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# Genotyping and Phylogenetic Analysis of Cystic Echinococcosis Isolated from Camels and Humans in Egypt

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**Abstract** The objectives of the present study were to investigate strain identification of *Echinococcus granulosus* infecting camel and human in Qalyubia, Egypt. Therefore partial sequences were generated after gel purification of nested PCR amplified products of mitochondrial NADH 1gene of *Echinococcus granulosus* complex. Sequences were further examined by sequence analysis and subsequent phylogeny to compare these sequences to those from known strains of *E.granulosus* circulating globally and retrieved from GenBank. All isolates are homologous to the camel strain, *E.* canadensis (G6) genotype. Nucleotide mutations generate polymorphism at position of 275 nucleotide, where a thymine replaced a cytosine and at the levels of 385 and 386 nucleotides, where two cytosine substituted a guanine and a thymine respectively. KF815488 Egypt showed typical identity (99.5%) with JN637176 Sudan, HM853659 Iran, AF386533 France and AJ237637 Poland with 0.5% diversion.. Phylogenetic analysis showed a robust tree clustering all isolates with sequences belonging to the camel genotype (G6) variant with strong bootstrap values at relevant nodes and the evolutionary distance between groups is very short. There are two mutations in the sequences of amino acids at the position of 92, where an Alanine is changed to a Valine and at the position of 129, where a Valine is transformed to a Proline. Our record of a single genotype determined a strain which could be incriminated for camel and human infectivity and responsible for its persistence in the endemic areas. Such epidemiological data could guide the application of efficient control strategies of hydatidosis in Egypt.

***Keywords:*** *Echinococcus granulosus, sequences, phylogeny, nucleotide, mutation*

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## 1. Introduction

Cystic echinococcosis (CE) is an important zoonotic disease affecting various species of livestock and humans, caused by metacestodes of dog tapeworms of the *Echinococcus granulosus* complex (*Eg* complex). The adult worm lives in the small intestine of a carnivore (definitive host), while the larval stage develops in the internal organs of an intermediate host, mainly in the lung and liver [1,2,3] which acquires the infection through accidental ingestion of the tapeworm eggs. Hydatid cyst develops in the internal organs of human and herbivore intermediate hosts. CE represents an increasing public health and socio-economic concern in Egypt [4] and many areas of the world especially in many rural, grazing areas of Africa [1,5]. Asia (, and [6,9] Australia [10].

Hydatid infection often leads to a decline of health status that in turn translates into serious production losses to humans and livestock industries. Economic losses arise not only from the condemnation of infected viscera, but also from reduction in yield and quality of meat, milk, wool, hide value, birth rate, and fecundity [11] Humans are accidentally infected by ingestion of food or drinking of water contaminated with dog feces containing infective eggs [12] CE is considered an emerging and re-emerging disease in many parts of the world [13]. The global burden of CE is estimated at >1,000,000 DALYs (disability adjusted life years) lost, which gives CE a greater impact than onchocercosis, Dengue fever and Chagas disease, and approaches the burden caused by African trypanosomosis and schistosomosis [14] Human hydatidosis is typically a symptomatic because of the slow growth of metacestodes. Clinical symptoms usually do not become evident until 10 years or more after initial infection [15] (Sako *et al.* 2011).

Early diagnosis and treatment are important for reduction of morbidity and mortality[16] (Sarkari *et al*. 2007).

The disease is usually diagnosed in patients using imaging technique as ultrasonography [17] (Sako *et al.* 2002). Camels seemed to play an important role in the transmission cycle of the parasite and the epidemiology of the disease especially in rural communities, where dogs infected by eating infected camel carcasses containing the hydatid cysts[18].

Studies based on mitochondrial DNA analysis have demonstrated that *E. granulosus* is actually a complex of species/genotypes which exhibit a marked genetic variability. Therefore, at least ten distinct genotypes (G1–G10) have been identified within the *E. granulosus* complex[19]. These include two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), two pig strains (G7 and G9), and two cervid strains (G8 and G10). In addition, recent molecular evidence suggests that infections in wild carnivores are likely caused by a specific strain (G11) named *E. felidis*. This genotype has been documented in lions and hyenas[20] (Huttner *et al.* 2008). Genotypes G1–G3 cluster firmly together to form the taxon, *E. granulosus sensu stricto* (*E. granulosus s.s.*). These variants have broad geographical distributions and a wide range of host specificity and are responsible (particularly G1) for most human infections. The more distantly related genotype cluster G6–G10 (*E. canadensis*) includes strains that are all infective to humans, but to a much lesser extent than those from *E. granulosus s.s.* [19].

Studying the genetic characterization of the population structure of *E. granulosus* *[21]* has significant implications fo epidemiological and control studies. However, only one study has explored the population structure of *E. granulosus* from Cairo, Egypt [22]. Therefore, the objectives of the present study were to investigate profoundly the molecular characterization of *E. granulosus* isolates from camels and humans by sequence and phylogenetic analyses of a fragment of the mitochondrial NADH dehydrogenase 1 gene as well as the nucleotide and protein polymorphism in the circulating genetic variants in Qalyubia Governorate, Egypt, and to compare our findings to those related to known strains of *E. granulosus* circulating globally. Consequently, this study is regarded as the first attempt in Qalyubia Governorate, to the best of our knowledge, for determination of a strain which could be incriminated for camel and human infectivity.

# 2. Materials and Methods

## 2.1. E. granulosus Isolates

For continuation of our previous work, under publication, twenty- five fertile cyst fluids recovered form lungs and livers were used as follows. Twenty isolates were recovered form of camels slaughtered at the official slaughterhouses of Toukh and Benha (35 and 50 km apart north Cairo, respectively), Qalyubia Governorate, Egypt, during the period from October 2012 to September 2013. Five hydatid- cyst fluids were recovered, according to [12] from humans (45-55 years old) admitted to Toukh's hospital and Benha insurance hospital, Qalyubia Governorate, Egypt, during the last two years. Samples of protoscolices isolated form cysts were used for genetic characterization and stored at -20 °C until used according to [23] Samples were subjected to nested PCR using two pairs of oligonucleotide primers of mitochondrial NADH dehydrogenase 1 gene primers, the first amplification step was conducted through using the outer primer EGL1 and EGR2 and the second amplification step analyzed by using the inner primer EGL3 and EGR4, as a result, the expected fragments 435 bp and 276 bp were identified respectively.

## 2.2. Sequence Analysis

The PCR products were gel purified by using QIAquick gel extraction kit (Qiagen, Valencia, Calif.) following the manufacture's instruction The purified PCR product was sequenced by using BigDye Terminator v3.1 Cycle Sequencing Kit on an automatic sequencer (3500 Genetic Analyzer; Applied Biosystems, Foster City, CA**).** The nucleotide sequences were then algined with existing sequences of known genotypes from other countries in the GenBank databases using BLAST programs and databases of the NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

## 2.3. Phylogenetic Analysis

Phylogenetic analyses were based on alignments obtained from ClustalW method using Bioedit (DNA analysis program) of a partial sequence of 276 bp length of NADH dehydrogenase 1 gene of the Egyptian camel G6 strain was carried out using MEGA software v5.0 as cited by [24]. The Phylogenetic tree were constructed using the neighbour-joining of MegAlign program from LaserGene Biocomputing Software Package (DNASTAR, Madison, WI).

# 3. Results

Partial sequencing of the NADH dehydrogenase 1 gene produces a sequence of 399 bp for each sample and submitted to the GenBank database with the accession (KF 815488). Camel and human isolates are homologous to the camel strain (G6) E. Canadensis.

Sequence alignment was compared with previously reported nineteen references of E. granulosus G6 genotypes of the most similar sequences retrieved from GenBank to identify the genotype of the isolate (Figure 1). Nucleotide sequencing revealed the occurrence of nucleotide mutations generating a single nucleotide polymorphism at position of 275 nucleotide, where a thymine (T) replaced a cytosine (C) and at the levels of 385 and 386 nucleotides, where two cytosine (CC) substituted a guanine and thymine (G T) respectively (Figure 1).

The analysis of genetic diversity based on partial mitochondrial DNA sequencing represented the percent of diversion and identity between the new Egyptian isolate and nineteen selected sequences *E.granulosus* G6 circulating globally and retrieved from GenBank displayed in Table 1, it revealed that our isolate showed typical identity (99.5%) with JN637176 Sudan, HM853659 Iran, AF386533 France and AJ237637 Poland with 0.5% diversion while the percentage of identity reached its lowest degree 96.4% with HQ423292 Canada.

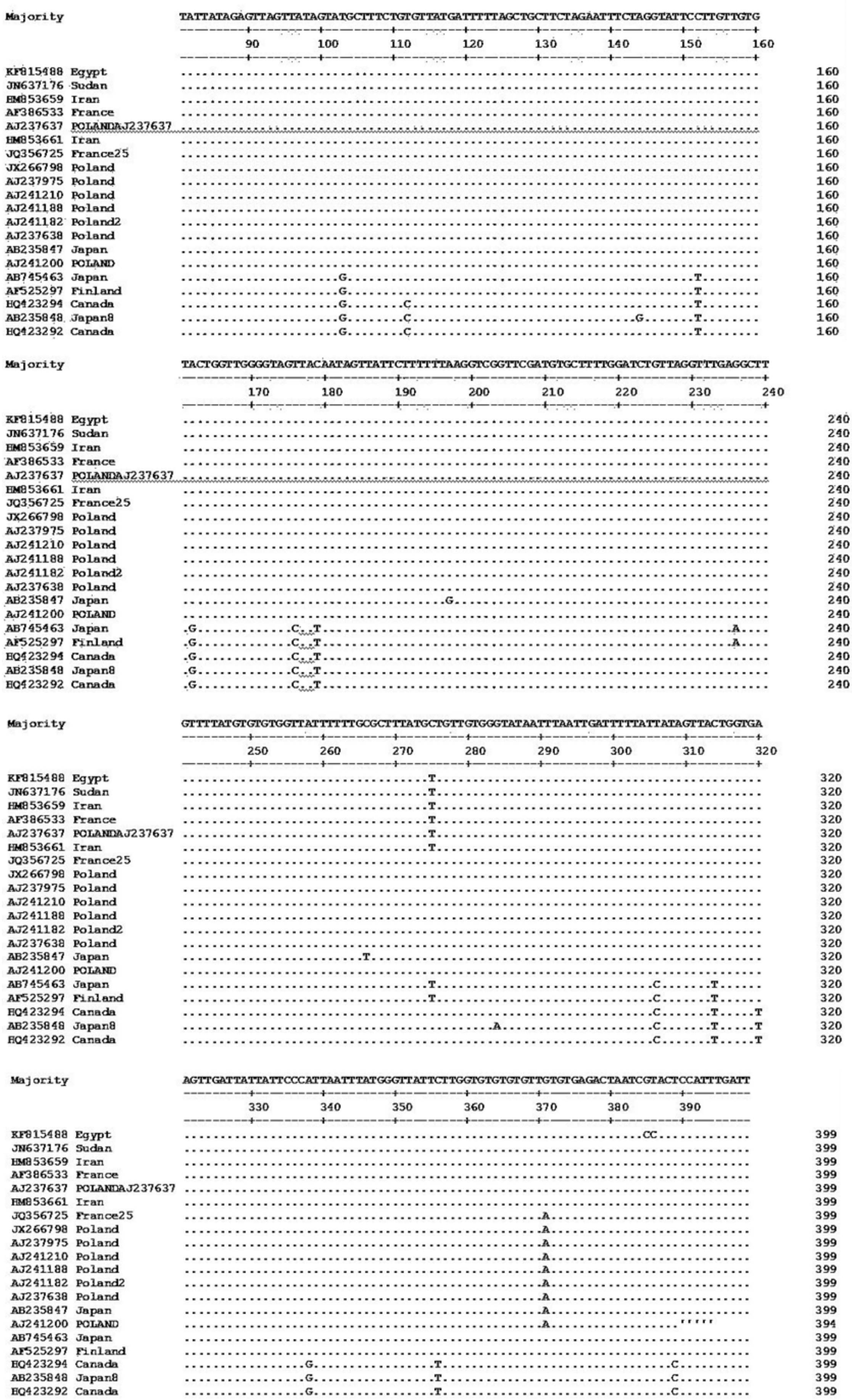


**Figure 1.** Nucleotide sequence alignment of NADH dehydrogenase 1 gene. Reference sequences for the NADH dehydrogenase 1 gene for the genotype G6 variant are shown with a random selection of isolate sequences beneath showing identity with the camel strain G6 genotype.Three nucleotides mutation in KF815488 Egypt at positions 275,385 and 386

**Table 1. The percent of diversion and identity between the new isolate sample from Egypt and nineteen selected sequences circulating globally from GenBank**

| | Percent Identity | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 27 | 18 | 19 | 20 | | |
| **1** | | 99.5 | 99.5 | 99.5 | 99.5 | 99.2 | 99.0 | 99.0 | 99.0 | 99.0 | 99.0 | 99.0 | 99.0 | 98.5 | 97.7 | 97.2 | 97.2 | 96.2 | 95.7 | 95.5 | **1** | KF825488 |
| **2** | 0.5 | | 100.0 | 100.0 | 100.0 | 99.7 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **2** | JN637176 |
| **3** | 0.5 | 0.0 | | 100.0 | 100.0 | 99.7 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **3** | HM853659 |
| **4** | 0.5 | 0.0 | 0.0 | | 100.0 | 99.7 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **4** | AF386533 |
| **5** | 0.5 | 0.0 | 0.0 | 0.0 | | 99.7 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **5** | AJ237637 |
| **6** | 0.8 | 0.3 | 0.3 | 0.3 | 0.3 | | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 98.7 | 98.0 | 97.5 | 97.5 | 96.5 | 96.0 | 95.7 | **6** | HM853661 |
| **7** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **7** | JO356723 |
| **8** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **8** | JX266798 |
| **9** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **9** | AJ237975 |
| **10** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **10** | AJ241210 |
| **11** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **11** | AJ241188 |
| **12** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **12** | AJ241182 |
| **13** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 99.5 | 98.7 | 97.2 | 97.2 | 96.7 | 96.2 | 96.0 | **13** | AJ237638 |
| **14** | 1.5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.3 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | | 98.2 | 96.7 | 96.7 | 96.2 | 95.7 | 95.5 | **14** | AB235847 |
| **15** | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | | 95.9 | 95.9 | 95.4 | 94.9 | 94.7 | **15** | AJ241200 |
| **16** | 2.8 | 2.3 | 2.3 | 2.3 | 2.3 | 2.6 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 3.4 | 2.9 | | 100.0 | 98.0 | 97.5 | 97.2 | **16** | AB745463 |
| **17** | 2.8 | 2.3 | 2.3 | 2.3 | 2.3 | 2.6 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 3.4 | 3.4 | 0.0 | | 98.0 | 97.5 | 97.2 | **17** | AF525297 |
| **18** | 3.9 | 3.4 | 3.4 | 3.4 | 3.4 | 3.6 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.9 | 3.4 | 2.0 | 2.0 | | 99.5 | 99.2 | **18** | HQ423294 |
| **19** | 4.4 | 3.9 | 3.9 | 3.9 | 3.9 | 4.2 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 4.4 | 4.0 | 2.6 | 2.6 | 0.5 | | 98.7 | **19** | AB235848 |
| **20** | 4.4 | 3.9 | 3.9 | 3.9 | 3.9 | 4.2 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 4.4 | 4.0 | 2.6 | 2.6 | 0.5 | 1.0 | | **20** | AQ423292 |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **27** | **18** | **19** | **20** | | |

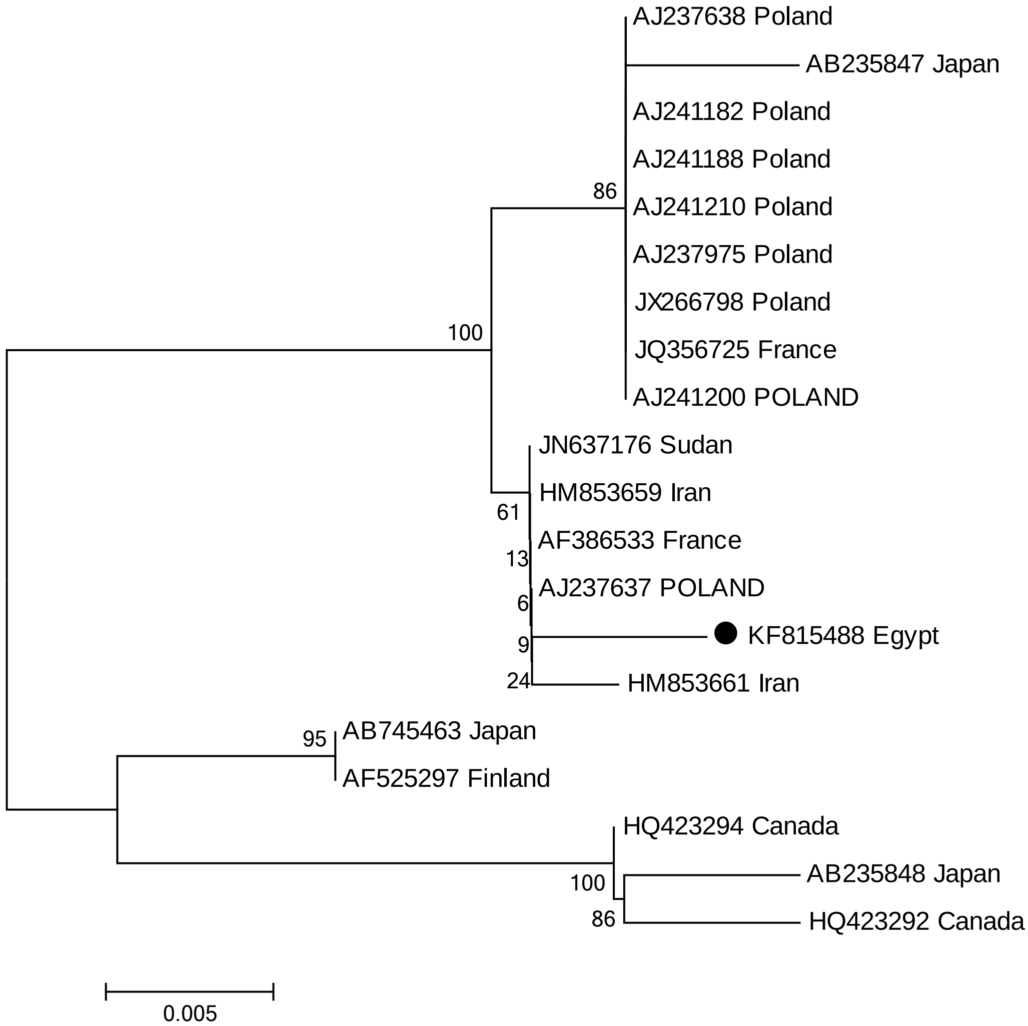


**Figure 2.** Phylogenetic tree sequences of Echinococcus granulosus (EG) from Egyptian camel and human and their relationship with reference sequences of other genotype G6 strain retrieved from GenBank. The tree analysis was obtained from partial sequence (276bp) from mitochondrial NADH dehydrogenase 1 gene. All isolates cluster with sequences belonging to the camel G6 genotype (Accession No. KF815488). A sequence aligned by Clustal W method and the tree was built by using MEGA5 software. Genetic distance is indicated below the tree

Phylogenetic analysis showed a robust tree clustering all isolates with sequences belonging to the camel genotype (G6) variant with strong bootstrap values at relevant nodes. Phylogenetic tree shows the evolutionary relationship of the sequences in which the length of the horizontal line was proportional to the estimated genetic distance between the sequences. Such tree indicated that the evolutionary distance between groups is very short (Figure 2).

Protein sequence analysis indicated the presence of two mutations at the position of 92, where an Alanine (A) is changed to a Valine (V) and at the position of 129, where a Valine ( V) replaced by Proline (P) (Figure 3).

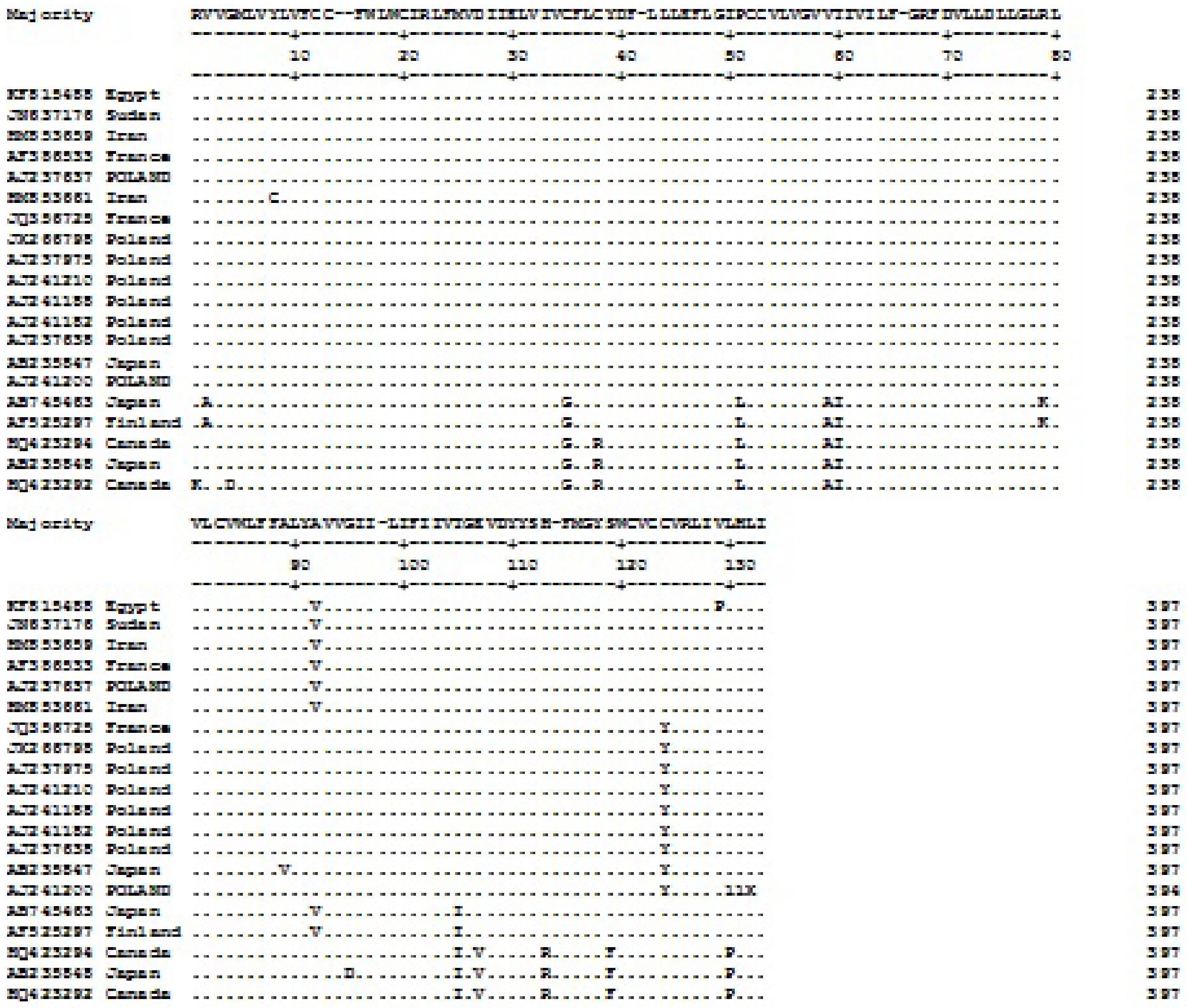

**Figure 3.** Protein sequence alignment of *E. granulosus* obtained from partial sequencing of NADH dehydrogenase 1 gene sequences aligned by MEGA5 with known strains sequences G6 in GenBank. Tow mutations in amino acids of KF815488 Egypt at positions 92 and 129

## 4. Discussion

Unilocular hydatidosis is a zoonotic parasitic disease representing a major public health problem in many countries around the world, including Egypt. Close relationships between dogs and humans appear to correlate with the high prevalence of the disease in endemic areas [18] and camel is an influential reservoir of the disease.

We selected the universal primers based on the highly conserved NADH dehydrogenase 1 gene [25] and our data indicated that the purified and partially sequenced PCR products generated 399 bp of NADH dehydrogenase 1 gene. The sequences were aligned by cluster grouping where the clusters aligned the most similar sequences firstly then progressively more distant groups of sequences until the global alignment was obtained. The NCBI-BLAST search found that our isolates are (100%) homologues to the genotype *E. canadensis* (G6) and its accession number is KF 815488.

Analogous to our findings, recent studies indicated that the camel strain was reported to be the most predominant (100%) among camels in Cairo, Egypt [22] and many African countries, such as Libya, Tunisia, Algeria, Sudan, and Mauritania (Table 2). In contrary, it has fewer existence in different countries worldwide (Table 3). *E. canadensis* G6 also infects sheep, goat, and cattle (Table 2 and Table 3). Taking together, these features provide strong evidence that camels play an important role for the maintenance of the *Echinococcus* life cycle in livestock intermediate hosts in Africa and Asia. On the contrary, camels appear to be suitable hosts for *E. granulosus* G1 infections (in Tunisia and Pakistan) and for G1, G3, G6, and G7 (in Iran) (Table 2 and Table 3).

**Table 2. Molecular epidemiology of cystic echinococcosis of animals reported in different African countries (2000 onwards)**

| Location | Origin | No. isolates | Gene markers | Genotype frequency (%) | Reference |
|---|---|---|---|---|---|
| Egypt | Camels | 20 | nad1 | G6 (100%) | The present study |
| | Camels | 47 | 12S rRNA | G6 (100%) | [22] |
| | Pigs | 6 | 12S rRNA | G6 (100%) | [22] |
| Libya | Camels | 83 | cox1 and nad1 | G6 (100%) | [27] |
| Algeria | Camels | 6 | bg 1/3, cox1 and nad1 | G6 (100%) | [27] |
| | Camels | 10 | *cox1*, *nad1*, act2 and hbx2 | G6 (66.6%); G1 (16.7%); G2 (16.7%) | [28] |
| Tunisia | Camels | 3 | cox1 | G6 (100%) | [29] |
| | Camels | 13 | cox1 | G1 (100%) | [30] |
| Sudan | Camels | 35 | 12S rRNA, *cox1* and *nad1* | G6-G7 (100%) | [31] |
| | Camels | 61 | nad1 | G6 (100%) | [23] |
| | Camels | 207 | 12S rRNA, cox1 and nad1 | G6-G7 (100%) | [1] |
| | Camels | 30 | cox1 and nad1 | G6 (100%) | [2] |
| | Sheep | 3 | 12S rRNA, *cox1* and *nad1* | G6-G7 (100%) | [31] |
| | Sheep | 111 | 12S rRNA, *cox1* and *nad1* | G6-G7 (100%) | [1] |
| | Sheep | 28 | cox1 and nad1 | G6 (100%) | [2] |
| | Goats | 65 | 12S rRNA, *cox1* and *nad1* | G6-G7 (100%) | [1] |
| | Cattle | 8 | 12S rRNA, *cox1* and *nad1* | G6-G7 (75.0%); G5 (25.0%) | [31] |
| | Cattle | 107 | 12S rRNA, *cox1* and *nad1* | G6-G7 (99.1%); G5 (0.9%) | [1] |
| | Cattle | 62 | cox1 and nad1 | G6 (100%) | [2] |
| Mauritania | Camels | 3 | bg 1/3, *cox1* and *nad1* | G6 (100%) | [32] |
| | Camels | 1 | 12S rRNA | G6-G7 (100%) | [33] |
| | Cattle | 20 | bg 1/3, *cox1* and *nad1* | G6 (100%) | [32] |
| | Camels | 17 | cox1, nad1, act2 and hbx2 | G6 (100%) | [33] |
| Kenya | Sheep | 69 | nad1 | G6 (1.4%) | [34] |
| | Goat | 15 | nad1 | G6 (26.7%) | [34] |
| | Pigs | 4 | 12S rRNA, *cox1* and *nad1* | G1 (50.0%); G6–G7 (25.0%); G5 (25.0%) | [31] |

Act2: nuclear actin 2; bg 1/3: Echinococcus genus-specific genomic DNA; *cox1*: mitochondrial cytochrome c oxidase subunit 1; *hbx2*: nuclear homeobox 2; ITS1: ribosomal internal transcribed spacer 1; *nad1*: mitochondrial NADH dehidrogenase subunit 1; 12S rRNA: mitochondrial 12S small subunit ribosomal RNA.

**Table 3. Molecular epidemiology of cystic echinococcosis of animals reported in different countries (2000 onwards).**

| Country | Origin | No. isolates | Gene markers | Genotype frequency (%) | Reference |
|---|---|---|---|---|---|
| **Asia** | | | | | |
| Iran | Camels | 32 | ITS1 DNA | G1 (25.0%); G6 (75.0%) | [35] |
| | Camels | 2 | ITS1 DNA | Likely G6–G7 (100%) | [36] |
| | Camels | 19 | *cox1 and nad1* | G1–G3 (68.4%); G6–G10 (31.6%) | [37] |
| | Camels | 18 | ITS1 DNA | G1 (66.7%); G6 (33.3%); | [37] |
| | Camels | 26 | *cox1*, *nad1*, ITS1 DNA | G1 (34.6%); G6 (65.4%) | [38] |
| | cattle | 14 | *cox1*, *nad1*, ITS1 DNA | G1 (64.3%); G6 (35.7%) | [38] |
| | Camels | 9 | *cox1* and *nad1* | G1 (44.4%); G3 (22.2%); G7 (33.3%) | [39] |
| | camels | 43 | *cox1*, *nad1*, *atp6* and 12S rRNA | G1 (88.4%); G6 (11.6%) | [40]) |
| | Camels | 19 | *cox1* and *nad1* | G1 (26.3%); G3 (42.1%); G6 (31.6%) | [41] |
| Pakistan | Camels | 5 | *cox1* | G1 (100%) | [42] |
| **America** | | | | | |
| Mexico | Pigs | 7 | *cox1*, ITS1 DNA | G6–G7 (100%) | [43] |
| Argentina | goats | 3 | *cox1*, *mdh* | G6 (100%) | [44] |
| **Europe** | | | | | |
| Lithuania | Cattle | 1 | *cox1* | G6–G7 (100%) | [45] |
| | Pigs | 7 | *cox1* | G6–G7 (100%) | [46] |

*Atp6*: mitochondrial ATP synthase subunit 6; *cox1:* mitochondrial cytochrome c oxidase subunit 1; ITS1: ribosomal internal transcribed spacer 1; *mdh:* cytosolic malate dehydrogenase; *nad1*: mitochondrial NADH dehidrogenase subunit 1; 12S rRNA: mitochondrial 12S small subunit ribosomal RNA.

Molecular epidemiological data in African pigs are recently only available from Egypt and Kenya. In Egypt, all swine isolates were identified as *E. canadensis* G6) [22], whereas Kenyan pigs were demonstrated to be predominantly infected with *E. granulosus s.s.* (genotype frequency: 50%), *E. canadensis* G6–G7, and *E. ortleppi* being responsible for 25% of the total infections each [31].

The exclusive finding of the G6 variant in all camel and human isolates in Qalyubia Governorate, Egypt indicates the presence of a predominant transmission cycle in which the camel strain exist. Our findings confirms a previous study done using RAPD-PCR for characterization of human and animal hydatid cysts, it has been shown that human and camel isolates were the most related pair and camels are important hosts for the transmission of human hydatidosis (Azab *et al.* 2004) [47]. Similarly, performing the cycle sequencing and nucleotide sequence analysis identified the G6 genotype in 30 (96.8%) out of 31 human isolates in Cairo, Egypt) [22].

Although the camel strain G6 is traditionally considered as less infective to humans [48,49] recent molecular findings [29], [23] and [22] as well as ours suggest that the prevalence of infection of this genotype may be higher than previously thought. Among the ten genotypes of *E. granulosus* (EG) recognized worldwide, only 5 strains were known to infect humans including G1, G2, G5, G6, and G7 strains (Table 4). The most frequent strain associated with human CE appears to be the sheep strain (G1) and the highest rates of infection are recorded in communities involved in extensive sheep farming [50].

**Table 4. Molecular epidemiology of cystic echinococcosis of humans in different countries (2000 onwards)**

| Country | No. isolates | Gene markers | Genotype frequency (%) | Reference |
|---|---|---|---|---|
| Egypt | 5 | *nad1* | G6 (100%) | The present study |
| | 31 | *nad1* | G1 (3.2%); G6 (96.8%) | [22] |
| Tunisia | 11 | *cox1* | G1 (100) | [29] |
| Sudan | 3 | *nad1* | G6 (100%) | [23] |
| Kenya | 59 | *cox1, nad1* | G1 (83%); G6 (17%) | [49] |
| South Africa | 32 | *nad1*, 125 rRNA | G1-G3 (81%); G6/G7 (16%); G5 (3%). | [51] |
| Iran | 31 | *cox1, nad1,* ITS1 DNA | G1 (80.6%); G6 (19.4%) | [39] |
| | 4 | ITS1 DNA | G1-G3 (100%) | [52] |
| Poland | 30 | *nad1* | G7 (100%) | [53] |
| Peru | 20 | *cox1* | G1 (95%); G6 (5%) | [48] |

*cox1:* mitochondrial cytochrome c oxidase subunit 1; ITS1: ribosomal internal transcribed spacer 1; *nad1:* mitochondrial NADH dehydrogenase subunit 1; 12S rRNA: mitochondrial 12S small subunit ribosomal RNA.

Sequencing of our samples revealed mutations in three nucleotides generating a change at the level of 275 nucleotide, where a T replaced a C Similar mutation had been recorded for strains isolated from Sudan, Iran, France, and Poland (Figure 1). In addition, our isolate revealed two other mutations at the levels of 385 and 386 nucleotides, where CC substituted GT. These mutations did not express in the previously mentioned international isolates. In contrary to our finding, the solely recorded Egyptian G6 strain isolated by [22] pointed out to the presence of a substitution of one nucleotide at the site number 207, in which a C is substituted by a T, after examining another mitochondrial gene, 12S rRNA.

Our isolate showed 99.5% identity with similar isolates from Sudan, Iran, France, and Poland. On the other hand, our isolate expressed 96.6% identity with that of the Canadian isolate (Table 1). On comparing the obtained nucleotide sequences (of mitochondrial 12S rRNA gene) of the only isolated Egyptian strain by [22] (Genbank ID: GQ476732–GQ476735) with that of the Argentinean G6 reference strain (GenBank accession no. AB208063), 100% identity was found.

Phylogenetic analysis showed that our isolates clustered with *E. canadensis* (G6) and revealed that KF815488 Egypt put in the same category with JN637176 Sudan, HM853659 Iran, AF386533 France and AJ237637 Poland. Phylogenetic tree indicated that the evolutionary distance between groups is very short, suggesting that the genetic divergence is recent.

Nucleotide mutations are translated to mutations in the protein sequence as our data refer to the presence of a V instead of an A, at the level of 92, and a P instead of a V, at the position 129. Similar V replacement at the site of number 92 had been recorded in Sudan, France, Poland, and Iran (Figure 3).

Our findings of nucleotide and protein mutations explain the higher human infectivity (100%) of G6 as all collected hydatid cysts of camels and humans were fertile. This is of great epidemiological importance as the fertile hydatid cysts are responsible for progression of the life cycle and acting as a reservoir for human [54] The occurrence of mutations explains why the camel strain (G6 genotype) appears to affect humans in certain geographical areas but not others. Similar finding had been recorded [55]

The extensive intraspecific variation in *E. granulosus* is associated with change in the life cycle pattern, host specificity, geographical distribution, transmission dynamics, infectivity to human, antigenicity, and sensitivity to chemotherapy [21,56].

## 5. Conclusion

For the first time in Qalyubia, Egypt, we successfully investigated the molecular characterization of *Echinococcus* genotype and highlighted the polymorphism of nucleotide and protein mutations of *E. canadensis* (G6) in camels and human patients which could explain the increased infectivity to humans. Our record of a single genotype, G6, suggests that similar mechanisms are responsible for its persistence in the endemic areas. Such epidemiological data could guide the application of efficient control strategies of CE in Egypt.

# 6. Further Studies

Our study may provide a foundation for future epidemiological studies on the transmission dynamics of the parasite as well as studying the function of malformed proteins and their efficacy on the infectivity of CE in different intermediate hosts as well as their effect on the sensitivity to chemotherapeutic agents.

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