

Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



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doi: [10.12681/jhvms.29408](https://doi.org/10.12681/jhvms.29408)

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To cite this article:

OGUZ, B., SELCIN, O., DEGER, M., BICEK, K., & OZDAL, N. (2022). A Case Report of *Echinococcus granulosus sensu stricto* (G1) in a Domestic Cat in Turkey. *Journal of the Hellenic Veterinary Medical Society*, 72(4), 3537–3542. <https://doi.org/10.12681/jhvms.29408>

A Case Report of *Echinococcus granulosus sensu stricto* (G1) in a Domestic Cat in Turkey

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ABSTRACT: *Echinococcus granulosus sensu lato* is a zoonotic helminth with a life cycle that include of definitive hosts (dogs and wild carnivores) and intermediate hosts (usually the even-toed ungulates, Artiodactyla). Intermediate hosts become infected by ingesting the parasite eggs in contaminated food and water. Accidental intermediate hosts acquire infection in a similar way as other intermediate hosts. A two-year-old female cat was presented to the Van (Turkey) Animal Care and Rehabilitation Center with abdominal tension. Multiple intraperitoneal vesicles, which were found to be *E. granulosus* (s.l.) metacestodes, were observed during the ultrasound imaging. Then, the animal was laparotomized. Phylogenetic analysis based the partial cytochrome c oxidase 1 (pcox1) mitochondrial gene region was performed on metacestode samples (hydatid cysts). The isolate was identified as sensu stricto genotype G1, which is most commonly found in Turkey.

Keywords: Cat, Cystic echinococcosis, *Echinococcus granulosus*, Mitochondrial gene, Turkey

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Date of initial submission: 18-08-2020
Date of acceptance: 27-03-2021

INTRODUCTION

Cystic echinococcosis (CE) is a neglected and zoonotic disease as it includes domestic animal species as definitive and intermediate hosts. This disease is caused by infection with *Echinococcus granulosus sensu lato* (s.l). *E. granulosus s.l* (Rudolphi, 1801) is classified under the kingdom Metazoa, phylum Platyhelminthes, class Cestoda, subclass Eucestoda, order Cyclophyllidea, family Taenidae and comprise different genotypes: *E. granulosus sensu stricto* (genotypes G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6-G10) and *E. felidis* (lion strain) (Scott et al., 1997; Nakao et al., 2007; Thompson, 2008; Romig et al., 2015). A large number of reports on the incidence of *E. granulosus* (s.l) have displayed that G1 (common sheep strain) is the most common genotype in worldwide (Bonelli et al., 2018). Turkey is also located in the Mediterranean countries which are widespread of genotype G1-G3 (Cardona and Carmena, 2013). Especially, the incidence of CE in humans, sheep and cattle is more prevalence in Van, where the livestock industry is important (Oğuz and Değer, 2013; Sayır and Çobanoğlu, 2013).

The life cycle of the genus *Echinococcus* mainly occurs between domestic/wild carnivores and herbivorous animals, with humans and cats being accidental intermediate hosts involved in this cycle. Domestic and wild carnivores harbour the mature tapeworm in their small intestine. Intermediate hosts, usually herbivores, are infected by ingesting the parasite eggs which are spread in the stool of the definitive host. In a study on whether cats were final hosts or not, it was reported that *E. granulosus* was not fully adult worm (Konyaev et al., 2012). On the contrary, it was reported in a study that domesticated cats might be definitive hosts of *E. multilocularis* (Kapel et al., 2006). There are several case reports in which cats were intermediate hosts and which resulted in cystic echinococcosis (CE) disease (Burgu et al., 2004; Konyaev et al., 2012; Armua-Fernandez et al., 2014; Bonelli et al., 2018). This case report provides on *E. granulosus sensu stricto* (genotype G1) in a domestic cat in Turkey.

CASE HISTORY

Abdominal enlargement, loss of appetite, and loss of weight were determined in the physical examination of a 2-year old stray cat brought to the Van province . Animal Care and Rehabilitation Center, eastern Turkey, in January 2020 (Fig. 1C). It was observed in abdominal palpation that the cat had pain and multi-

ple round masses were felt by hand. Ultrasound imaging showed an image characterized by an anechoic content that contained a multi-leaf structure and multiple intraperitoneal vesicles at different sizes (Fig. 1A) and limited by a hyperechoic edge. After laparotomy, nearly 100 hydatid cysts (measured 0.58 cm and 2.96 cm) were removed, most of which were secondary cysts (translucent and thin-walled) that were commonly found in the entire abdominal cavity (Fig. 1B). Also, one burst primary cyst was seen in liver. The cat was alive after the operation.

The fertility of hydatid cysts was determined by the microscopic presence of protoscolexs, confirming that the parasite was able to complete full development in cats (Fig. 2B). DNA isolation from protoscolex was performed using genomic DNA tissue kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific, Waltham). Genomic DNA extracts from one cyst samples (average number of protoscolex was 80 in 100 µl cyst liquid) were subjected to the JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT -3') and JB4.5 (5'- TAAAGAAAGAACAT AATGAAAATG -3') specific primer PCR reactions amplifying the partial mitochondrial cox1 gene region approximately 450 bp of *E. granulosus* (Bowles et al., 1992). The reaction mixture was prepared at the final concentration of 25µl (Bowles et al., 1992). PCR was carried out in a final volume of 25 µL, containing 7.5 µL DNase- and RNase-free steril distilled water (Biobasic, Canada), 10 µL 5X MyTaq Reaction buffer, 1 µL of each primer (20 pmol), 5 µL of template DNA (100-200 ng), and 0.5 µL of Taq DNA polymerase (1.25 IU) (MBI Fermentas, Lithuania). The PCR conditions were as follows: 5 min at 94 °C (initial denaturation), 35 cycles of 30 s at 94 °C, 45 s at 50 °C, 35 s at 72 °C, and finally 10 min at 72 °C (final extension). The PCR products were separated on agarose gels (1.5 %), stained with ethidium bromide and visualized and photographed on an gel documentation system (Avegene, Taiwan). The positive sample was purified using a commercial purification kit (High Pure PCR Cleanup Micro Kit, Roche, Germany) before index analysis, and then, was subjected to capillary electrophoretic separation in a private laboratory (Sentebiolab, Ankara, Turkey) and index analysis of the products was carried out. Sequence chromatograms were checked and arranged using the BioEdit software (Hall, 1999). "Nucleotide BLAST" (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/BLAST) analysis was applied to the final consensus sequences of the isolates in the GenBank database and similarity rates



Figure 1. A: Ultrasound image of hydatid cysts, **B:** Hydatid cysts removed from the abdominal cavity, **C:** Abdominal distension due to peritoneal cystic echinococcosis

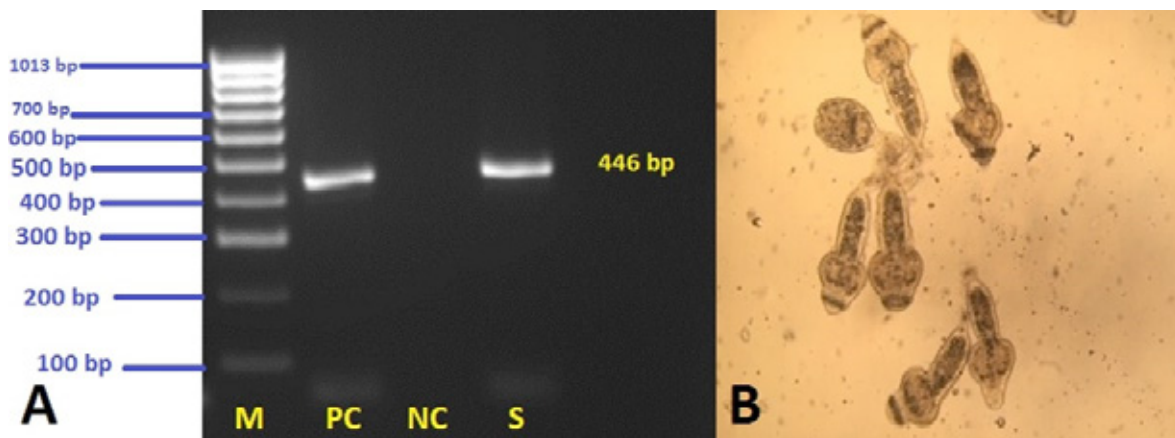


Figure 2. A: Gel electrophoresis from cox1-PCR amplification of *E. granulosus*. (M; molecular marker 100 bp DNA ladder (Hyper-Ladder, Bioline, London, United Kingdom), NC; negative control (no DNA), PC; positive control, S; specific product for *E. granulosus* isolated from cat cyst. **B:** Fertile cysts were indicated by the presence of protoscolices

of the isolates were compared with the ones reported from different countries. The partial Cox 1 phylogenetic analysis data set was composed of nucleotide sequences of a total of 23 isolates. *Taenia multiceps* (AB792725) was used as the “outgroup.” About a portion of 450 bp was used for phylogenetic analysis.

Phylogenetic analyses and tree creation were carried out using the “maximum likelihood” method with a 1000-iteration bootstrap in the MEGA 7.0 (Kumar et al., 2016) software. The nucleotide sequence obtained in the study was recorded in the GenBank as MN990735. *E. granulosus* positive control DNAs and negative control, which are available in our laboratory, were used for all processes. ~450 bp-bands expected for *Echinococcus granulosus* were successfully amplified (Fig. 2A). Phylogenetic analysis

(Fig. 3) revealed that the sequence isolated from the cat belonged to the G1 cluster unlike the other genotypes and was %100 similar to common haplotypes (MG722980 and MF544127) that had been previously reported to be dominant in Europe and Turkey.

DISCUSSION

Cystic Echinococcosis (CE) is one of the most common zoonotic diseases and occurs in people who have close contact with livestock and dogs, especially in rural areas (Orsten et al., 2018). While the lifecycle of *Echinococcus granulosus sensu lato* is observed between dogs and sheep in Europe, Spain, Italy, former Yugoslavian countries, Greece, and Turkey according to the geographic distribution of the animals that are intermediate hosts; it is observed between dogs and horses in Western Europe and Ireland and

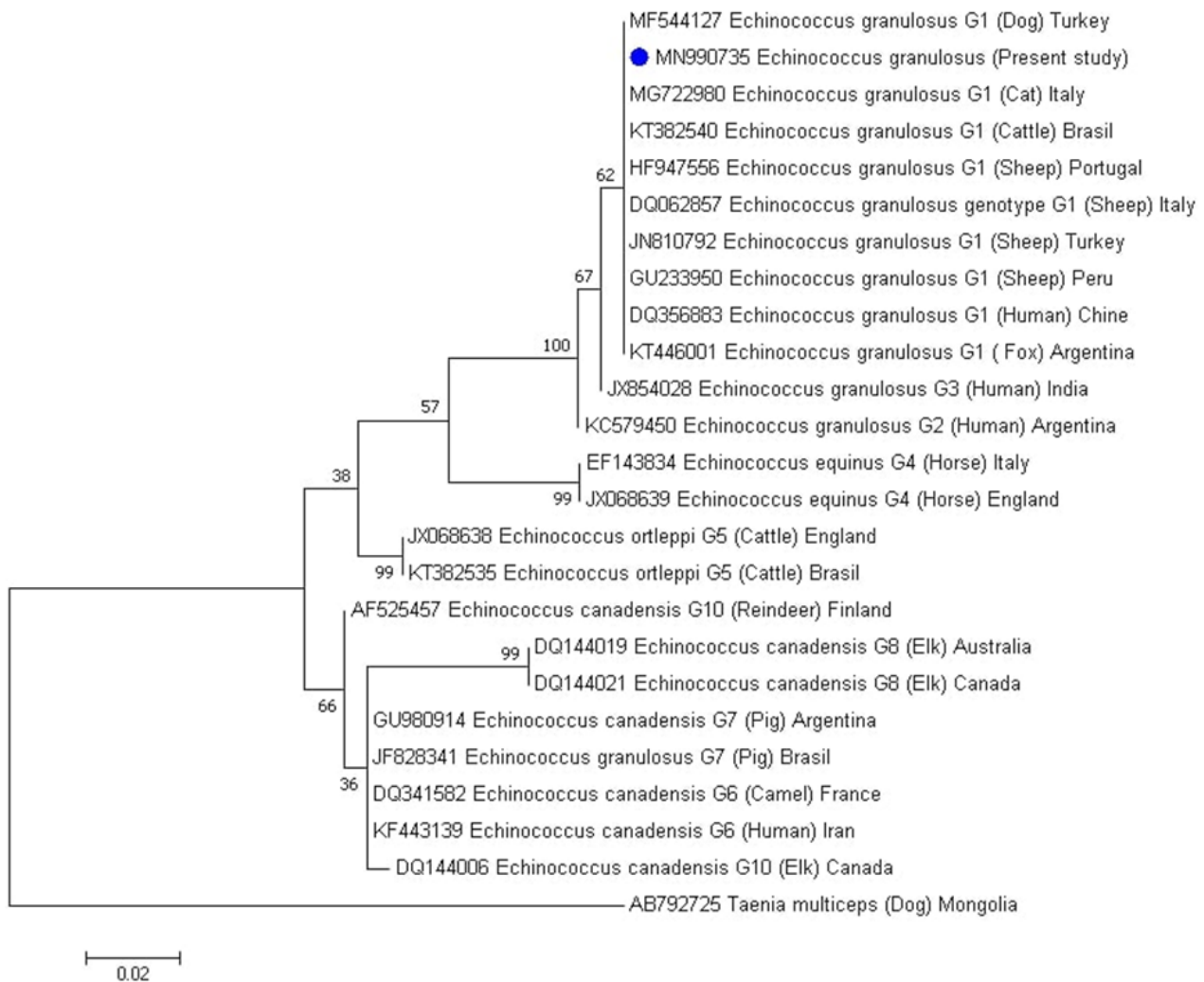


Figure 3. Phylogenetic analysis of *Echinococcus granulosus* cat isolate from Van, Turkey (Blue dot) and reference sequences for *E. granulosus* and *Taenia multiceps* as the outgroup. The relationships were inferred based on phylogenetic analysis of partial cox1 sequence data using Maximum likelihood

mostly between dogs and cattle in Belgium, Germany, and Switzerland (Grosso et al., 2012; Otero-Abad and Torgerson, 2013; Ozcel et al., 2007). In the studies conducted in South Africa and Africa, wild cats were reported to be the definitive hosts of *E. felidis* and *E. oligarthrus* species (Lizardo-Daudt et al., 1993; Kapel et al., 2006). Studies on domestic cats proved that they are intermediate hosts and could not be definitive hosts. However, it was found for *E. multilocularis* that adult tapeworm that could lay eggs at low rates could be formed (Lizardo-Daudt et al., 1993; Kapel et al., 2006).

Some researchers could not detect primary cysts or any scars in cats with cystic echinococcosis during operations (Von der Ahe, 1967; McDonald and Campbell, 1993). Also, Armua-Fernandez et al. (2014) claim that cysts that are observed in the free hydatid cyst during operations are secondary cysts. They stated that the reason for this was that primary cysts burst during climbing and jumping of cats and in this way, protoscoleces in primary cysts produce secondary cysts. In our case, a burst primary cyst was observed in the liver of the cat, and similarly, we think that secondary cysts that were detected were formed in consequence of the bursting of this cyst. We did not carry out any morphological examination of secondary cysts related to the cyst wall (such as cuticular or germinal layer).

The diagnosis of cystic echinococcosis rests mostly on imaging (ultrasound images) for humans (Higuaita et al., 2016). However, cat ultrasounds have been in more use throughout the veterinary medical community for many decades. Ultrasounds use sound waves to examine and photograph internal organs in real time. To determine whether the cat infected with cystic echinococcosis (CE), the cat's abdominal re-

gion was analyzed for hydatids of large dimension by Bonelli et al., (2018). The study demonstrated that multiple intraperitoneal vesicles of different dimensions and anechoic content by ultrasound imaging. According to the results of our study, such imaging systems can be used to determine free hydatid larvae.

Until now, G1, G3, G4, G5, G6 and G7 genotypes have been isolated in Turkey (Utuk and Simsek, 2008; Simsek et al., 2011; Oguz et al., 2018; Avcioglu et al., 2021). G1 is the dominant strain also in Turkey, and in consequence of the DNA sequence analyses of the mitochondrial *cox1* regions of the isolates obtained from sheep, goats, cattle, camels, humans, and dogs, G1 strain was identified in all of these hosts (Utuk and Simsek, 2008). In all of the case reports in which domestic cats are intermediate hosts in Russia, Italy, and Uruguay, domestic sheep strain of *E. granulosus*, i.e., genotype G1 was found. The fact that genotype G1 was found in also our study shows again that we are in accord with the literature in both Turkey and the rest of the world and it is the most common strain. Also, genotype G1 of *E. granulosus* was reported in a study on dogs in the Van province of Turkey, where coincides with this case report (Oguz et al., 2018).

In conclusion, this report of a clinical case of cystic echinococcosis in a domestic cat. Molecular analysis suggest that sequence isolated from the cat is 100% identical to the common haplotype (MG722980 and MF544127) previously reported as the *E. granulosus* G1 genotype. Cystic echinococcosis still continues to be a major public health problem and environmental contamination.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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