

# Molecular characterization of Hydatid Cyst from Egyptian one humped Camels (*Camelus dromedaries*)

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## Abstract

Hydatidosis or cystic echinococcosis (CE) is caused by *Echinococcus granulosus* (EG) complex, which infects humans especially children and several species of wild and domestic animals including camels, cattle, equines, sheep and goats. Infection come from the consumption of *Echinococcus granulosus* eggs with contaminated food, and therefore the syndrome is of public health significance. There are several genotypes nearly ten from genotype 1 to genotype 10, which are found all over the world based on genetic diversity. Several studies are conducted on the genetic diversity of hydatid cyst on Egyptian camels (*Camelus dromedaries*) at Qalyubia Governorate, Egypt, and this study is the continuation of previous works. In this study a total of 55 hydatid cysts samples were collected from the lungs of camels admitted to abattoir of Qalyubia Governorate, Egypt. Brood capsules, free solitary scolices, and sediment of hydatid fluid, were the source of DNA, which was extracted by the commercial kit. For polymerase chain reaction (PCR) amplification, two gene primers, the cytochrome C oxidase subunit 1 (cox1 gene), and the mitochondrial NADH dehydrogenase subunit 1 (NADH1 gene) were used and the partial sequences obtained were analyzed by gene sequencing and then phylogenetic analysis was carried out. Results of this study showed that 90.5% (n = 49/55) of the samples were identified as *Echinococcus canadensis* genotype 6 (G6), and 9.5 % (n=6/55) were identified as *Echinococcus ortleppi* (G5) which newly imported from Sudan for slaughtering. The presence of *Echinococcus ortleppi* of the cattle isolate (G5) in the Egyptian camels was concluded for the first time.

**Keywords:** *Echinococcus ortleppi*, *Camelus dromedaries*, Gene sequencing, Qalyubia Governorate.

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## INTRODUCTION

Livestock comprises of cattle, buffaloes, sheep, goats, camels, horses, asses and mules. There are a large number of parasites which infect the domesticated animals and are responsible for production loss (Iqbal *et al.*, 2013; Iqbal *et al.*, 2014; Muhammad *et al.*, 2015; Omar *et al.*, 2013). Several studies on the parasitic infections in domestic animals including sheep, camels, cattle, equines were done all over the world, and also was reported from one humped camels, especially in Egypt (Amer *et al.*, 2015; Iqbal *et al.*, 2014; Khalifa *et al.*, 2014; Muhammad *et al.*, 2015). Hydatid cyst infection involve the vital organs in the body of animals and human, so it is considered public health problem causing economic loss due to the condemnation of these organs at abattoirs. It is distributed worldwide especially the Sub-Saharan Africa (Wahlers *et al.*, 2012). A number of studies were carried out depending on the mitochondrial DNA examination showed that *Echinococcus granulosus* complex having a high genetic

diversity and variability and at least ten known strains (G1-G10) were identified as follows; G1 and G2 of them belonging to the ovine strain, G3 and G5 for bovine strains, G4 for the equine strain, G6 for camel strain, both G7 and G9 were for pig and the last two G8 and G10 strains for cervid strains (Cardona and Carmena, 2013).

The genetic studies of *E. granulosus* population has remarkable significance for epidemiological and control studies (Thompson *et al.*, 2002). One previous study has explained the population structure of *E. granulosus* from Cairo, Egypt (Abdel-Aaty *et al.*, 2012), molecular characterization of *E. granulosus* isolates was done from camels and humans by sequence and phylogenetic analyses in Qalyubia Governorate, Egypt (Omar *et al.*, 2013).

The aim of this study was the investigation of the strains of *E. granulosus* present in camels in slaughter houses in Egypt using primers for mitochondrial and cytochrome oxidase gene in Qalyubia Governorate. It was

considered the second attempt to identify the circulating genetic strains from one humped camels in this governorate.

## MATERIALS AND METHODS

### Samples collection, and preparation

Fifty five hydatid cyst samples were taken only from the lung over a period of nearly one month from one humped camels from the Toukh's abattoir (Northern to Cairo), Qalyubia Governorate, Egypt. Cysts were collected directly after evisceration of the slaughtered animals into flasks (thermo flasks) and transferred to the Molecular Biology unit, Assiut University for processing and molecular intervention. Hydatid fluids were collected into several test tubes after opening the cyst wall and then identified, centrifuged and supernatant excluded. The sediment (hydatid sand) which contains free scolices, brood capsules

was collected into a clean sterile bottle having 70% ethyl alcohol for preservation and stored at -20°C (Osman *et al.*, 2009).

### DNA Extraction

Hydatid sand was washed by nucleic acid free water for removing ethyl alcohol preservative. DNA was extracted using the QIAamp tissue kit (QIAGEN, Germany) according to instructions on the kit. DNA was obtained by spinning in centrifuge at 12,000 RPM for 60 sec. at 37°C. 10 µl of the suspended DNA was used in the amplification reaction (McManus and Thompson, 2003).

### Primers

The primers for mitochondrial NADH dehydrogenase subunit 1 and cytochrome C oxidase subunit 1 gene for EG isolates (Bowles and McManus, 1993; Ibrahim *et al.*, 2011) were used (Table 1).

**Table 1. The primers *Echinococcus granulosus* isolates.**

Gene	Primer Type	Primer Sequence	Primer Size
(NADH 1) gene	Outer primers JBI1	5' AGATTCGTAAGGGGCCTAATA 3'	530 bp
	Outer primers JB12	5' ACCACTAACTAATTCACCTTC 3'	
	Internal primers JB11.5	5' TTATGGTAGATATTATAG 3'	
	Internal primers JB12.5	5' CACACACATAAAACAAGC 3'	
(cox1) gene.	Forward primer CO1	5' GAG GTT TTT TAT TTT GGG CAT CCT 3'	415 bp
	Reverse primer CO1	5' TAA AGA ATA AAG ATG AAA ATG 3'	

### PCR, Gene Sequence analysis and phylogeny

Polymerase chain reaction was done on the extracted DNA according to previous study (Nakao *et al.*, 2007). The PCR product was purified by QIAquick PCR purification kit (QIAGEN) and then sent to Assiut university of Egypt the molecular biology unit, for the purpose of sequencing and then the sequence alignment was obtained by Local Alignment Search Tool (BLAS). It was compared to strains of known genotype from other countries of the world through NADH1 and cox1 subunit of genes. By using MEGA software version 5.0 (Tamura *et al.*, 2011), the phylogenetic tree were then constructed as out groups (Ahmed *et al.*, 2013).

## RESULTS AND DISCUSSION

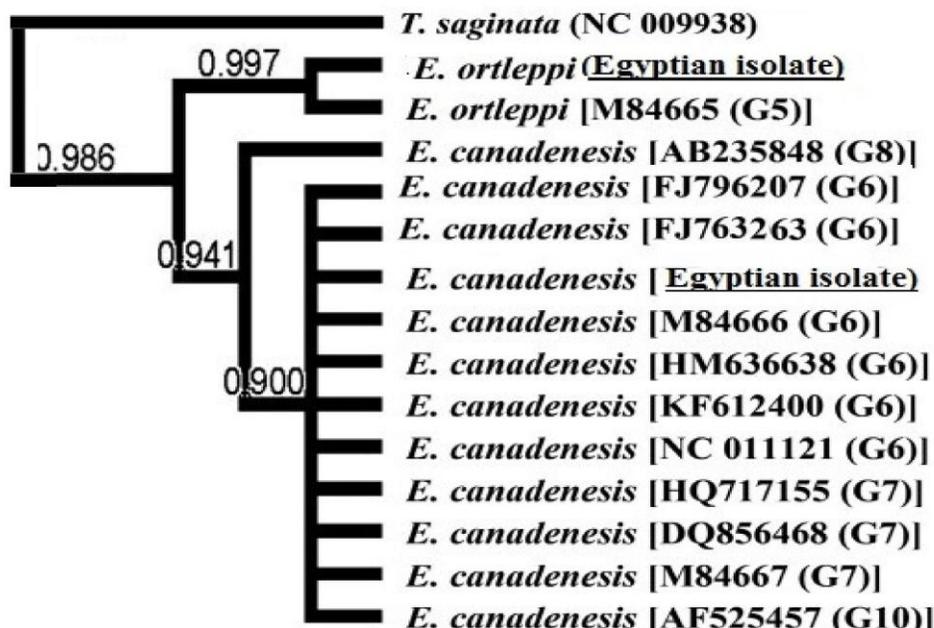
Results of the present study confirmed that the isolates under this study belong to the EG complex. It was found that 90.5% (49 out of 55 EG isolates) of samples had 100% homology with *E. canadensis* genotype 6 (G6) found mostly in camels genotype. However, only six EG isolates (9.5%) belonged to *E. ortleppi* which is the cattle genotype (G5) (Figure 1).

In Egypt, the camel genotype (G6) is the most common genotype of EG and the one humped camels

(*Camelus dromedaries*) play a crucial role in the epidemiology (Omar *et al.*, 2013). But from our point of view, this assumption needs more and more investigation. Some studies stated that this genotype is considered of low public health significance (Santivaney *et al.*, 2013; Casulli *et al.*, 2010). Recent works illustrated that the incidence of this genotype is higher than described in the previous studies (M'Rad *et al.*, 2005). Several studies were organized on the biology and molecular characterization of *Echinococcus granulosus* strains found in different parts of Egypt to identify the circumstances around the epidemiology of the hydatid infection in one humped camels. The first step in prevention and clearance of the infections from specific area is to identify the strain(s) incriminated in the dissemination of infection and determine its life cycle. In a previous study on hydatid cyst of lung from Qalyubia governorate, Egypt, different genotypes including G6 were identified by gene sequencing of the NADH 1 genes (Khalifa *et al.*, 2014). However, no studies were done for determining the phylogeny of EG5 in Egypt. The circulation of major variant (G6) in Egyptian camels suggests that specific mechanisms are responsible for its persistence in this area. This is probably due to close relationships between dogs and camels in the study area (Dinkel *et al.*, 2004).

The previous theory about the relation between dogs and abattoirs was confirmed. In poor communities as in this province, people perform the slaughtering process in the open space, so by this condition dogs can be easily fed on

slaughtered animals viscera that could carry the hydatid cyst completing the life cycle to become adult inside intestine of dogs. This practice can easily maintain the camel strain (G6) inside the study area.



**Figure 1:** Phylogeny tree of EC-complex strains from one humped camels from Egypt and other globally. Based on the NADH dehydrogenase subunit 1 gene and Partial Cox 1 gene sequences generated from EG isolates that used in this study.

Further studies and EG strain genotyping and phylogeny can help for identifying the epidemiology of this isolate in Egypt. Availability of more data can help the public health authorities for management and prevention protocol for the infection. Our results coincided with the previous studies which were carried out for molecular identification of animal and human hydatid cysts. It showed that camel and human strains are the most similar pairs so camels are important hosts for the dissemination of human hydatidosis (Khalifa *et al.*, 2014; Azab *et al.*, 2004).

## CONCLUSION

In conclusion, this study explored the presence of *E. orteppi* the cattle genotype (G5) in the newly imported one humped camel from Sudan in Egypt for the first time at Qalyubia governorate, Egypt. It was the second study that revealed *Echinococcus* genotype of *E. canadensis* (G6) in camels. Therefore *E. orteppi* strain must be considered during future studies especially the imported camels from Sudan that may provoke it to become endemic in Egypt.

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## AUTHORS' CONTRIBUTIONS

Rasha A. El Meghanawy collected samples, extracted the DNA and optimized PCR assay; analyzed the sequences; Amer R. Abdel Aziz designed the experiment and prepared the final manuscript. All authors read and approved the final version of the manuscript.

## CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

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