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RESEARCH ARTICLE

Molecular Characterization of *Echinococcus granulosus* Isolates Found in Cattle, Buffaloes, Sheep and Goats in Afyonkarahisar, Turkey

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ABSTRACT

This study has been carried out to determine the genotypes of *Echinococcus granulosus* cysts in cattle, buffaloes, sheep and goats raised in Afyonkarahisar region. Cysts were collected from the internal organs of 258 animals, including 65 goats, 71 sheep, 119 cattle and 3 buffaloes infected with hydatid cysts. DNA was isolated from a total of 78 cysts from germinal membranes and protoscoleces extracted from cysts to identify the genotypes of *E. granulosus* in infected animals. PCR-RFLP was carried out using the Hin6I and StuI restriction enzymes in the ND1 gene and no polymorphism could be determined in all isolates. In the COX1 gene analysis, G1 strain known as domestic sheep strains and 18 different haplotypes were found in all isolates from cattle, buffaloes, sheep and goats. As a result, it was concluded that all isolates of the cattle, buffaloes, sheep and goats grown in Afyonkarahisar region determined in the analyses carried on the COX1 gene were G1 strain.

Keywords: Ruminants, *Echinococcus granulosus*, ND1, COX1, PCR-RFLP, DNA Sequencing.

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Afyonkarahisar'da Sığır, Manda, Koyun ve Keçilerde Bulunan Echinococcus granulosus İzolatlarının Moleküler Karakterizasyonu

ÖZ

Bu çalışma, Afyonkarahisar yöresinde yetiştirilen sığır, manda, koyun ve keçilerde bulunan *Echinococcus granulosus* kistlerinin genotiplerinin belirlenmesi amacıyla yapılmıştır. Araştırmada, hidatik kistle enfekte 65 keçi, 71 koyun, 119 sığır ve 3 manda olmak üzere toplam 258 hayvanın iç organlarından kistler toplanmıştır. Enfekte hayvanlardaki *E. granulosus* genotiplerini belirlemek amacıyla kistlerden çıkarılan germinal membran ve protoskolekslerden toplam 78 kistten DNA izole edilmiştir. ND1 gen bölgesi *Hin*6I ve *Stu*I restriksiyon enzimleri kullanılarak PCR-RFLP yapılmış ve tüm izolatlarda polimorfizm belirlenememiştir. COX1 gen bölgesi analizinde sığır, manda, koyun ve keçilerden elde edilen izolatların tümünde evcil koyun suşu olarak bilinen G1 suşu ve 18 farklı haplotip bulunmuştur. Sonuç olarak, Afyonkarahisar yöresinde yetiştirilen sığır, manda, koyun ve keçilerde bulunan izolatların COX1 geninde yapılan genetik analizler sonucunda tüm izolatların G1 suşu olduğu kanısına varılmıştır.

Anahtar Kelimeler: Ruminantlar, *Echinococcus granulosus*, ND1, COX1, PCR-RFLP, DNA Dizileme

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INTRODUCTION

Cystic echinococcosis is common worldwide, especially in underdeveloped and developing countries (Köse and Sevimli 2008). Cystic echinococcosis is a common and important parasitic zoonosis caused by the larval stage of the genus Echinococcus (Cadona and Carmena 2013). Adult parasites are found mainly in the small intestine of the canids including dogs, foxes, wolves, jackals while the larval form is found mainly in the liver, lungs and sometimes the spleen of mostly goats, cattle, pigs, camel, deer, rabbits, monkeys, kangaroos and sometimes settling into the liver, lungs, spleen, heart, kidneys, brain and bone marrow of humans as well as poultry to cause serious public health problems as well as important economic losses. *E. granulosus* and *E. multilocularis* species are the causes of echinococcosis in Turkey. Most cases of hydatidosis encountered in Turkey are caused by the *E. granulosus* species (Merdivenci 1963, Unat et al. 1995, Barış et al. 1989, Markel et al. 1999, Toparlak and Tüzer 2000, Dalimi et al. 2002, Thompson and McManus 2002, Gıcık et al. 2004, Ayaz and Tınar 2006). The genetic diversity of Echinococcus species is evaluated as 10 different genotype strains (Nakao et al. 2007, Thompson 2008, Saarma et al. 2009, Nakao et al. 2010). These strains are G1 (sheep strain), G2 (Tasmanian sheep strain), G3 (buffalo strain), G4 (horse strain), G5 (bovine strain), G6 (camel strain), G7 (pig strain), G8 (deer strain G9 (human strain), G10 (Fennoscandian deer strain) (Eckert and Thompson 1997, Haag et al. 1997, Scott et al. 1997, Thompson and McManus 2002, Lavikainen et al. 2003, Romig et al. 2006). Full mitochondrial genome analysis of Echinococcus species led to taxonomic revision and G1-G3 genotypes were grouped as *Echinococcus granulosus* sensu stricto, G4 *Echinococcus equinus*, G5 *Echinococcus ortleppi* and G6-G10 *Echinococcus canadensis*. (Nakao et al. 2007, Thompson 2008, Saarma et al. 2009, Nakao et al. 2010). The domestic sheep strain (G1) is the most common strain in the world and host specificity is not limited to sheep. Cysts that develop in cattle are mainly sterile while those in mammals such as buffalo, camel and kangaroo are fertile (Bowles and McManus 1993, Eckert and Thompson 1997). It has been demonstrated by many molecular studies that the source of human infections is often the domestic sheep strain. Examinations of isolates obtained from different hosts in Turkey have determined that domestic sheep strains are the active strains (Utuk et al. 2008, Vural et al. 2008, Snabel et al. 2009).

The objective of this study was to determine the strains and genetic affinity of ND1 and mt-COX1 gene zones of *E. granulosus* isolates obtained from cattle, buffaloes, sheep and goats raised in central Afyonkarahisar province and its districts by PCR-RFLP and DNA sequence analysis.

MATERIAL and METHODS

Collecting the samples

In order to determine *E. granulosus* strains in cattle, buffaloes, sheep and goats raised in Afyonkarahisar City center and Emirdağ, Bolvadin, Şuhut, Dinar, İhsaniye districts, hydatid cysts were collected from the internal organs of 258 animals, including 65 goats, 71 sheep, 119 cattle and 3 buffaloes slaughtered in slaughterhouses between March 2010 and April 2012. The contents of the cysts from the same organ were numbered separately. Both germinal membranes and cyst fluids were examined by microscopy for protoscoleces and evaluated as fertile or sterile. The germinal membranes and protoscoleces were washed with PBS and stored at −20°C in microcentrifuge tubes with 70% alcohol until use.

DNA isolation and PCR

Protoscoleces were used primarily in the samples stored in alcohol (70%) at −20°C while germinal membranes were used as necessary. The samples were washed with PBS before DNA extraction. DNA extraction was carried out according to Boom et al. (1990) and Höss&Paabo (1993). The DNA samples were checked for integrity on a 0.6% agarose gel, the amount and quality were measured using spectrophotometer devices (Multiscan GO and Qubit). DNA samples were adjusted to 20 ng / µl and stored at −20°C until analysis.

The primers required to amplify mitochondrial NADH dehydrogenase 1 (ND1) and cytochrome oxidase 1 (COX1) genes were designed using the FastPCR software (Kalendar et al. 2009) (Table 1).

Table 1. Primers, Tm and length of genes.

Gene	Primer 5′→3′	Tm (°C)	Base pair
ND1 F	gtagttactcttatgttggt	56	1038
ND1 R	cttgaagttaacagcatcacg		
COX1 F	tacgttgcctgttttggctgc	57	550
COX1 R	ccagtaatcaaaggccatcacc		

The total of the PCR mixture which was 25 µl contained 50 ng DNA, 1x PCR buffer (supplied), 2 mM $MgCl_2$, 0.2 mM dNTP set (Fermantas), 3 pmol each primer (Alpha DNA), and 1 Unit Platinum Taq DNA polymerase (Invitrogen). Reactions were carried out in an Eppendorf EpGradientS Thermal Cycler. The PCR was programmed for ND1 and COX1 at 95°C for 2 min pre-denaturation, followed by 35 cycles at 94°C for 30 s denaturation, binding at 56 - 60°C for 45 s (Table 1), elongation at 72°C. for 1

min, final elongation step at 72°C for 10 min. The PCR products were checked under UV by using 1% agarose gel and GelRed (Biotium, 41003).

PCR-RFLP

ND1 gene PCR products were cut separately with Hin6I (Thermo) and StuI (Thermo) restriction enzymes. For this, 8 µl of PCR product, 1 µl of restriction enzyme, 2 µl of restriction buffer and 9µl of distilled water were used. Subsequently a 14-hour incubation at 37ºC was carried out. After incubation the Hin6I and the StuI enzymes were inactivated at 65ºC and 80ºC respectively for 20 min. The products subjected to cutting were examined under UV with 2.5% agarose gel supplemented with GelRed and the band patterns of the samples were displayed (Figure 1 and 2).

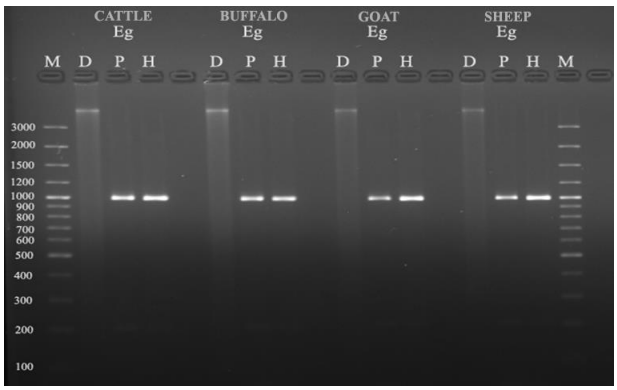

Figure 1. Agarose gel electrophoresis image of PCR-RFLP products of the ND1 gene cut with Hin6I. M: Marker D: DNA P: PCR product H: PCR products treated with Hin6I restriction enzyme.

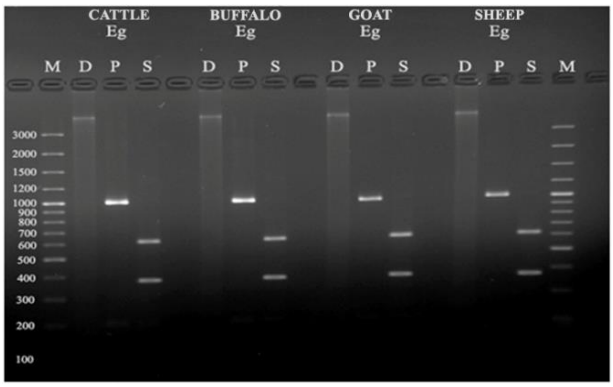

Figure 2. Agarose gel electrophoresis image of PCR-RFLP products of the ND1 gene cut with StuI M: Marker D: DNA P: PCR product S: PCR products treated with StuI restriction enzyme.

Sequence analysis

DNA double-sided sequence analysis of the PCR products of 78 isolates for the COX1 gene was carried out. PCR products were purified using 0.5µl Exo I and 1µl FastAp mixture prior to DNA sequence analysis. The mixture was kept at 37ºC for 15 minutes and at 85ºC for 15 minutes. All of samples sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies).
Sequence PCR products were purged with ethanol / EDTA / sodium acetate and the reactions were run on an ABI 3500Genetic Analyzer. The DNA sequences were edited with the Sequencher 5.4.1 computer program (Gene Code Corporation, Ann Arbor, Michigan, USA) and were aligned with BioEdit 7.0.9 Sequence Alignment (Hall 1999) programs.

Phylogenetic and Statistical Analysis

The nucleotide differences between haplotypes (π), the haplotype mutation rate (Θ) and the Tajima D value were calculated and UPGMA dendrogram was created with the Mega 4 computer package program (Tamura et al. 2007).

RESULTS

As a result of DNA isolation from the internal organs of 258 animals, including 65 goats, 71 sheep, 119 cattle and 3 buffaloes infected with hydatid cysts, DNA was isolated from 78 cysts (30 goats, 26 sheep, 19 cattle and 3 buffaloes).

ND1 gene

DNA obtained from 78 isolates was used for PCR-RFLP and after the PCR analysis, a DNA sequence with the length of 1038 bp was obtained. As a result of cutting the PCR products with Hin6I and StuI restriction enzymes, two bands (Figure 1) with a 1038 bp band and two bands measuring 391 and 647 bp were observed (Figure 2), respectively. All samples showed an exemplary band structure in terms of the ND1 gene.

COX1 Gene

After PCR analysis, a length of 550 bp DNA sequence was obtained in the 1608 bp mt-COX1 gene zone. As a result of the comparison of sequence analysis, 18 different haplotypes (GenBank ID: MT318680-MT318697) were found for domestic sheep strain G1 and variants of isolates. When the distribution of these 18 haplotypes in goats, sheep, cattle and buffaloes and percentage ratios are observed, it is evident that TR_AF001 (MT318680) haplotype is more common (Table 2).

The total number of polymorphic zones(S) for the nucleotide sequences of *E. granulosus*, polymorphic siteratio (ps), nucleotide differences (π), population mutation rate (Θ) and Tajima D value are given in Table 3.

Table 2. Haplotype Distributions and Percentage Ratios in Goats, Sheep, Cattle and Buffaloes.

(GenBank Accession No) Haplotype		GOAT		SHEEP		CATTLE		BUFFALO		TOTAL
		n	%	n	%	n	%	n	%	%
(MT318680)	TR_AF001	6	20,0	19	73,1	4	21,1			37,2
(MT318681)	TR_AF002	1	3,3							1,3
(MT318682)	TR_AF003	6	20.0							7,7
(MT318683)	TR_AF004	1	3,3							1,3
(MT318684)	TR_AF005	10	33,3							12,8
(MT318685)	TR_AF006	4	13,3							5,1
(MT318686)	TR_AF007	1	3,3							1,3
(MT318687)	TR_AF008	1	3,3			3	15,8			5,1
(MT318688)	TR_AF009			2	7,7	3	15,8			6,4
(MT318689)	TR_AF010			2	7,7					2,6
(MT318690)	TR_AF011			1	3,8					1,3
(MT318691)	TR_AF012			2	7,7					2,6
(MT318692)	TR_AF013							3	100	3,8
(MT318693)	TR_AF014					1	3,8			1,3
(MT318694)	TR_AF015					3	15,8			3,8
(MT318695)	TR_AF016					1	3,8			1,3
(MT318696)	TR_AF017					1	3,8			1,3
(MT318697)	TR_AF018					3	15,8			3,8
TOTAL		**30**		**26**		**19**		**3**		

Accordingly, the polymorphism of the 20 COX1 gene of 78 *E. granulosus* was determined and the polymorphism rate was approximately (ps) 3.6%, the population mutation rate (Θ) 0.7%, nucleotide difference (π) 0.4% and Tajima D value was calculated as 1.1067 (Table 3). The average evolutionary differentiation coefficient and standard error of the studied *E. granulosus* cysts was estimated to be 0.351 ± 0.042 (Table 4).

Table 3. Tajima Neutrality Test Results of Samples.

m	S	p_s	Θ	π	D
78	20	0.036	0.007	0.004	-1.1067

Table 4. Mean evolutionary differentiation between DNA sequences belonging to *E. granulosus* in species.

Species	d	S.E.
Goat	0.005	0.002
Sheep	0.001	0.001
Buffalo	0.000	0.000
Cattle	0.005	0.002

The nucleotide differences of the haplotypes in the mt-COX1 gene are given in Table 5. When the genetic relationships between haplotypes are examined, it is observed that TR_AF013 (MT318692) haplotype which is formed by buffalo isolates and TR_AF003 (MT318682) haplotype consisting of goat isolates (Figure 3) and evolutionary distance value is calculated as 0,007 (Table 6). When the haplotypes are evaluated in terms of evolutionary distances, the highest TR_AF018 (MT318697) haplotype was found between the TR_AF004 (MT318683) haplotype and TR_AF018 (MT318697) haplotype and TR_AF014 (MT318693) haplotype (0.015) (Table 6).

Table 5. Nucleotide differences of haplotypes in mt-COX1 gene zone

	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
	6	6	6	6	6	7	7	7	8	8	8	9	9	9	0	0	0	0	0	0	0
(GenBank	1	3	6	6	8	1	2	8	0	1	5	1	7	8	0	0	2	5	5	8	9
Accession No) Haplotype	6	3	7	9	4	7	3	4	0	0	5	8	2	6	1	8	0	6	9	9	9
NC_008075.1	**A**	**C**	**T**	**A**	**T**	**C**	**T**	**A**	**C**	**C**	**T**	**C**	**A**	**A**	**T**	**T**	**A**	**G**	**A**	**G**	**A**
(MT318680) TR_AF001	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318681) TR_AF002	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318682) TR_AF003	.	.	.	.	.	T	.	.	.	T	C	T	.	.	C	.	.	.	.	.	.
(MT318683) TR_AF004	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318684) TR_AF005	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318685) TR_AF006	.	.	.	.	.	.	.	.	G	T	C	.	.	.	C	.	G	.	.	.	.
(MT318686) TR_AF007	.	.	.	.	.	.	G	.	G	T	C	.	.	.	.	.	G	.	.	.	.
(MT318687) TR_AF008	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.
(MT318688) TR_AF009	.	.	.	.	.	.	.	.	.	T	C	.	.	.	C	.	.	.	.	.	.
(MT318689) TR_AF010	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	.
(MT318690) TR_AF011	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318691) TR_AF012	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
(MT318692) TR_AF013	.	.	.	.	.	.	.	G	.	T	C	.	.	.	C	C	.	.	.	.	.
(MT318693) TR_AF014	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.
(MT318694) TR_AF015	.	.	C	.	.	.	.	.	.	.	C	.	G	.	.	.	.	T	.	.	.
(MT318695) TR_AF016	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.
(MT318696) TR_AF017	.	.	.	.	.	.	.	.	T	T	C	.	.	.	.	.	.	.	.	.	.
(MT318697) TR_AF018	.	T	.	.	.	.	.	.	T	.	C	.	.	G	.	.	.	.	G	.	.

Table 6. Evolutionary distances between haplotypes

	TR_AF001	**TR_AF002**	**TR_AF003**	**TR_AF004**	**TR_AF005**	**TR_AF006**	**TR_AF007**	**TR_AF008**	**TR_AF009**	**TR_AF010**	**TR_AF011**	**TR_AF012**	**TR_AF013**	**TR_AF014**	**TR_AF015**	**TR_AF016**	**TR_AF017**	**TR_AF018**
TR_AF001	***																	
TR_AF002	0.002	***																
TR_AF003	0.007	0.009	***															
TR_AF004	0.002	0.004	0.009	***														
TR_AF005	0.002	0.004	0.009	0.004	***													
TR_AF006	0.007	0.009	0.007	0.009	0.009	***												
TR_AF007	0.007	0.009	0.011	0.009	0.006	0.004	***											
TR_AF008	0.002	0.004	0.009	0.004	0.004	0.007	0.007	***										
TR_AF009	0.004	0.005	0.004	0.005	0.005	0.004	0.007	0.005	***									
TR_AF010	0.004	0.005	0.011	0.002	0.006	0.011	0.011	0.005	0.007	***								
TR_AF011	0.002	0.004	0.009	0.004	0.002	0.009	0.007	0.004	0.005	0.005	***							
TR_AF012	0.002	0.004	0.009	0.004	0.004	0.009	0.009	0.004	0.005	0.006	0.004	***						
TR_AF013	0.007	0.009	0.007	0.009	0.009	0.007	0.011	0.009	0.004	0.011	0.009	0.009	***					
TR_AF014	0.002	0.004	0.009	0.004	0.004	0.009	0.009	0.004	0.005	0.006	0.004	0.004	0.009	***				
TR_AF015	0.006	0.007	0.013	0.007	0.007	0.013	0.013	0.007	0.009	0.009	0.007	0.007	0.013	0.007	***			
TR_AF016	0.002	0.004	0.005	0.004	0.004	0.006	0.006	0.004	0.002	0.005	0.004	0.004	0.005	0.004	0.007	***		
TR_AF017	0.004	0.005	0.007	0.005	0.005	0.006	0.006	0.002	0.004	0.007	0.005	0.005	0.007	0.005	0.009	0.002	***	
TR_AF018	0.007	0.009	**0.015**	0.009	0.009	0.013	0.013	0.005	0.011	0.011	0.009	0.009	**0.015**	0.009	0.013	0.009	0.007	***

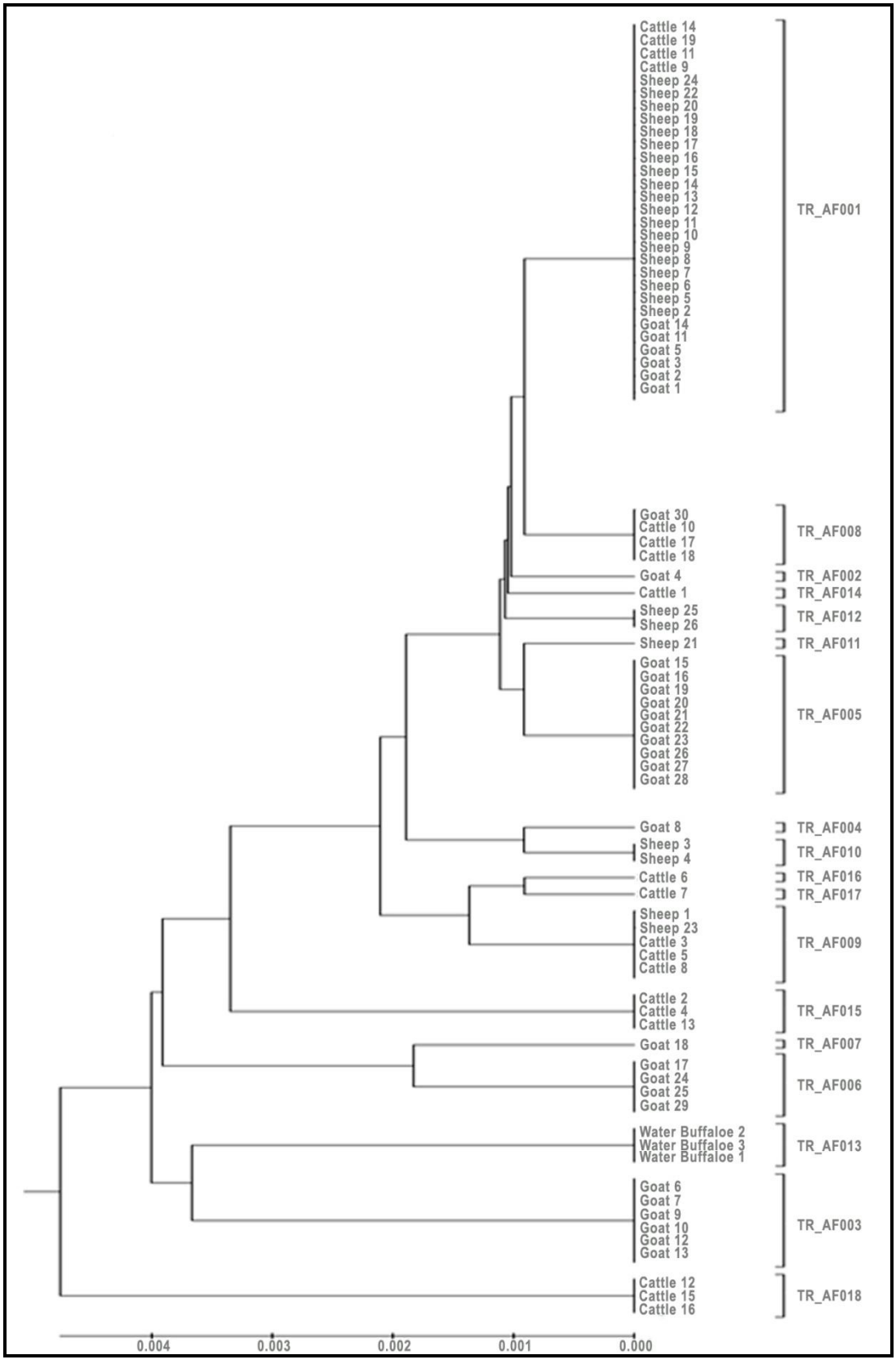

Figure 3. UPGMA dendrogram showing relationships of genetic distances between haplotypes

DISCUSSION

Cystic echinococcosis is an important and widespread parasitic zoonosis all over the world and mainly in less developed and developing countries observed in humans and animals caused by the larval stage of the genus Echinococcus (Köse and Sevimli 2008, Cadona and Carmena 2013). Although the last host is usually carnivores like dogs, foxes, jackals, wolves, different strains of the agent can be found in different geographical regions in numerous intermediate host mammals such as cattle, sheep, goats, deer, camels, buffaloes, rabbits, kangaroos, pigs, horses, donkeys able to infect humans (McManus et al. 2003).
Studies on *E. granulosus* species have been carried out in different regions of the world by using molecular techniques. These studies are shown in Table 7.

Table 7. DNA sequence analysis studies on *E. granulosus*.

Country	Source	Gene	Strain
Spain	Gonzalez et al., 2002	mt-COX1, ND1	G1,G7
Bulgaria	Breyer et al., 2004	ND1	G1
Italy	Capuano et al., 2006	mt-COX1	G1,G3
China	Bart et al., 2006a	mt-COX1	G6
Romania	Bart et al., 2006b	mt-COX1, ND1	G1,G2,G7
Greece	Varcasia et al., 2007	mt-COX1, ND1	G1,G3,G7
Turkey	Vural et al., 2008	mt-COX1	G1,G3
Turkey	Snabel et al., 2009	mt-COX1, ND1	G1,G3,G7
Kenya	Casulli et al., 2010	mt-COX1, ND1	G1,G6
Pakistan	Latif et al., 2010	mt-COX1	G1,G3
Argentina	Soriano et al., 2010	mt-COX1	G1,G3,G6,G7
Turkey	Simsek et al., 2010	mt-COX1	G1,G3
Turkey	Beyhan and Umur, 2011	mt-COX1	G1,G2,G3
Iran	Pour et al., 2011	mt-COX1	G1,G3
Japan	Guo et al., 2011	mt-COX1, ND1	G1,G2,G3
Mongolia	Jabbar et al., 2011	mt-COX1, ND1	G1,G3,G6,G10
India	Singh et al., 2012	mt-COX1	G1,G3
Peru	Sanchez et al., 2012	mt-COX1, ND1	G1,G7
Egypt	Aboelhadid et al., 2013	mt-COX1, ND1	G1,G7
Palestine	Adwan et al., 2013	mt-COX1	G1,G2,G3

In this study, the ND1 gene zone of the isolates was examined by PCR-RFLP technique and shows that the isolates may have a similar genetic structure and the same strain. The DNA sequence analysis of the mt-COX1 gene of the isolates suggests that all isolates are G1 genotype. As a result of the mutations in the COX1 gene region, 18 different haplotypes have been manifested. Table 1 shows that most haplotypes are from goat and cattle species with eight haplotypes. Five haplotypes have been found in sheep. The presence of fewer haplotypes in sheep is probably explained by sampling in nearby regions. In the analyzes, the TR_AF005 (MT318684) haplotype (33.3%) found in goats and the frequency of the TR_AF001 (MT318680) haplotype in sheep and cattle was 21.1% and 73.1%, respectively. The fact that haplotype frequencies vary according to species suggests that the examined animals may have come from the same location. Results in Table 6 confirm this. When Table 6 is examined, it is noted that the frequency of TR_AF001 (MT318680) haplotype in sheep and cattle is high because of the animals in Şuhut district and the high frequency of the TR_AF005 (MT318684) haplotype in goats is caused by animals from Dinar district. The presence of only one haplotype in buffaloes (TR_AF013 (MT318692)) suggests that the number of buffaloes is low and that the samples may have been collected from the same area or from the same herd. The prevalence of the TR_AF001 (MT318680) haplotype over the entire Afyonkarahisar province (37.2%) can be explained by the fact that this haplotype is more likely to produce different types of infection than other haplotypes, or that it may be more widely distributed by animal movements.

The results of the study show that *E. granulosus* is a dominant genotype of domestic sheep strain in Afyonkarahisar and the limited number of studies (Vural et al. 2008, Utuk et al. 2008, Beyhan and Umur 2011, Eryıldız and Şakru 2012) in this subject support the study results.

CONCLUSION

A sequencing analysis of the mt-COX1 gene zone of *E. granulosus* was carried out in this study and as a result of the evaluation of the obtained sequence analysis information, the intermediate hosts were found to be infected with the domestic sheep strain (G1) which is accepted to be the most common and most pathogenic strain in Afyonkarahisar region and the World.

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REFERENCES

Aboelhadid SM, El-Dakhly KM, Yanai T, Fukushi H, Hasanin KM. Molecular Characterization of Echinococcus granulosus in Egyptian. Vet Parasitol. 2013; 193: 292-296.

Adwan G, Adwan K, Bdir S, Abuseir S. Molecular Characterization of Echinococcus granulosus Isolated from Sheep in Palestine. Exp Parasitol. 2013; 134: 195-199.

Ayaz E, Tınar R. Cestoda. In: Tınar R (ed) Helmintoloji, 1st edn. Nobel yayınevi, Ankara. 2006; pp 103-213.

Barış İ, Şahin A, Bilir N, Kalyoncu F, Emri S, Barış B, Çapur S, Selçuk T. Hidatik Kist Hastalığı ve Türkiye'deki Konumu. Türkiye Akciğer Hastalıkları Vakfı Yayını No: 1 Ankara. 1989

Bart JM, Abdukader M, Zhang YL, Lin RY, Wang YH, Nakao M, Ito A, Craig PS, Piarroux R, Vuitton DA, Wen H. Genotyping of Human Cystic Echinococcosis in Xinjiang, PR China. Parasitology. 2006a; 133(5): 571-579.

Bart JM, Morariu S, Knapp J, Ilie MS, Pitulescu M, Anghel A, Cosoroaba I, Piarroux R. Genetic Typing of Echinococcus granulosus in Romania. Parasitol Res. 2006b; 98: 130-137.

Beyhan YE, Umur Ş. Molecular Characterization and Prevalence of Cystic Echinococcosis in Slaughtered Water Buffaloes in Turkey. Vet Parasitol. 2011; 181: 174-179.

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-Van Dillen PME, Van Der Noorda J. Rapid and Simple Method for Purification of Nucleic Acids. J Clin Microbiol. 1990; 28(3): 495-503.

Bowles J, McManus DP. Rapid discrimination of *Echinoccus* species and strains using a polymerase chain reaction-based RFLP method. Mol Biochem Parasitol. 1993; 57: 231–240.

Breyer I, Georgieva D, Kurdova R, Gottstein B. Echinococcusgranulosus Strain Typing in Bulgaria: The G1 Genotype is Predominant in Intermediate and Definitive Wild Hosts. Parasitol Res. 2004; 93: 127-130

Cadona GA, Carmena D. A Review of The Global Prevalence, Molecular Epidemiology and Economics of Cystic Echinococcus In Production Animals. Vet Parasitol. 2013; 192: 10-32.

Capuano F, Rinaldi L, Maurelli MP, Perugini AG, Veneziano V, Garippa G, Genchi C, Musella V, Cringoli G. Cystic echinococcocosis in water buffaloes: epidemiological survey and molecular evidence of ovine (G1) and buffalo (G3) strains. Vet Parasitol. 2006; 137: 262-268.

Casulli A, Zeyhle E, Brunetti E, Pozio E, Meroni V, Genco F, Filice C. Molecular Evidence of the Camel Strain (G6 Genotype) of *Echinococcusgranulosus* in humans from Turkana, Kenya. Trans Roy Soc Trop Med Hyg. 2010; 104: 29-32.

Dalimi A, Motamedi GH, Hosseni M, Mohammadian B, Malaki H, Ghamari Z, Far GF. Echinococcosis/ hydatidosis in western Iran. Vet Parasitol. 2002; 105: 161–171.

Eckert J, Thompson RCA. IntraspesificVarition of Echinococcus granulosus and Related Species with Emphises on their Infectivity to Humans. Acta Trop. 1997; 64: 19-34.

Eryıldız, C., Şakru, N. Molecular Characterization of Human and Animal Isolates of Echinococcus granulosus in the Thrace Region, Turkey. Balkan Med J. 2012; 29: 261-267.

Gıcık Y, Arslan MÖ, Kara M, Köse M. Kars ilinde Kesilen Sığır ve Koyunlarda Kistik Ekinokokkozisin Yaygınlığı. Türkiye Parazitol Derg. 2004; 28(3): 136-139.

Gonzalez LM, Daniel-Mwambete K, Montero E, Rosenzvit MC, McManus DP, Garate T, Cuesta-Bandera C. Further Molecular Discrimination of Spanish Strains of Echinococcus granulosus. Exp Parasitol. 2002; 102: 46-56

Guo ZH, Kubo M, Kudo M, Nibe K, Horii Y, Nonaka N. Growth and Genotypes of Echinococcus granulosus Found in Cattle Imported from Australia and Fattened in Japan. Parasitol Int. 2011; 60: 498-502.

Haag KL, Zaha A, Araujo AM, Gottstein B. Reduced Genetic Variability within Coding and Non-coding Regions of the Echinococcus multilocularis Genome. Parasitology. 1997; 115: 521-529.

Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999; 41: 95-98.

Höss M, Paabo S. DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Res. 1993; 21(16): 3913-3914. (Bu kaynak metin içinde kullanılmamıştır.)

Jabbar A, Narankhajid M, Nolan MJ, Jex AR, Campbell BE, Gasser RB. A First İnsight into the Genotypes of

Echinococcus granulosus from Humans in Mongolia. Mol Cell Probe. 2011; 25: 49-54.

Kalendar R, Lee D, Schulman AH. Fast PCR Software for PCR Primer and Probe Design and Repeat Search. G3. 2009; 3(1): 1-14.

Köse M, Sevimli FK. Prevalence of Cystic Echinococcosis in Slaughtered Cattle in Afyonkarahisar. *Türkiye Parazitol Derg.* 2008; 32(1): 27-30.

Latif AA, Tanveer A, Maqbool A, Siddiqi N, Kyaw-Tanner M, Traub RJ. Morphological and Molecular Characterisation of Echinococcus granulosus in Livestock and Humans in Punjab, Pakistan. Vet Parasitol. 2010; 170: 44-49.

Lavikainen A, Lehtinen MJ, Meri T, Hirvela-Koski V, Meri S. Molecular genetic characterization of the Fennoscandiancervidstrain, a new genotypic group (G10) of *Echinococcus granulosus*. Parasitology. 2003; 127: 207-215.

Markel EK, Voge M, John DT. Medical Parasitology. 8th Edn, WB Saunders Company, USA. 1999; pp 234-268.

McManus DP, Wenbao Z, Li J, Bartley PB. Echinococcosis. Lancet. 2003; 326: 1295-1304.

Merdivenci A. İstanbul Sokak Köpeklerinde Echinococcus granulosus (Batsch, 1786) Rudolphi, 1805. Mikrobiol Derg. 1963; 16(1): 23-28.

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: 713-722.

Nakao M, Yangida T, Okomoto M, Knapp J, Nkouawa A, Sako Y, Ito A. State-of-the-Art Echinococcus and Taenia: Phylogenetic Taxanomy of Human Pathogenic Tapeworms an Its Application to Molecular Diagnosis. Infect Genet Evol. 2010; 10: 444-452.

Pour AA, Hosseini SS, Shayan P. Comparative Genotyping of Echinococcus granulosus Infecting Buffalo in Iran Using Cox1 Gene. Parasitol Res. 2011; 108: 1229-1234.

Romig T, Dinkel A, Mackenstedt U. The Present Situation of Echinococcus in Europe. Parasitol Int. 2006; 55: 187-191.

Saarma U, Jogisalu I, Moks E, Varcasia A, Lavikainen A, Oksanen A. A Novel pylogeny for the Genus Echinococcus, Based on Nuclear Data, Challenges Relationships Based on Mitochondrial Evidence. Parasitology. 2009; 136: 317-328.

Sanchez E, Caceres O, Naquira C, Miranda E, Samudio F, Fernandes O. Echinococcus granulosus Genotypes Circulating in Alpacas (Lama pacos) and Pigs (Susscrofa) from an Endemic Region in Peru. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2012; 107: 275-278.

Scott JC, Stefaniak J, Pawlowski ZS, McManus DP. Molecular Genetic Analysis of Human Cystic Hydatid Cases from Poland: Identification of a New Genotipic Group (G9) of Echinococcus granulosus. Parasitology. 1997; 114: 37-43.

Şimşek S, Balkaya I, Köroğlu E. Epidemiological Survey and Molecular Characterization of Echinococcusgranulosus in Cattle in an Endemic Area Eastern Turkey. Vet Parasitol. 2010; 172: 347-349.

Singh BB, Sharma JK, Ghatak S, Sharma R., Bal MS, Tuli A, Gill JPS. Molecular Epidemiology of Echinococcosis from Food Producing Animals in North India. Vet Parasitol. 2012; 186: 503-506.

Snabel V, Altıntaş N, D'amelio S, Nakao M, Romig T, Yolasığmaz A, Güneş K, Turk M, Busi M, Huttner M, Sevcova D, Ito A, Altıntaş N, Dubinsky P. Cystic Echinococcosis in Turkey: Genetic Variability and First Record of the Pig Strain (G7) in the Country. Parasitol Res. 2009; 105(1): 145-154.

Soriano SV, Pierangelii NB, Pianciola L, Mazzeo M, Lazzarini LE, Saiz MS, Kossman AV, Bergagna HFJ, Chartier K, Basualdo JA. Molecular characterization of Echinococcus canadensis G6 Genotype in Neuquen, Patagonia Argentina. Parasitol Int. 2010; 59: 626-628.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Molecular Biology and Evolution 10.1093/molbev/msm092. 2007.

Tajima F. Statistical Methods to Test for Nucleotide Mutation Hypothesis by DNA Polymorphism. Genetics. 1989; 123: 585-595.

Thompson RCA. The Taxanomy, Phylogeny and Transmission of Echinococcus. Exp parasitol. 2008; 119: 439-446.

Thompson RCA, McManus DP. Towards a Taxonomic Revision of the Genus Echinococcus. Trends Parasitol. 2002; 18: 452-457.

Toparlak M, Tüzer E. Veteriner Helmintoloji. İ.Ü. Veteriner Fakültesi Parazitoloji Anabilim Dalı. 2000; pp 50-56.

Unat EK, Yücel A, Atlaş K, Samastı M. Unat' ın Tıp Parazitolojisi (5. Baskı). İ.Ü Cerrahpaşa Tıp Fak. Vakfı Yayın no: 15, İstanbul. 1995.

Utuk AE, Şimşek S, Köroğlu E, McManus DP. Molecular Genetic Characterization of Different İsolates of *Echinococcus granulosus* in east and southeast regions of Turkey. Acta Trop. 2008; 107: 192-194.

Varcasia A, Canu S, Kogkos A, Pipia AP, Scala A, Garippa G, Seimenis A. Molecular characterization of Echinococcus granulosus in sheep and goats of Peloponnesus, Greece. Parasitol Res. 2007; 101: 1135-1139.

Vural G, Unsal BA, Gauci CG, Bagci O, Gıcık Y, Lightowlers MW. Variability in the Echinococcus granulosus Cytochrome c oxidase 1 Mitochondrial Gene Sequencefrom Livestock in Turkey and a Re-appraisal of the G1-3 Genotype Cluster. Vet Parasitol. 2008; 154(3-4): 347-350.