kilis Province of southeast Turkey. These polymorphisms may be related to global warming and the geographical situation of Kilis Province. The definition of molecular epidemiology applies to a discipline overlapping with both public health and experimental science. It is useful to consider molecular epidemiology within the framework of epidemiological studies in general. Molecular epidemiology aims to identify determinants of disease and to quantify their role (29). Our study showed that the molecular epidemiology of echinococcosis is changing due to migration and global warming.

The *Echinococcus* species requires two mammalian hosts to complete its life cycles. Segments containing eggs or free eggs are passed in the feces of the definitive host, a carnivore such as dog. The eggs are ingested by an intermediate host such as sheep in which the metacestode stage and protoscoleces develop. The cycle is completed if such an intermediate host in eaten by a suitable carnivore. The distribution of CE follows closely the occurrence of its most important intermediate host, the sheep. In some areas, the hydatid is also a common parasite in dogs and cattle. We investigated the molecular epidemiology of *Echinococcus* in sheep and dogs in the Kilis Province after the Syrian War. Although the life cycle patterns of the two strains overlapped both geographically and in intermediate and definitive hosts, the strains maintained their heterogeneous genetic identify (unpublished data).

Conclusion

AE and CE are generally severe lethal diseases, but these diseases are neglected in our country. The control of AE and CE is more complex due to the involvement of wild animal species as both definitive and intermediate hosts. The identification of risk areas and estimation of the population at risk with the associated molecular factors are important for the design of control programs. The molecular

diagnosis can reveal the diversity of etiological agents of these diseases, so molecular diagnosis is very important for the prevention and control of AE and CE. The molecular epidemiology of echinococcosis is related to migrations in the southeast region of Turkey. However, new epidemiological investigations are necessary to obtain more information about the incidence of echinococcosis in the southeast region of Turkey.

Acknowledgements

The authors thank Direcorate of Kilis State Hospital, Kilis, Turkey.

Funding Statement

No financial support from any institution.

Conflict of interest

The authors declare no conflict of interest related to this study.

References

1. Siyadatpanah A, Anvari D, Emami Zeydi A, et al. A systematic review and meta-analysis for genetic characterization of human echinococcosis in Iran, an endemic country. Epidemiol Health. 2019; 41:e2019024.
2. Avcioglu H, Guven E, Balkaya I, et al. First detecton of *Echinococcus multilocularis* in rodent intermediate hosts in Turkey. Parasitology. 2017; 144(13):1821-1827.
3. Due Q, Wang Y, Zhang M, et al. A new treatment strategy for end-stage hepatic alveolar echinococcosis: IVC resection without reconstruction. Sci Rep. 2019; 9:9419.
4. Khan A, Ahmed H, Simsek S. War, migration and cystic echinococcosis. Travel Med Infect Dis. 2019; 28:111-112.
5. Kohansal MH, Nourian A, Rahimi MT, et al. Natural products applied against hydatid cyst protoscolices: A review of past to present. Acta Trop. 2017; 176: 385-394.
6. Salamatin R, Kowal J, Nosal P, et al. Cystic echinococcosis in Poland: genetic variability and the first record of *Echinococcus granulosus* sensu stricto (G1 genotype) in country. Parasitol Res. 2017; 116(11),3077-3085.
7. Simsek S, Roinioti E, Eroksuz H. First report of *Echinococcus equinus* in a Donkey in Turkey. Korean J Parasitol. 2015; 53(6):731-5.
8. Akcam AT, Ulku A, Koltas IS, et al. Clinical characterization of unusual cystic echinococcosis in southern part of Turkey. Ann Saudi Med. 2014; 34(6),508-516.
9. Ebrahimipour M, Budke CM, Najjari M et al. Surgically managed human cystic echinococcosis in north-eastern Iran: a single center's experience from 2001 to 2008. J Parasit Dis. 2017; 41(3),883-887.
10. Tamarozzi F, Hou A, Morales ML, et al. Prevalence and risk factors for human cystic echinococcosis in the Cusco Region of the Peruvian highlands diagnosed using focused abdominal ultrasound. Am J Trop Med Hyg. 2017; 96(6),1472-1477.
11. Eybpoosh S, Haghdoos AA, Mostafavi E, et al. Molecular epidemiology of infectious diseases. Electronic Physician. 2017; 9(8):5149-5158.
12. Hoffmann S, Samuk S. Turkish immigration politics and the Syrian refugee crisis. Working Paper Research Division Global Issues. SWP. Berlin. 2016.
13. Nakao M, Xiao N, Okamoto M, et al. Geographic pattern of genetic variation in the fox tapeworm *Echinococcus multilocularis*. Parasitol Int. 2009; 58(4):384-9.
14. Roinioti E, Papathanassopoulou A, Theodoropoulou I, et al. Molecular identification of *Echinococcus granulosus* isolates from ruminants in Greece. Vet Parasitol. 2016; 15, 226:138-44.
15. Matera G, Loria MT, Peronace C, et al. Increase of vascular endothelial growth factor and decrease of MCP-1 and some updated epidemiology aspectes of cystic Echinococcosis human cases in Calabria region. Mediators Inflamm. 2018; 2018: 4283672.
16. Knapp J, Millon I, Mouzon I, et al. Real-Time PCR to detect the environmental faecal contamination by *Echinococcus multilocularis* from red fox stools. Vet Parasitol. 2014; 201(1-2):40-7.
17. Pestechian N, Hosseini Safa A, Tajedini M. Genetic diversity of *Echinococcus granulosus* in

center of Iran. Korean J Parasitol. 2014; 52(4): 413–418.
18. http://www.who.int/mediacentre/factsheets/fs377/en/ (last accessed date: 07.03.2019).
19. Rencuzogulları A, Koltas IS, Akcam AT, et al. Challenges, prognosis and outcomes of surgical resection for hepatic alveolar echinococcosis: a single centre experience. Diclemedj. 2017; 44(2):183-190.
20. Ozgur T, Kaya OA, Hakverdi S, et al. Retrospective evaluation of the echinococcosis cases regarding histopathological aspects. Diclemedj. 2013; 40(4):641-644.
21. Knapp J, Umhang G, Poulle ML, et al. Development of a Real-Time PCR for a sensitive one-step coprodiagnosis allowing both the identification of carnivore feces and the detection of *Toxocara* spp. and *Echinococcus multilocularis*. Appl Environ Microbiol. 2016; 82(10):2950-2958.
22. Schweiger A, Ammann RW, Candinas D, et al. Human alveolar echinococcosis after fox population increase, Switzerland. Emerg Infect Dis. 2007; 13(6): 878–882.
23. Federer K, Armuda-Fernandez MT, Gori F, et al. Detection of taeniid (*Taenia* spp., *Echinococcus* spp.) eggs contaminating vegetables and fruits sold in European markets and the risk for metacestode infections in captive primates. Int J Parasitol Parasites Wildl. 2016*;* 5(3),249-53.
24. Boufana B, Umhang G, Qiu J, et al. Development of three PCR assays for the differentiation between *Echinococcus shiquicus*, *Echinococcus granulosus* (G1 genotype), and *Ehinococcus multilocularis* DNA in the co-endemic region of Qinghai-Tibet plateau, China. Am J Trop Med Hyg. 2013; 88(4): 795–802.
25. Umhang G, Bastien M, Renault C, et al. A flotation/sieving method to detect *Echinococcus multilocularis* and *Toxocara* spp. eggs in soil by Real-Time PCR. Parasite. 2017; 24:28.
26. Ahmadi N, Dalimi A. Characterization of *Echinococcus granulosus* isolates from human, sheep, and camel in Iran. Infect Genet Evol. 2006; 6(2),85-90.
27. Icduyu A. Syrian refugees in Turkey the long road ahead. Transatlantic council on migration, Washington, DC: Migration Policy Institute. 2015.
28. Ütük AE. Molecular distinction of *Echinococcus granulosus* isolates in eastern and southeastern Anatolia region. PhD Thesis. Fırat University. 2008.
29. Ütük AE, Piskin FC, Dalkılıc B. Molecular characterization of sheep isolates of *Echinococcus granulosus* in Kilis province. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2012; A35-A38.