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G1 the common *Echinococcus granulosus* genotype infected domestic cat (*Felis catus*) in Iraq

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ABSTRACT

Background: Infections of cats with Echinococcus granulosus is uncommon because the cat is not part of the parasite life cycle that a carnivorous and another herbivore represent. Nevertheless, it occurs incidentally when eating food or drinking water contaminated with the worm's larva, especially with the presence of the definitive host (dogs), in this case, the infections are concentrated in stray or outside cats. For this reason, this study examined the possibility of cat infection with E. granulosus and diagnosed the common genotype of this infection.
Objective: This study examined the possibility of cat infection with E. granulosus and diagnosed the common genotype of this infection.
Methods: Four of the 37 cats that had died in different accidents developed cystic echinococcosis (CE). The cytochrome c oxidase subunit I (COX1) gene was initially amplified and sequenced to determine if these cysts belonged to E. granulosus, in beginning. The DNA fragments resulting from sequencing were then compared and aligned with other sequences using the Gene Bank database. Finally, a phylogenetic tree was drawn according to the sequence data obtained from cox1 genes sequencing, and the MEGA 7.0 phylogenetic analysis program was utilized.
Results: Four different sequences were deposited in the Gen Bank with accession numbers (ON795961 to ON795964), all of which belong to the G1 genotype. Approximately 84% and 100% of these sequences aligned with G1 (AB622277.1) and G1 (MG722980.1), respectively.
Conclusions: G1 is the dominant genotype that causes cat infections, even though the cat's EC infection was incidental.

Keywords: Cystic echinococcosis; *Echinococcus granulosus*; Hydiat cysts; *Felis catus*; genotyping

INTRODUCTION

Hydatid disease is one of the oldest human diseases, also known as cystic echinococcosis [1]. It is a widespread epidemic disease. It is prevalent throughout Iraq, many Mediterranean countries, and the rest of the world. The main endemic areas are those with a greater tendency for sheep and cattle breeding, particularly in southern Australia, New Zealand, the northern part of Africa, and some South American countries [2]. Human infections constantly occur on the European continent and in North and West Asia. The disease causes health, economic, and social problems. Iraq is one of the countries most affected by this disease [3].

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The cysticercus larval of *Echinococcus granulosus* causes hydatid disease. *E. granulosus* is from the genus of tapeworms *Echinococcus* spp. [4]. This genus contains two main species: *E. granulosus* and *Echinococcus multilocularis*. They are the most well-known worldwide because they cause cystic echinococcosis and alveolar echinococcosis, respectively [5]. The other lower famed species are *Echinococcus vogeli* and *Echinococcus oligarthrus*, which are endemic to Central and South America [6].

The life cycle of *E. granulosus* passes into two stages. The adult stage is the first stage. it lives in the intestines of the final host (Canidae family, which includes dogs, wolves, and jackals) [7]. The larval stage is the second stage, which can infect various hosts, including humans, livestock, and other animals [8]. Raising dogs for grazing or other purposes contributes to the infection chain between the intermediate and definitive host, resulting in parasite growth and spread, particularly in the presence of livestock animals [2].

Cats are the definitive hosts of *E. multilocularis* [9]. On the other hand, even though they can be an intermediate host for *E. granulosus* [10,11], they are not affected by the adult parasite because the parasite cannot resist stomach acids or attach to other parts of the digestive system [12]. This genus has ten genotypes (G1–G10) that affect different hosts [13]. The G1 genotype is the generality common throughout the world, particularly in the Middle East [14]. The G1 strain affects sheep, whereas the G3 strain affects cows [15]. The coexistence of livestock animals and cats in the same habitat facilitates the spread of these genotypes and the infection of many intermediary hosts [16].

The acquisition of *E. granulosus* mitochondrial genome sequencing supported research into identifying and constructing the parasite phylogenetic tree. The most sensitive genes for identifying the various genotypes of this parasite are the mitochondrial cytochrome C oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes [17].

MATERIALS AND METHOD

Between December 2021 and June 2022, 37 stray cats (eight months to three years old) that had perished in various incidents were picked up from the Al-Qadisiyah Governorate (in the southeast of Iraq). They were inspected at the Al-Hamza veterinary clinic, where a skilled veterinarian assisted in dissecting the cats. After the inspection, four of them were infected with Cystic echinococcosis.

The cysts were separated, rinsed three times with distilled water (phosphate buffered saline, PBS), and stored at 4°C in 70% ethanol [16]. A microscopic examination of the cysts revealed an infection. The majority of the cysts had three walls and hydatid sand within. The fertility of these cysts was then assessed by counting the protoscolex using the procedure reported by Muhaidi et al. [18], and the protoscolex was separated.

Five viable cysts from each animal, totaling 20, were selected for molecular analysis. The DNA was extracted using the kit provided by Bioneer Company (Korea), according to the manufacturer's instructions. The DNA samples were kept at −20°C.

The extracted DNA fragments were amplified using the (789 bp) *COX1* gene primers JB3 (5′-TTTTGGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′-TAAAGAAAGAACATAATGAA-

AATG-3′), according to Mardani et al. [19]. The DNA products were purified using the EZ EZ-10 Spin Columnar Gel Extraction Kit (Biobasic, Canada), which was supplied by The Bioneer Company (Korea). Eight samples (two samples from each animal) were sent to the same company for sequencing.

The sequences were added to the Gene Bank database and the National Center for Biotechnology Information on the NCBI (http://www.ncbi.nlm.nih.gov) database, where they were aligned and compared with the obvious sequences [20]. The development pattern from Molecular Evolutionary Genetic Analysis (MEGA) version 7.0, Maximum Likelihood, estimated using the Tamura-Neirange, was used to build the phylogenetic tree [21]. Statistical analysis was performed to extract the incidence rate, *p* value, and SD using the SPSS ver. 24 program (IBM Corp., USA).

Ethics approval

The Ethics Committee at Ministry of Agriculture - Veterinary Department No. 99 at 15/12/2020, accepted ethic statements for the collection of samples from animals.

RESULTS

Four (10.8%) of the 37 cats that perished in separate incidents had cystic echinococcosis. **Table 1** displays the details, including the proportion of infection. Every cyst (4–21 mm) was located above the liver. Thirty-two developed protoscoleces were present, and the fertility rate was 21%. The infection validity was determined using molecular techniques after utilizing the gene (*cox1*) after a microscopic investigation had determined the infection and the kind of cysts (**Fig. 1**).

The National Center for Biotechnology Information (NCBI) received a deposit of DNA products sequencing results and the accession number (ON795961 to ON795964). When these sequences were aligned to the G1 sequences using the Gene Bank data, all samples were found to be *E. granulosus* under the G1 genotype (**Fig. 2**). The sequence (G1: MG722980.1) from Russian cats and *E. granulosus* (G1: AB622277.1) from Italian cats were used to obtain alignment rates of 100% and 84%, respectively. **Fig. 3** presents a phylogenetic tree.

DISCUSSION

Infection of cats with *E. granulosus* is uncommon because it is not part of the parasite life cycle represented by carnivorous and other herbivorous animals [22]. Nevertheless, cats can be

Table 1. Details of infections with CE in cats

Parameters	No. of case	SD	Infected	OR	95% CI	*p* value
Sex						
Male	15 (40.5%)	1.17	0 (0%)			
Female	22 (59.5%)	1.12	4 (18%)	6.2	0.311–0.123	0.023
Age (yr)						
> 1	14 (37.8)	1.42	2 (14.3%)			
1–2	10 (27%)	1.33	1 (10%)			
< 2	13 (35.2%)	2.06	1 (7%)	0.6154	0.05–0.065	0.068
Total	37 (100.0%)		4 (10.8%)			

OR, odds ratio; CI, confidence interval.

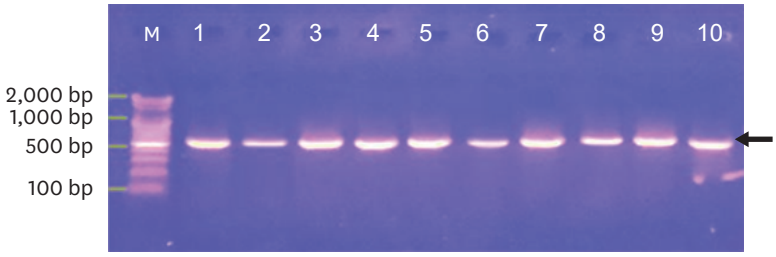

Fig. 1. DNA products of the *COX1* gene *cox1* using 2% agarose.
cox1, cytochrome C oxidase subunit 1.

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Identities:457/457(100%),  Gaps:0/457(0%), Strand: Plus/Plus

Query  2    TGAGAGTGGTGTGATTAGGTAGATGGGTGTTTACTTTAGATCATAAGCGCATAGGTGTGA  61
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGAGAGTGGTGTGATTAGGTAGATGGGTGTTTACTTTAGATCATAAGCGCATAGGTGTGA  60

Query  62   TTTATAGTTTATTGGGTATATGATCTGGTTTTGTGGGTTTGAGATTTAGTTTGTTGATTC  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTTATAGTTTATTGGGTATATGATCTGGTTTTGTGGGTTTGAGATTTAGTTTGTTGATTC  120

Query  122  GTGTTAATTTTTTGGAGCCTTATTATAATGTTATACCTTTGGATTGTTATAATTTTTTGG  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GTGTTAATTTTTTGGAGCCTTATTATAATGTTATACCTTTGGATTGTTATAATTTTTTGG  180

Query  182  TTACAAACCATGGTATAATAATGAttttttttttCTTGATGCCTATATTGATTGGGGGGT  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTACAAACCATGGTATAATAATGATTTTTTTTTTCTTGATGCCTATATTGATTGGGGGGT  240

Query  242  TTGGGAATTATTTATTGCCTTTGTTAGGTGGGTTGTCTGATTTGAATTTACCGCGTTTGA  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTGGGAATTATTTATTGCCTTTGTTAGGTGGGTTGTCTGATTTGAATTTACCGCGTTTGA  300

Query  302  ATGCTTTGAGTGCTTGACTTTTGATTCCTTCGTTGGttttttttGTTGGTTAGTATGTGTT  361
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATGCTTTGAGTGCTTGACTTTTGATTCCTTCGTTGGTTTTTTTGTTGGTTAGTATGTGTT  360

Query  362  TAGGGGCTGGTGTTGGTTGGACATTTTATCCGCCGTTGTCCTCGTCGTATTTTTCTAGTA  421
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TAGGGGCTGGTGTTGGTTGGACATTTTATCCGCCGTTGTCCTCGTCGTATTTTTCTAGTA  420

Query  422  GTTGTGGTGTTGATTTTTTGATGTTTTCTCTGCATTT  458
            |||||||||||||||||||||||||||||||||||||
Sbjct  421  GTTGTGGTGTTGATTTTTTGATGTTTTCTCTGCATTT  457
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Fig. 2. Alignment of the *COX1* gene of *E. granulosus* using Gene Bank.

infected accidentally by consuming food or drinking water contaminated with worm eggs, particularly in the presence of the host (dogs) [8]. The infections in cats are prevalent in stray or outdoor cats. As a result, this study examined the incidence of *E. granulosus* infection in cats to identify the genotype commonly seen in these infections.

The number and vitality of the cysts were low, which is consistent with the findings reported by Avila et al. [12], who recorded 10% fertility of the cysts. By contrast, Konyaev et al. [11] reported that the fertility of the cysts might reach 46%. Armua-Fernandez et al. [16] observed that there were no primary cysts, and the larvae contained only secondary cysts. They explained that the loss of the primary cysts occurs when cats jump or climb, and the transformation of the primary cysts transform into secondary cysts [16]. According to Oguz et al. [23], there is no distinct phenotypic difference between primary and secondary cysts [8]. On the other hand, several investigations have found that these cysts have poor pathogenic consequences. Burgu et al. [24] validated the capacity of cysts recovered from cats to infect dogs but found no substantial efficacy or pathogenic consequence [24].

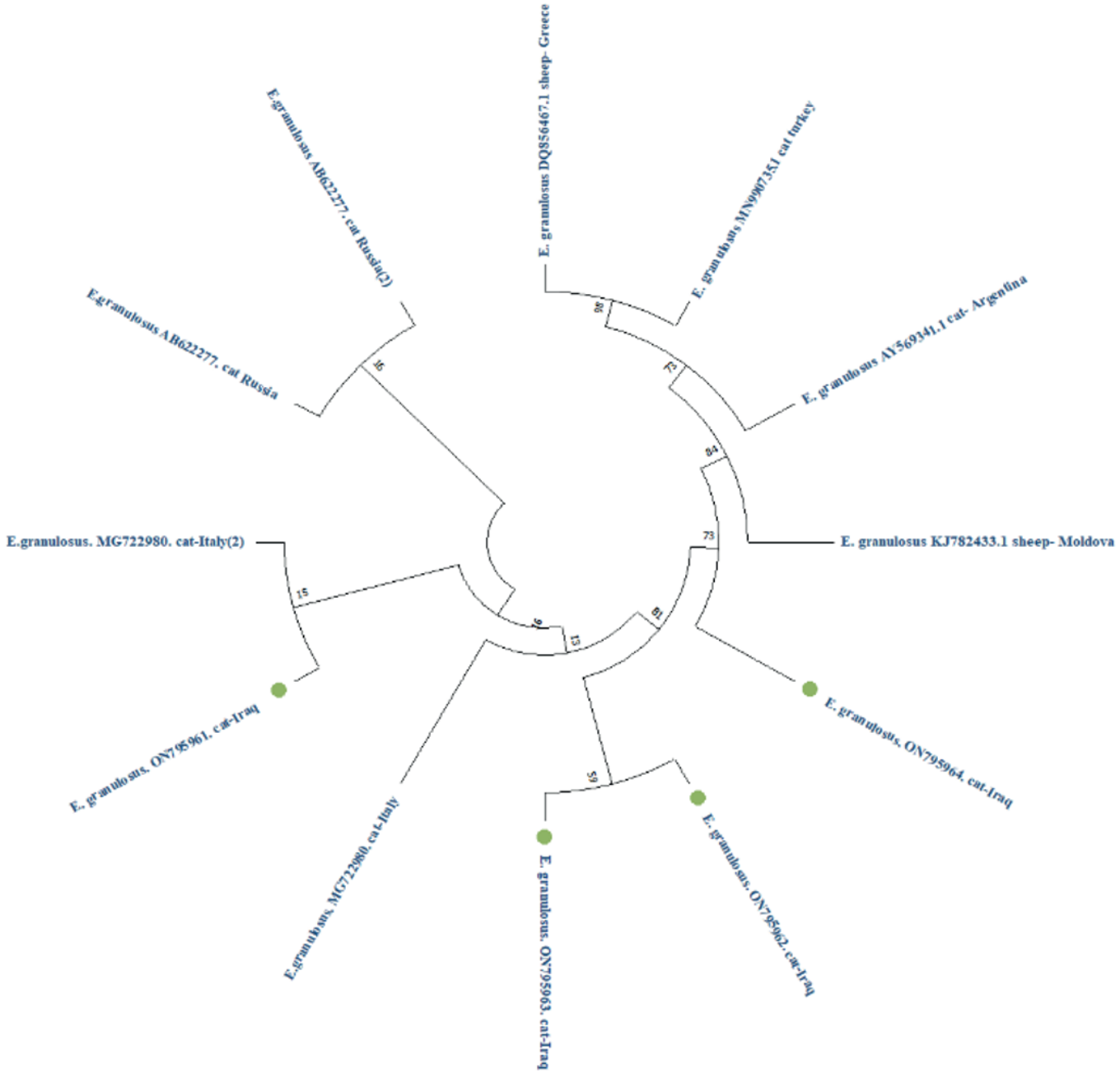

Fig. 3. Phylogenetic tree analysis using the Maximum Likelihood test.

The mitochondrial genome sequencing process of *E. granulosus* made it easy to study its genotypes and enabled the selection of genes that carry distinct variations between genotypes. The *cob* and *COX1* genes are most commonly used to differentiate these genotypes [25]. Many studies in humans [26, 27] and livestock animals [28, 29] used these genes to establish the genotype of the hydatid cyst worm. These investigations found the G1, G3, and G7 genotypes in humans and G1 and G3 genotypes in sheep and cattle. The most common genotypes in Iraq are G1 and G3, affecting humans, sheep, and cattle [30]. Al-Asadi et al. [31] addressed a human infection with the G1 genotype, whereas Fadhil and A'aiz [32] investigated animal infections with the genotypes (G1-G3). The *NAD1* gene was utilized in all experiments

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[32]. No study has been conducted thus far on the genotyping of *E. granulosus* in cats. The current study is the first report in this field. This study showed that cats carry the genotype (G1). Previous comprehensive studies in other countries revealed the existence of the G1 genotype of hydatid cyst in domestic cats [12,16].

Cats can accidentally be infected with hydatid cysts. The current study discovered that genotype (G1) affects cats, and molecular analysis aligned it with previously common haplotypes (G1: AB622277.1 and G1: MG722980). The infection of cats adds a new burden to public health, particularly if these cats are in direct contact with humans and livestock animals.

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