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Genotyping and Identification of Antigen B Gene Polymorphism of *Echinococcus granulosus* in Edirne, Thrace, and the First Report of Genotype G2 (Tasmanian Sheep Strain) in Turkey

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Background: *Echinococcus granulosus* is the causative agent of cystic echinococcosis in humans and livestock. It is common worldwide. Cystic echinococcosis is still an important public health problem in Turkey, which is an endemic region.

Aims: To genotype *Echinococcus granulosus* isolates and investigate antigen B gene polymorphism in Thrace, Turkey.

Study Design: A cross-sectional study.

Methods: Seventy-five hydatid cyst materials obtained between June 2020 and May 2021 were included in the study. Hydatid cyst materials were collected from 12 humans from various hospitals in Edirne and 63 from slaughterhouse animals during the same period. Cyst materials were localized in 8 livers and 4 lungs in humans, 23 livers and 17 lungs in cattle, and 13 livers and 10 lungs in sheep. In the first step, the 12S ribosomal RNA gene was amplified by polymerase chain reaction for all samples and run on an agarose gel. Band patterns were used for strain typing. Then, the selected samples that represented each of the band patterns obtained by single-strand conformation polymorphism analysis were sequenced for AgB1, AgB2, mt-CO1, and mt-ND1 genes.

Results: Three different genotypes in Edirne, Thrace, Turkey, were observed for *Echinococcus granulosus*: G1 (domestic sheep strain), G2 (Tasmanian sheep strain), and G3 (buffalo strain). G1 was the dominant genotype in Edirne, and G3 was the second most common. Additionally, polymorphism in AgB1 and AgB2 gene regions was found.

Conclusion: This study is the first to report on *Echinococcus granulosus* G2 (Tasmania sheep strain) in Turkey and G3 (buffalo strain) and antigen B polymorphism in Thrace. The study results will contribute to the prevention and control programs for cystic echinococcosis in Turkey and worldwide.

INTRODUCTION

Cystic echinococcosis (CE) is a zoonotic infection caused by the larval (metacestode) stages of cestodes of the genus *Echinococcus* of the *Taeniidae* family. Carnivores are the definitive hosts of the parasite, and various herbivorous and omnivorous animals are intermediate hosts.[1] Humans acquire the parasite in the same way as other intermediate hosts, but they do not play a role in transmitting the infection to the definitive host, so they are referred as random, dead-end intermediate hosts.[2] Hydatid cysts of *Echinococcus granulosus* (*E. granulosus*) develop as fluid-filled unilocular cysts in the internal organs of humans and other intermediate hosts, especially in the liver and lungs.[1]

The World Health Organization (WHO) estimates that over one million people worldwide have echinococcosis annually.[2] Most of Africa, the Middle East, the Mediterranean countries, Central Asia, South America, and Western China are high-endemic areas. Turkey and Iran are among the countries that are most highly affected by livestock and human CE. Both countries are considered as zones of CE endemicity, where high rates of infection with various species and genotypes of *E. granulosus* sensu lato have been reported in livestock.[3] In these endemic areas, the incidence is estimated between 1 and 200/10^5, whereas the mortality rate is generally low (2%-4%).[4] A cross-sectional study conducted in Bulgaria, Romania, and Turkey in 2014-2015 found that the actual burden of CE was poorly understood, and many cases remained asymptomatic. In

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the studied parts of Turkey, in rural areas of Edirne (our region), Balıkesir, Ankara, Aksaray, Şanlıurfa, and Bitlis, 8618 people were screened by ultrasonography, and 46 new CE cases were detected.[5]

Molecular analyses showed that *E. granulosus* can be divided into 10 genotypes (G1-G10). *E. granulosus* sensu stricto (G1-G3) is the most common globally, and G1 and G3 genotypes are the most reported genotypes. G1 is the most common genotype detected in humans in Turkey, followed by G3, G6, and G7 genotypes. In 2012, a study conducted in our region also showed the dominance of the G1 genotype.[6]

As regards the lipoprotein structure, the cyst fluid has two main antigens, antigen 5 and antigen B (AgB). AgB, a 160 kDa thermostable polymeric lipoprotein first described in 1971, consists of 8, 12, 16, and 24 kDa subunits.[7] AgB is a critical antigenic component of the metacestode stage of the parasite. Studies have shown that a multigene family encodes AgB with at least five gene loci (B1-B5).[8,9] Genes encoding AgB often have various polymorphisms. This genetic variation can alter the produced B antigens and significantly affect their effectiveness in serodiagnostic tests used in CE diagnosis. The WHO recommended that the natural B antigens that will be used for diagnostic purposes should be prepared separately for each one of the disease-endemic areas.[10]

The molecular analyses of the antigen B2 (AgB2) gene of cattle, sheep, and human isolates have not been previously studied in Turkey. Thus, this study aimed to define types of *E. granulosus* isolates at the molecular level and determine AgB polymorphisms by AgB1 and AgB2 subunits using single-strand conformation polymorphism (SSCP) and DNA-sequence analyses in common intermediate hosts in Turkey.

MATERIALS AND METHODS

Ethical approval was obtained from the Trakya University Faculty of Medicine Scientific Research Ethics Committee (TUTF-BAEK-2020/152).

Sample Collection and Microscopic Examination

Between 01.06.2020 and 30.05.2021, 12 hydatid cyst materials were obtained from patients who were operated on or applied puncture-aspiration-injection-respiration (PAIR) procedure in Edirne and 63 materials from animals slaughtered in Edirne slaughterhouse during the same period. A total of 75 hydatid cyst materials were included in the study. Cyst fluid and/or germinal membrane were examined under a light microscope for the presence of protoscolex or free rostellar hook. Viability was determined using 0.1% eosin and 0.03% methylene blue dyes. The cyst fluid and/or germinal membrane were stored in 70% ethanol at -20 °C until used for further analyses.

DNA Extractions

Genomic DNAs of samples (germinative membrane and/or cyst fluid) were extracted using the EcoPURE Genomic DNA Kit (EcoTech Biotechnology, Erzurum, Turkey) according to the manufacturer's recommendations. DNA amounts were measured by Nanodrop Spectrophotometer (Allsheng, China).

Amplification of 12S rRNA Gene by Polymerase Chain Reaction (PCR)

At the first step of genotyping the isolates, the 12S rRNA gene region was amplified in PCR as described by Dinkel et al. [11] The G1-G3 complex (*E. granulosus* sensu stricto) was determined by visualizing PCR product under UV light in agarose gel as 254 bp band.

Amplification of AgB1 and AgB2 Genes by PCR and SSCP Procedures

The method described by Frosch et al.[12] was used for AgB1, and the method described by Fernández et al.[13] was used for AgB2. AgB1 and AgB2 have 129 bp and 350 bp band sizes in agarose gel, respectively. PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific, Vilnius, Lithuania), according to the manufacturer's recommendations, for the SSCP. SSCP analyses were performed according to the method of Orita et al.[14], with minor modifications (12% for AgB1 and 10% for AgB2 using 49:1 polyacrylamide gel), and silver staining was also performed with minor modifications (0.1% silver nitrate and 1.5% NaOH were used) as described by Byun et al.[15]

According to the results of the SSCP, 12 isolates with different band patterns from the control strains and five isolates that have the same pattern as the control strains, in a total of 17 isolates, were selected for further analyses. Mitochondrial cytochrome c oxidase subunit (mt-CO1) gene region and mitochondrial NADH dehydrogenase subunit 1 (mt-ND1) gene region were amplified according to the PCR protocols described elsewhere[16,17] for more detailed genotyping of the selected samples. Sanger sequencing was performed using ABI 3730XL (Applied Biosystems, Foster City, CA, USA) and BigDye Terminator v3.1 Cycle (Applied Biosystems, Foster City, CA, USA) kit (BM Laboratory Systems, Ankara, Turkey). Sequencing raw data were aligned using ProSeq (v2) software, and BioEdit (v7.2.5) software was used to compare the nucleotide sequences of the samples and reference sequences.

Phylogenetic Analysis

Phylogenetic analysis was performed on the partial mt-CO1, mt-ND1, AgB1, and AgB2 sequence data using the Genbank reference sequences indicated in Table 1 (BM Laboratory Systems, Ankara, Turkey). Phylograms were created separately for each sequence dataset with the MEGA 11 program using the maximum likelihood method of Tamura and Nei.[18]

Haplotype Analysis

The mt-CO1 and mt-ND1 gene datasets were loaded separately into DnaSP v6 for haplotype analysis. Population diversity values (haplotype numbers, haplotype diversity, and nucleotide diversity) were calculated using DnaSP v6.[19] Output formats, such as resource description framework, were generated for the next analysis using DnaSP v6. Subsequently, networks were created with the median-joining network method using Network 10.2 software.[20]

RESULTS

Microscopic Examination

Of the hydatid cysts included in the study, 44 (58.7%) were located in the liver and 31 (41.3%) were located in the lung. Moreover, 31 (41.3%) of hydatid cysts were fertile, of which 24 (76.2%) were viable. Detailed data were given in Table 2.

12S rRNA PCR Results

All PCR products of 75 isolates have a 254 bp band on an agarose gel. Because of these findings, all the samples were genotyped as G1-G3 complex.

SSCP for AgB1 and AgB2

AgB1 and AgB2 band patterns were visualized in all samples on an agarose gel. All samples were compared with the G1 control strain in the SSCP analysis. Findings were as follows: a) Three different band patterns were detected for AgB1. One isolate was excluded because there was no visible band in the SSCP analysis. Sixty of the isolates exhibited pattern I (consistent with control strain G1), 12 samples exhibited pattern II, and two samples exhibited pattern III. b) Two distinct band patterns were detected for AgB2. One isolate was excluded because there was no visible band in the SSCP analysis. Sixty-one isolates exhibited pattern A (consistent with control strain G1), and 13 exhibited pattern B. Following SSCP evaluation, the selected samples were sequenced for AgB1, AgB2, mt-CO1, and mt-ND1 genes.

Sequence Analyses

Two polymorphisms were determined in the comparison of AgB1 sequences with reference sequences. While 14 isolates were homologous with reference sequences (AY773092 and M36774), 105G/T variation was detected in two isolates, and 104A/T variation was detected in one isolate. Two isolates with polymorphism were cattle isolate and G2/G3 genotype, and one was sheep isolate and G3 genotype.

Given the insufficiency of raw sequencing data, just seven samples were evaluated for AgB2, and four polymorphic sequences were determined. While two samples have the same sequence with the reference sequence AY496952, three of the samples have 236G deletion, one of the samples has 8G/C, 15C/T substitution, and 236G deletion, and finally one of the samples has 58G and 112C deletions. Newly described three polymorphic sequence data were recorded in DNA Data Bank of Japan (DDBJ) (Table 1). Two isolates with polymorphism were human isolates with G1 genotype, one sheep isolate had G1 genotype, and one sheep isolate had G3 genotype.

In PCR amplification of the mt-CO1 and mt-ND1 gene regions of 17 selected samples, 400 bp and 400 bp length DNA fragments were observed, respectively. As a result of comparing the sequences of samples with reference sequences for mt-CO1 and mt-ND1, 10 samples were G1, four were G3, two were G2/G3, and one was G2 (Table 1).

When DNA sequences of samples for mt-CO1 and reference sequences were compared, 11 haplotypes were detected, and three of them were identical to reference sequences HQ703429, M84662, and M84663. In the evaluation for mt-ND1, 10 haplotypes were detected, and two of them were identical to reference sequences HQ717151, AJ237633, and AJ237634. Newly determined sequences were registered in DDBJ.

Phylogenetic Analysis

The mt-CO1 and mt-ND1 trees with the highest log likelihood (-981.08 and -1019.53 respectively) were shown. The phylograms obtained from the phylogenetic analysis of the haplotypes of the mt-CO1 and mt-ND1 gene regions found in this study and reference sequences are shown in Figure 1. The AgB1 and AgB2 trees with the highest log likelihood (-208.67 and -560.16, respectively) are shown. The circular phylograms shown in Figure 2 are obtained from the phylogenetic analysis of polymorphic sequences belonging to AgB1 and AgB2 gene regions and reference sequences.

Haplotype Analysis

In the analyzed mt-CO1 and mt-ND1 gene sequence, the lengths of the *E. granulosus* isolates were 366 bp and 373 bp, respectively. The mt-CO1 gene haplotype network had 11 haplotypes, arranged in a complex star-like configuration, along with the main haplotype separated from the other haplotypes by 1-7 mutational steps and covering 27% (4/15) of the total number of isolates. This main haplotype (H1) comprised three cattle isolates and one human isolate. The mt-ND1 gene haplotype network had 11 haplotypes, arranged in a star-like configuration, along with a central main haplotype separated from the other haplotypes by 1-5 mutational steps and covering 30% (5/17) of the total number of isolates. This main haplotype (H1) comprised four cattle isolates and one human isolate (Figure 3). Detailed haplotype analysis data were given in Table 3.

DISCUSSION

Parasites of the genus *Echinococcus* are prevalent worldwide, and they cause very important cestode infections that threaten public health and economic losses. Nowadays, 10 *Echinococcus* species have been determined, and significant strain variations were found in these species. Although some *Echinococcus* species have limited geographical distribution, *E. granulosus* strains are widespread.[1]

G1 is the most frequently detected genotype in humans in Turkey, followed by G3, G6, and G7 genotypes. *E. granulosus* sensu stricto (G1-G3) was frequently detected in farm animals.[3] In this study, all samples were identified as having G1-G3 complex by 12S rRNA PCR. Our results were partly compatible with those of previous studies.

AgB, widely used for the diagnosis of CE, is one of the most abundant antigens in hydatid cyst fluid.[7] Since genes encoding AgB often have many polymorphisms, this genetic diversity can change the produced B antigens. This situation can significantly affect the efficacy of serodiagnostic tests used in CE diagnosis.[21]

TABLE 1. *E. granulosus* mt-CO1, mt-ND1, AgB1, and AgB2 Gene Sequences Data From the Present Study Compared with Reference Sequences and Accession Numbers (The Parenthesis Next to the Accession Number Indicates the Article Number).

Isolate number	Host	Localization	Cyst viability	Sequence analysis and bioinformatics evaluation results				
				mt-CO1				mt-ND1
				Result of alignments	Haplotype	Genbank BLAST	Access no.	Result of alignments
1	Hu	Lu	No	G1	H1	Identical to HQ703429	LC680960	(356T/G, 358T/G)
2	Ca	Li	No	G1	H1	Identical to HQ703429	LC680960	G1
3	Ca	Li	No	(56C/T, 111C/T, 246G/A, 248G/C)	H2	Some base changes are present for MG808306	LC680961	G1
4	Ca	Lu	No	G2	H7	Identical to M84662	LC681751	G2/G3
5	Sh	Li	No	(66C/T, 123T/C, 251T/C, 257T/C)	H9	Some base changes are present for MK319871	LC681753	G2/G3
6	Ca	Lu	No	(66C/T, 246G/T, 257T/C)	H10	Some base changes are present for MN807919	LC681754	(5T/G, 346A/G)
7	Ca	Li	No	No data		No data	No data	G2/G3
8	Ca	Lu	No	G1	H1	Identical to HQ703429	LC680960	G1
9	Ca	Li	No	(40A/G, 66C/T, 257T/C)	H11	Identical to MG548751	LC681755	G1
10	Hu	Li	Yes	(56C/T, 125A/T, 223T/C, 325A Ins, 337G Ins, 341A Ins, 355A Ins)	H3	Some base changes are present for MT537160	LC681747	G1
11	Ca	Li	No	(220A/G)	H4	Identical to MK975915	LC681748	(53T Ins, 54T Ins)
12	Ca	Lu	No	G1	H1	Identical to HQ703429	LC680960	(356T/G, 358T/G)
13	Ca	Li	No	(56C/T, 228A/C, 248G/C, 298T/C, 299T/C, 307T/C)	H5	Some base changes are present for KM576189	LC981749	(5T/G, 57G Ins, 66T/C, 356T/G)
14	Sh	Li	Yes	No data		No data	No data	(31G Ins, 57G Ins, 333T/G, 356T/G, 358T/G)
15	Sh	Lu	Yes	G3	H8	Identical to M84663	LC681752	(321C Ins, 345T/A)
16	Ca	Lu	Yes	(3T/C, 16A/G, 56C/T, 66C/T)	H6	Some base changes are present of KT184866	LC681750	G2/G3
17	Ca	Li	Yes	(3T/C, 16A/G, 56C/T, 66C/T)	H6	Some base changes are present for KT184866	LC681750	(5T/G)
Reference sequences				mt-CO1			HQ703429 (6)	mt-ND1
							M84662 (16)	
							M84663 (16)	
							M84664 (16)	
							M84665 (16)	
							M84666 (16)	
							HQ717155 (6)	
							AB235848 (16)	
							AF525457 (16)	

Hu, human; Ca, cattle; Sh, sheep; Lu, lung; Li, liver; A, adenine; G, guanine; T, thymine; C, cytosine; Ins, insertion; Del, deletion. (*) indicates the sequences that are not entered in DDBJ because they are short.

Sequence analysis and bioinformatics evaluation results									
mt-ND1				AgB1			AgB2		
Haplotype	Genbank BLAST	Access no.	Final Genotype	Result of alignments	Genbank BLAST	Access no.	Result of alignments	Genbank BLAST	Access no.
H2	There is no base change	LC681757	G1	Identical to AY773092 and M367741		*	Identical to AY496952		LC682195
H1	Identical to HQ717151	LC681756	G1	Identical to AY773092 and M367741		*	No data		No data
H1	Identical to HQ717151	LC681756	G1	Identical to AY773092 and M367741		*	No data		No data
H9	Identical to AJ237633	LC681764	G2	Identical to AY773092 and M367741		*	236G Del		LC682194
H9	Identical to AJ237633	LC681764	G3	105G/T	There is no base change	*	8G/C, 15C/T and 236G Del	Some base changes are present for AY569356	LC682196
H10	Some base changes are present for MN269994	LC681765	G3	Identical to AY773092 and M367741		*	No data		No data
H9	Identical to AJ237633	LC681764	G2/G3	104A/T	There is no base change	*	No data		No data
H1	Identical to HQ717151	LC681756	G1	Identical to AY773092 and M367741		*	No data		No data
H1	Identical to HQ717151	LC681756	G3	Identical to AY773092 and M367741		*	No data		No data
H1	Identical to HQ717151	LC681756	G1	Identical to AY773092 and M367741		*	58G and 112C Del	There is no base change	LC682197
H3	There is no base change	LC681758	G1	Identical to AY773092 and M367741		*	No data		No data
H4	There is no base change	LC681759	G1	Identical to AY773092 and M367741		*	No data		No data
H5	Some base changes are present of MH557950	LC681760	G1	Identical to AY773092 and M367741		*	No data		No data
H6	There is no base change	LC681761	G1	Identical to AY773092 and M367741		*	Identical to AY496952		LC682195
H7	There is no base change	LC681762	G3	Identical to AY773092 and M367741		*	236G Del	There is no base change	LC682194
H9	Identical to AJ237633	LC681764	G2/G3	105G/T	There is no base change	*	236G Del	There is no base change	LC682194
H8	Base changes are not present	LC681763	G1	Identical to AY773092 and M367741		*	No data		No data
mt-ND1		HQ717151 (6)	G1	AgB1		AY773092 (9)	AgB2		AY569347(21)
		AJ237633 (37)	G2			M367741 (24)			AY496952 (8)
		AJ237634 (37)	G3						
		AJ237635 (37)	G4						
		AJ237636 (37)	G5						
		AJ237637 (37)	G6						
		HQ717154 (6)	G7						
		AJ237643 (38)	G8						
		AF525297 (38)	G10						

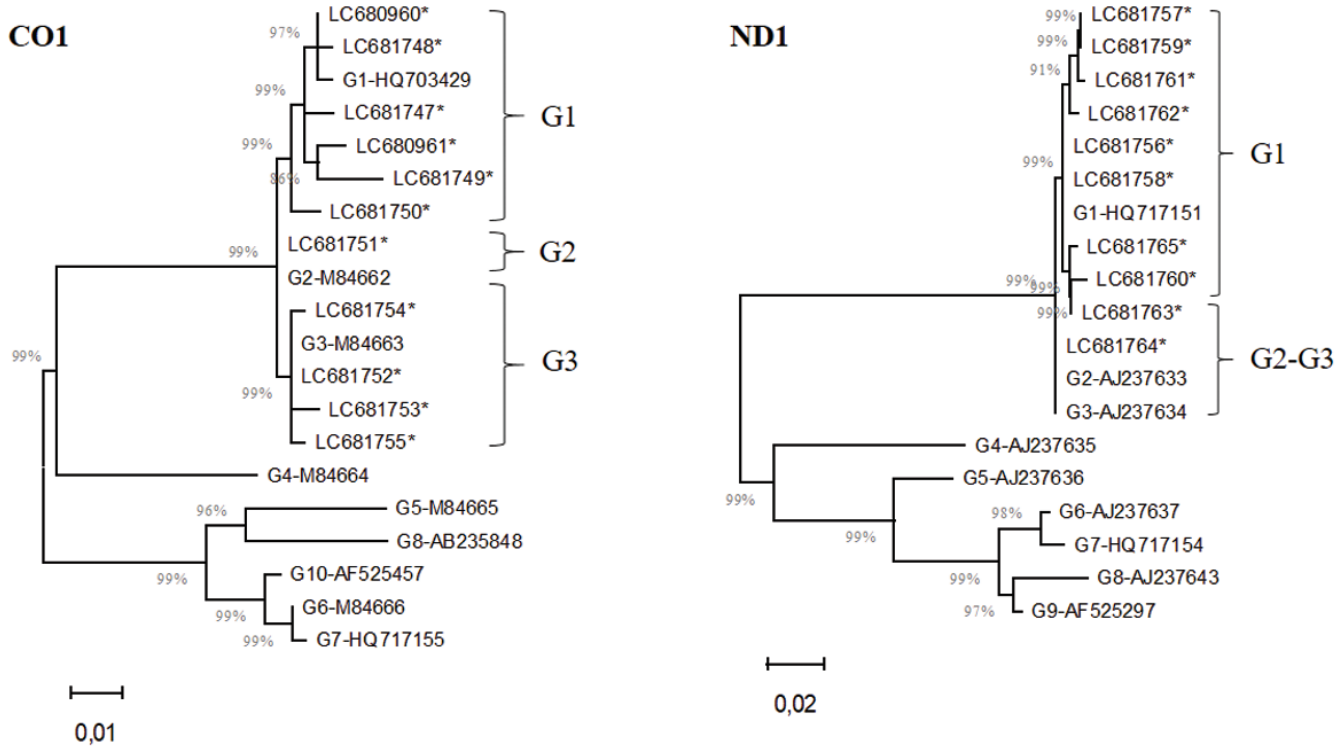

FIG. 1. Genetic relationships of haplotypes (11 haplotypes in mt-CO1 and 10 haplotypes in mt-ND1) from *E. granulosus* isolates in the present study with reference sequences selected from previous studies. The relationships were inferred based on the phylogenetic analysis of mt-CO1 and mt-ND1 sequence data (Table 1) using the maximum likelihood method. (*) indicates the DDBJ accession numbers of the sequences obtained in the present study. Evolutionary analyses were conducted in MEGA11.

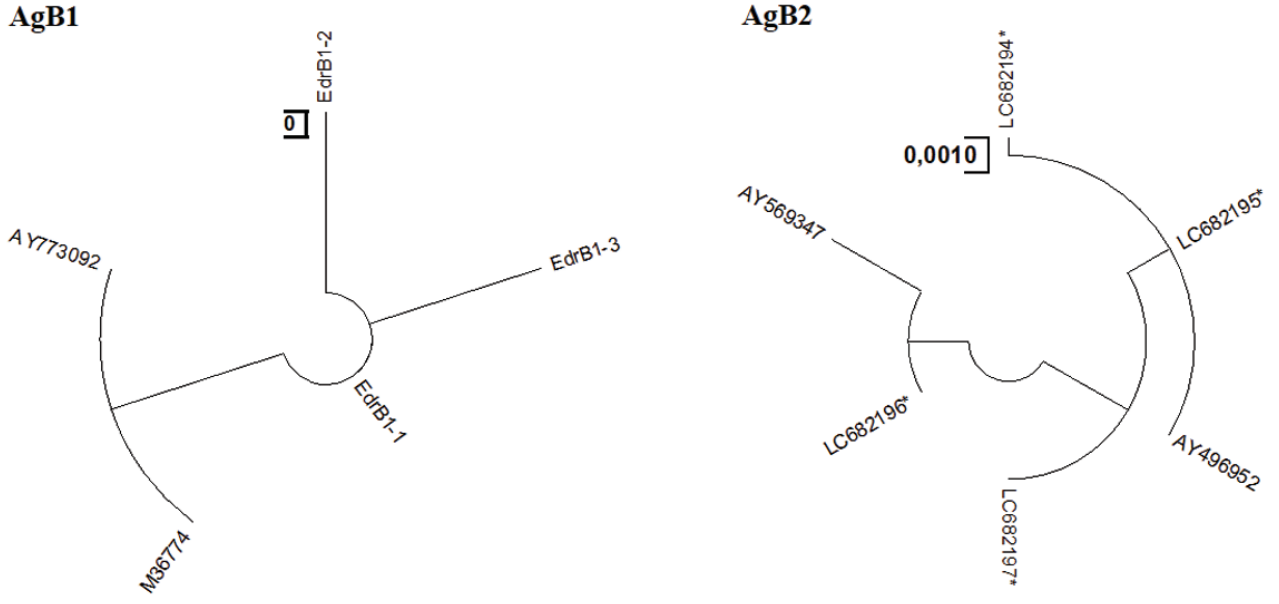

FIG. 2. Genetic relationships of polymorphic sequences AgB1 and AgB2 genes (three sequences and four sequences, respectively) from E. granulosus isolates in the present study with reference sequences selected from previous studies. The relationships were inferred based on the phylogenetic analysis of AgB1 and AgB2 sequence data (Table 1) using the maximum likelihood method. (*) indicates the DDBJ accession numbers of the sequences obtained in the present study. Evolutionary analyses were conducted in MEGA11.

A study examining the subunits of AgB showed that the AgB1 subunit was well represented in all samples analyzed, and AgB2 and antigen B4 (AgB4) subunits were well represented in cattle and human samples.[22] Virginio et al.[23] stated that the AgB2 subunit showed high diagnostic performance for CE in human cysts. Frosch et al.[12] defined the first nucleotide polymorphisms in cDNA sequences encoding the AgB1 subunit in their study, with isolates obtained from different intermediate hosts from different geographical regions. Shepherd et al.[24] examined the nucleotide sequence of the AgB subunit gene fragment and found a different gene sequence displayed in the AgB subunit in the sequence data obtained.

In the study by Pan et al.[25], as a result of PCR-SSCP and DNA-sequence analysis of AgB1 gene region of 110 intermediate host isolates, 16 polymorphic sites were found in the 129 bp length gene fragment. Simsek et al.[26] analyzed 99 intermediate host isolates for AgB1 and obtained 76 assessable bands. The results of the SSCP analysis revealed that two isolates from a human and cattle have different band patterns, and the remaining samples have the same band patterns. Four randomly selected isolates from

TABLE 2. Cyst Localization, Fertility and Viability Rates of Isolates

Host		Cyst location (n/%)	Fertility rate (n/%)	Viability rate (n/%)
Human	Liver	8 (66.7%)	6/8 (75.0%)	4/5 (80.0%)
	Lung	4 (33.3%)	2/4 (50.0%)	0/2 (0.0%)
Cattle	Liver	23 (57.5%)	5/23 (21.7%)	3/5 (60.0%)
	Lung	17 (42.5%)	1/17 (5.8%)	1/1 (100.0%)
Sheep	Liver	13 (56.5%)	9/13 (69.2%)	8/9 (88.8%)
	Lung	10 (43.5%)	8/10 (80.0%)	7/8 (87.5%)
Total		**75**	**31/75 (41.3%)**	**23/30 (76.6%)**

n, number of samples; %, percentage of total

TABLE 3. Haplotype Analyzes Data: DnaSP Output Showing Diversity Indices

Gene region	n	H	hd ± SD	πd ± SD	Number of polymorphic sites	Singleton variable sites (nucleotid positions)	Parsimony informative sites (nucleotid positions)
mt-CO1	15	11	0.933 ± 0.054	0.01255 ± 0.0198	18	3, 16, 40, 111, 123, 125, 220, 223, 228, 246, 251, 298, 399, 307	56, 66, 248, 257
mt-ND1	17	10	0.8824 ± 0.070	0.24256 ± 0.07742	7	66, 333, 345	5, 346, 356, 358

n, number of isolates; H, number of haplotypes; hd, haplotype diversity; πd, nucleotide diversity; SD, standard deviation

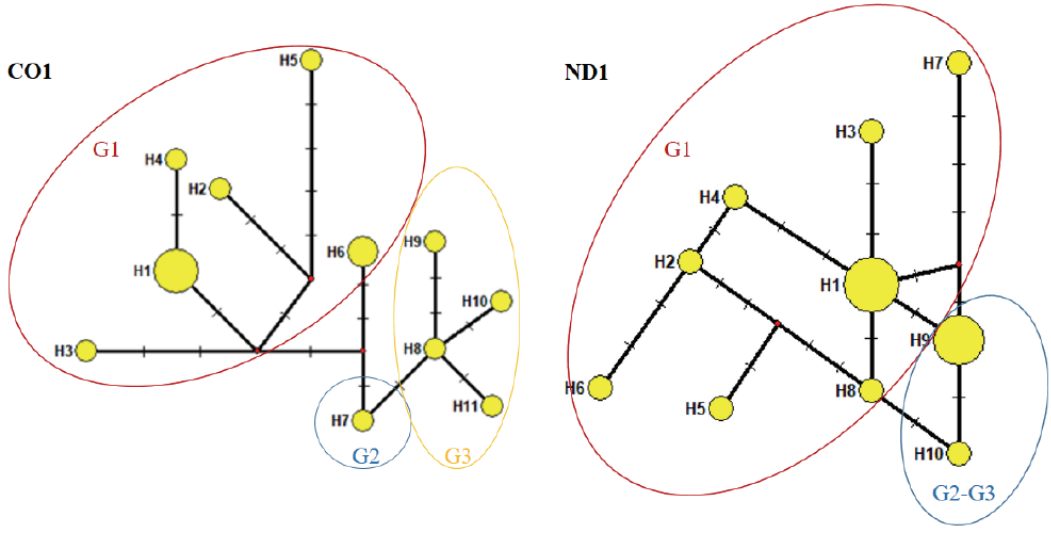

FIG. 3. Haplotype network of mt-CO1 and mt-ND1 genes. Circle sizes are related to haplotype frequency. Hatch marks show the number of mutations distinguishing the haplotypes.

74 samples, and the cattle and the human samples, which have different band patterns, were sequenced. As a result of comparison with the reference sequence (AY773092), polymorphic sites were detected in cattle and human isolates, but no sequence variation was found in sheep isolates. While a 44T/C substitution was detected in human isolates, cattle isolates showed 12G/A and 13A/G substitutions of nucleotide sequence and a 12A nucleotide deletion. In our study, 105G/T variation was found in two of three isolates (one cattle and one sheep isolates), and 104A/T changes were detected in one of the three isolates (one cattle isolate). Human isolates have no polymorphism. Sequences containing 105G/T and/or 104A/T polymorphic sites were not found in the Genbank BLAST search.

In the study by Tawfeek et al.[27] in Egypt, 20 isolates obtained from hydatid cysts of four isolates from different host origins (humans, camels, pigs, and sheep) showed similar band patterns for AgB2 in PCR_RFLP. The results of sequencing and phylogenetic analysis emphasized that human isolates were 100% identical to sheep isolates, 96% with pig isolates, and 99% with camel isolates in terms of the AgB2 gene region. In the study by Kamenetzky et al.,[21] seven isolates (two isolates from G1, sheep strain; one from G2, Tasmanian sheep strain; one from G5, cattle strain; one from G6, camel strain; and two from G7, pig strain) were analyzed for AgB2 nucleotide sequence. As a result of the sequence analysis, different levels of variation were detected in three regions of AgB genes. While only five polymorphic regions were observed in exon 1, they detected differences in a few nucleotides in intron and exon 2. In addition, in the light of their findings, they concluded that the distribution of polymorphisms across the gene is not random. SSCP and DNA-sequence analyses performed in the present study for the AgB2 gene region is the first for Turkey. In the present study, 236 G deletion in three of five isolates (two cattle and one sheep); 8G/C, 15C/T, and 236 G deletion in one of the five isolates (one sheep); and 58G deletion and 112C deletion in one of five isolates (one human) were detected in the sequencing of the AgB2 gene region. 8G/C and 15C/T changes frequently seen in Kamenetzky's study and reference sequences such as AY569356 and AY569351 were detected in one sample with 236 G deletion. This haplotype was not found in the research conducted in the Genbank database. In addition, sequences containing only 236 G deletion and only 58G and 112C deletions were not found in the database.

Manterola et al.[28] reviewed genotyping studies conducted worldwide. Their evaluation revealed that mt-CO1 (91.8%) and mt-ND1 (40.0%) genes were used in genotype determination studies, and in some cases, they were simultaneously sequenced. Considering the geographical distribution of the examined samples, the studies were conducted in many countries, especially in Iran, Turkey, Argentina, Chile, Brazil, and China. In addition, the most frequently identified genotypes were G1 (45.3%), G1-G3 complex (33.0%), and G6 (5.7%). Only 48 isolates were defined as G2 genotype in 12 studies. When evaluated in terms of our country's neighbours, in the east, in Iran, the G2 genotype was first reported in 2012 by Parsa et al.[29] In the following years, many G2 genotypes were reported.[30,31] In the west, eight isolates genotyped as G2 were reported from Romania, and two and eight isolates were reported by two studies performed in Italy.[32-34] Any studies originating from Turkey have no reported G2 genotype until now. It appears that the G2 genotype was defined for the first time in our country.

In our study, the mt-CO1 haplotype search in the Genbank database with BLAST® revealed that four isolates (Haplotype 1) showed homology with the G1 reference sequence HQ703429, one isolate (Haplotype 7) with the G2 reference sequence M84662, and one isolate (Haplotype 8) with the G3 reference sequence M84663. In the same way, the search for the mt-ND1 haplotypes in Genbank showed that five isolates (Haplotype 1) were homologous with the G1 reference sequence HQ717151, and four isolates (Haplotype 9) were homologous with the G2 and G3 genotype reference sequences AJ237633 and AJ237634. The BLAST® results of other mt-CO1 and mt-ND1 haplotypes are given in Table 1.

When genotyping studies were examined, the mt-CO1 gene region was used more frequently than the mt-ND1 gene region.[28,35] In our study of haplotype analysis, we found more nucleotide changes in the mt-CO1 gene region than in the mt-ND1 gene (18 and 7 polymorphic regions, respectively). As there are several reports about the G3 genotype in Turkey, there are no reports from Thrace.[35,36] As a result of our study, the G3 genotype was reported for the first time in the Thrace region. There are no reports from Turkey for the G2 genotype, and the G2 genotype detected in our study is the first data reported from Turkey.

In the samples genotyped with sequence analysis, a total of 5 isolates were viable, which included two bovine isolates (one lung and one liver) two sheep isolates (one lung and one liver), and one human (one liver) isolate. The genotypes of the viable isolates were three G1, one G3, and one G2/G3. No differences were found between viability and the genotypes or location of cyst or the intermediate host from which it was obtained.

This study investigated the genotype diversity in Edirne. Firstly, all samples were genotyped for the 12S rRNA by PCR, and all samples were found as G1-G3. Then, the AgB1 and AgB2 gene regions were analyzed by SSCP for all samples and sequencing method for the selected samples. Two polymorphic sites 105 G/T and 104A/T were identified for AgB1, 236G deletion, 8G/C, and 15C/T substitution with 236G deletion, and 58G and 112C deletion sites were identified for the AgB2 gene region. Sequencing analysis data of mt-CO1 and mt-ND1 gene regions were identical in some samples with various reference sequences previously reported. These data were also investigated bioinformatically.

The results of this study revealed that the G1 genotype (domestic sheep strain) is a common genotype in Edirne. In addition, the G3 genotype (buffalo strain) in the Thrace region and the G2 genotype (Tasmanian sheep strain) in Turkey were detected for the first time. In the light of the findings, we think that the biological and developmental characteristics of the domestic sheep strain (G1), Tasmanian sheep strain (G2), and buffalo strain (G3) of *E. granulosus* should be considered in the control programs of CE

in Turkey. The determined polymorphic sites in the nucleotide sequences of the AgB1 and AgB2 gene regions did not cause changes in the amino acid sequences. However, studies with a large number of isolates collected from different geographical regions and various hosts are needed to reveal the relationship between AgB gene sequence heterogeneity and various morphological, biochemical, and genetic characteristics of the parasite. In addition, the obtained AgB polymorphism results will guide the development of appropriate diagnostic kits and early diagnosis of CE. We believe that the results of our study will contribute to CE prevention and control programs.

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Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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