

Occurrence and phylogenetic description of cystic echinococcosis isolate from Egyptian camel (*Camelus dromedarius*)

I. S. ELSHAHAWY^{1,*}, M. A. EL-SEIFY², Z. K. AHAMED³, M. M. FAWAZ¹

¹Department of Parasitology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, *E-mail: dr.ismail_para@yahoo.com, ismail-saad@vet.svu.edu.eg; ²Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt; ³Department of Parasitology, Animal Health Research Institute (Aswan Branch), Aswan, Egypt

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Summary

Cystic echinococcosis is one of the most significant cyclo-zoonotic diseases of major economic and public health significance worldwide. The current study was carried out to determine the epidemiological profile of cystic echinococcosis as well as to investigate its molecular and phylogenetic status from one-humped camel (*Camelus dromedarius*) in the southern region of Egypt. In the present work, 110 camels freshly slaughtered at Daraw abattoirs, Aswan governorate were inspected for the presence of Hydatid cysts (HCs) visually and manually by palpation and incision, over a period of one year (June, 2018 – May, 2019). Furthermore, fourteen fertile hydatid cyst samples were collected from lungs of slaughtered camels. DNA extraction from two fertile samples was successfully achieved followed by phylogenetic analysis on two mitochondrial genes (*cox1* and *nad1*). Out of 110 camels slaughtered 11 (10 %) were found harboring hydatid cysts. The infection was found to prevail throughout the year, with the highest peak encountered in winter (45.5 %). The lungs were the most frequently infected organs (72.7 %) with liver cysts occurring at a significantly lower rate (27.3 %). The mean value of total protein, glucose, urea, cholesterol, magnesium, potassium, copper and creatinine was higher in cystic fluid from camels as compared to cattle. Blast and phylogenetic analysis on sequenced genes showed the presence of *Echinococcus intermedius*, originally the pig genotype (G7) in camels for the first time in Egypt. To the best of our knowledge, the current research provides a description of the current epidemiological and molecular situation of camel hydatidosis in the southern region of Egypt. Furthermore, the current results may have significant implications for hydatid disease control in the studied region.

Keywords: Cystic echinococcosis; *Camelus dromedarius*; Egypt; Epidemiological status; Molecular characterization

Introduction

In Egypt, camels (*Camelus dromedarius*) play an important role in the national economy and are considered as the best useful addition to the food supply chain in terms of milk, meat and other products. Parasitism is a unique problem and considered as a ma-

ajor obstacle in the health and product performance of livestock. Among those, cystic echinococcosis is a conceivably common (Getachew *et al.*, 2012).

Cystic echinococcosis (CE) or hydatidosis is one of the most dangerous zoonotic diseases, it can be fatal if untreated. It occurs due to infection with the larval stage of the common dwarf dog tape-

* – corresponding author

worm, *Echinococcus granulosus* (Elmahdi *et al.*, 2004). Furthermore, it causes great economic loss due to increased mortality, forced slaughter, condemnation of infected offal, reduced breeding value, reduced quality and yield of meat, milk or wool and high costs of hygienic procedures (Bessonov, 2007).

Echinococcus granulosus sensu lato (s.l.) generally implies a typical taeniid cestode life cycle involving two mammalian hosts. Carnivorous animals such as dogs and wild canids are the definitive hosts in which sexual reproduction occurs, while different herbivore species act as intermediate hosts for the larval stage of the parasite. Humans represent incidental dead-end hosts becoming infected with food or water contaminated with feces of domestic dogs containing eggs of the parasite or through direct contact with dogs (Craig *et al.*, 2007).

Conventionally, different isolates of *E. granulosus sensu lato* (s.l.) were categorized on the basis of differences in morphological features, biochemical composition and intermediate host specificity (Jenkins *et al.*, 2005). In recent years, various molecular tools as PCR-RFLP and mitochondrial DNA sequencing are broadly used. These techniques were proved to be very useful in understanding of the population genetics, epidemiology and taxonomy of the parasite (Thompson, 2008).

Detailed evidence about the diversity of *E. granulosus* (s.l.) is accessible worldwide. Ten genotypes of *E. granulosus* have been described using mitochondrial DNA sequences, as confirmed by previous studies (Thompson & McManus, 2002; Lavikainen *et al.*, 2003). on the basis of the phenotypes and molecular data, *E. granulosus* (s.l.) was divided into five species: *E. granulosus* (sensu stricto) (sheep strain, genotypes G1-G3); *Echinococcus equinus* (horse strain, genotype G4); *Echinococcus ortleppi* (cattle strain, genotype G5); *Echinococcus canadensis* (camel strain, genotype G6; pig strain, genotype G7; cervid strains, genotypes G8 and G10); and *Echinococcus felidis* (lion strain, no genotype assigned) (Nakao *et al.*, 2013a, 2013b; Alvarez Rojas *et al.*, 2014; Romig *et al.*, 2015), and the proposed species *E. intermedius* (G6, G7) are the most recent taxonomic revisions in the genus *Echinococcus* (Lymbery, 2017; Ali *et al.*, 2020).

Until now, however, few studies have reported the presence of genotypes of the *E. granulosus* complex (G6, the most predominant isolate, G1 and G5) in Egyptian camels based on data using the *cox1* gene sequences (Amer *et al.*, 2015; Abdel-Aziz & El-Meghanawy, 2016; Mousa *et al.*, 2020), and data on their distribution patterns and molecular characterization in the southern region of Egypt are scarce (Abdelbaset *et al.* 2021). Besides camels, G4 was also found in Egyptian donkeys among the genotypes of *E. granulosus* s.l. (Aboelhadid *et al.*, 2013). Therefore, the current study primarily conducted to achieve the frequency of infection, localization, fertility/sterility rates of hydatid cysts and the viability of their protoscoleces and also to determine the biochemical profile of hydatid cyst fluid (HCF). Molecular and phylogenetic strain typing of *E. granulosus* in camels in studied area was the second aim.

Materials and Methods

Study area and design

A cross sectional study was conducted in Aswan city, which is located at a latitude of 24° 5' 20.18" N and a longitude of 32° 53' 59.39" E. Aswan is famously for its beautiful Nile Valley scenery, important archaeological sites and peaceful atmosphere. The climate is warm throughout the year, making it an ideal winter destination. Furthermore, agriculture is the main source of employment opportunities in the province, with approximately 29 % of the province's population working in agriculture.

Study animals

The sample size was calculated according to the method previously described by Charan (2013). The parameters of the formula ($n = Z^2 \times P(1-p) / d^2$) were as follows: Z = 1s standard normal variate at 5 % type 1 error (P < 0.05), it is 1.96, P = the expected prevalence of HC based on previous studies was 9.7 % (Zienab 2021), and d = is ±5 % desired level of precision. The formula was $n = (1.96)^2 \times 0.097 \times (1 - 0.097) / 0.05$. The required sample size n was determined to be 110 camels. To determine the prevalence of hydatid cyst infection in the camels of the southern region of Egypt, the visceral organs (liver, lungs, kidneys, and spleen) of 110 slaughtered camels were inspected over the course of one year from June, 2018 to May, 2019 in Daraw Abattoir, Aswan, Egypt. During the sampling gender, age, and the seasonal dynamics were recorded.

Sampling and parasitological assessment

All organs or tissues containing HCs were collected and all HCs found were carefully removed and separately collected, kept in sterile saline solution, 70 % ethanol and transported within 2 hours to the Parasitology laboratory, Faculty of Veterinary Medicine, South Valley University in ice box for further cyst characterization to assess their status and for genetic strain typing of *E. granulosus sensu lato* (s.l.). Data related to the cyst size, distribution, seasonal dynamics, viability and fertility was recorded. A total of 35 HCs were randomly selected from lungs and livers and was measured and categorized as small (< 4 cm in diameter), medium (4 – 8 cm in diameter) and large (>8 cm) (Oostburg *et al.* 2000). The cyst fertility was assessed based on the presence or absence of brood capsules containing protoscolices in hydatid fluid examined microscopically (Kebede *et al.*, 2009). Additionally, fertile cysts were subjected to viability test. A drop of sediment containing the *protoscoleces* was placed on the microscopic glass slide and a drop of 0.1 % eosin solution was added and covered with cover slip and then examined under a high power microscopy (40x) after 5 min. with the principle that viable *protoscoleces* should completely or partially exclude the dye, while the dead ones take it up (Daryani *et al.*, 2007). During these investigations, the biosecurity rules were strictly observed and all examined biological material and the remains of hydatid cysts were incinerated.

Biochemical analyses of the cyst fluids

Five samples of hydatid fluids were collected from the lung cysts of the screened hosts (camels and cattle), approximately 25ml of the collected fluids (HCF) was centrifuged at 15000 rpm at 4 °C for 30 min and the supernatant was analysed for various biochemical parameters. The flame photometry method was utilized to measure the amount of Sodium and Potassium, copper and magnesium by the spectrophotometry method. Creatinine, Calcium and Protein were analyzed by the colorimetric method. The measurements of Glucose, cholesterol, triglycerides, and urea was made by enzymatic methods (SYNCHRON CX 7 PRO, USA 2009). These parameters were estimated by a commercially available diagnostic Kits from Sigma, Germany. Each electrolyte and biochemical profile was prepared and quantified according to the manufacturer's instructions.

Data analysis

Statistical significance differences were assessed with a Chi-square using a statistical package program (Sigma Plot version 11.0). Statistical significance was considered with P values <0.05.

Molecular analysis

DNA was successfully extracted from two fertile HCs out of fourteen cysts using QIAamp DNA Mini Kit (Qiagen, Germany, Catalogue no.51304,) according to the manufacturer's recommendation. DNA was stored at -20 °C until being used for DNA amplification. For all DNA extracts, PCR were conducted on the gene encoding *cytochrome c oxidase subunit 1 (cox1)* and for the gene

encoding *NADH dehydrogenase subunit 1 (nad1)*. Fragments of mitochondrial genes amplified with specific primers that previously described as JB3 primer (5'-TTTTTTGGGCATCCTGAGGT-TTAT-3') and JB4.5 (5'-TAAAGAAAGAACATAATG AAAATG-3') for *cox1* and JB11 (5'-AGATTCGTAAGGGGCCTAATA-3') and JB12 (5'-ACCACTAACTAATTCACCTTC-3') for *nad1*, as forward and reverse primers respectively (Aboelhadid *et al.*, 2013). PCR products were visualized using electrophoresis with 1.5 % Agarose gel in TAE buffer and stained with 0.5 µg/ml ethidium bromide (cat. no. SM0243). A 100-bp molecular ladder was used as DNA size marker in each gel for estimating the size of the bands. Gels were observed and photographed using a gel documentation system and the data was analyzed through computer software. The obtained DNA sequences were subsequently subjected to BLASTn (www.ncbi.nlm.nih.gov/BLAST/) analysis to check for similarity of the sequence against the GenBank database and compared with verified sequences of *E. granulosus* strains available in GenBank. Sequences with higher identity were downloaded from GenBank for phylogenesis and *Taenia saginata* (GenBank accession number MK644934) was used as an outgroup species (Table 1). Sequences were aligned using the Muscle alignment tool in MEGA X software (<https://www.megasoftware.net/>) as per Kumar *et al.* (2018). A phylogenetic tree was constructed using the maximum likelihood method and the Tamura Nei model (Tamura & Nei, 1993). The sequences analyzed in the present study were finally deposited in GenBank. Furthermore, reference sequences were compiled from previous studies (Table 1), with *Taenia saginata* as an outgroup.

Table 1. GenBank accession numbers of *cox1* and *nad1* of *Echinococcus* genotypes used in phylogenetic analysis in the current study

Accession No. for NAD1	Locality	Host origin	Genotype	Accession No. for Cox1	Locality	Host origin	Genotype
MW183240	Egypt	Camel	G7*	MW173485	Egypt	Camel	G7*
MW183239	Egypt	Camel	G7*	MW173484	Egypt	Camel	G7*
MH301013	France	Pig	G7	MH301013	France	Pig	G7
KX010897	Hungary	Pig	G6/7	MH301019	Italy	Pig	G7
KX010890	France	Pig	G6/7	MH301010	France	Pig	G7
KX010889	Namibia	Oryx	G6/7	MH301009	France	Pig	G7
KX010886	Sudan	Camel	G6/7	MH301008	France	Pig	G7
MH301016	France	Pig	G7	MH300939	Sudan	Camel	G6
KX510135	Serbia	Pig	G6/7	KX010833	Kenya	Camel	G6/7
KX231668	Armenia	Pig	G6/7	MT166286	Nigeria	Camel	G6
KX010880	Ethiopia	Cattle	G6/7	MT166284	Nigeria	Cattle	G6
MT525964	Kenya	Cattle	G6/7	MH301021	Ukraine	Pig	G6
MT166290	Nigeria	Camel	G6	MH301006	Poland	Pig	G7
MT166289	Nigeria	Cattle	G6	MH301005	Poland	Homosapiens	G7
JN191326	Egypt	Donkey	G4	JN191319	Egypt	Donkey	G4
MK644934	South Korea	Human	<i>Taenia saginata</i>	MK644934	South Korea	Human	<i>Taenia saginata</i>

*Sequences generated in the current study

Ethical Approval and/or Informed Consent

All applicable institutional, national and international guidelines for the care and use of animals were followed regarding the National Research Ethics Committee of the South Valley University and Veterinary authorities in South Valley University, Egypt.

Results

Survey finding

Out of 110 dromedaries examined, only 11 were found to be infected with one or more cysts giving an overall prevalence of 10 %. In respect to cyst distribution, the current finding declared that lungs were the most commonly infected organs. The current survey revealed that infection varied according to the camel's age, with the highest prevalence of infection in older animals of age 3 years and

over (12.3 %, 8/65), while animals with mostly 1 – 2 years have less infection rate (6.7 %, 3/45). The statistical analysis, however, confirmed that these differences were not statistically significant ($P = 0.332$). Similarly, gender-wise prevalence indicated a non-significantly ($P = 0.915$) higher infection rate in adult male (10.1 %, 10/99) in comparison with female animals (9.1 %, 1/11). Overall, 72.7 % of infected camels harboured cysts in the lungs, while only 27.3 % had cysts in the liver. Lung infections were significantly more common than those in the liver ($\chi^2 = 4.545$, $P = 0.03$). The range in the number of cysts was 1 – 7 in infected animals. The fertility rate of 35 examined hydatid cysts selected from slaughtered camels was found to be 54.3 %, while 45.7 % of the cysts were sterile. Further microscopic observations indicated that, 54.3 % (19/35) cysts were found to be fertile in the lung that was characterized by the presence of protoscolices (hydatid sand) in the vesicular fluid, microscopically, whereas 31.4 % (11/35) were sterile cysts.

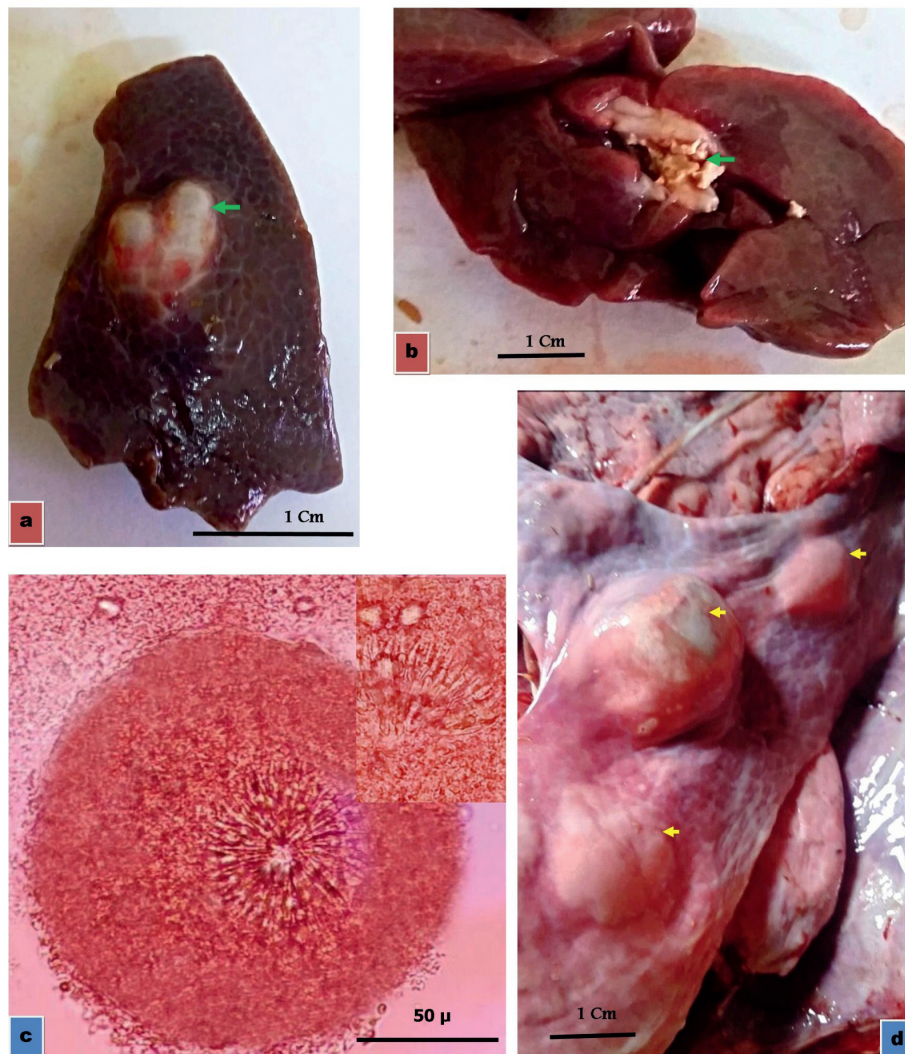


Fig. 1. a,b) Calcified cyst; c) Protoscolices; d) Multiple fertile cysts in the lung (yellow arrow head).

Table 2. Comparative biochemical analysis of hydatid fluids collected from dromedary camels and cattle infected with cystic echinococcosis (mean \pm SE, n = 5).

Biochemical profiles	Host	
	Camel	Cattle
Total protein (g/l)	3.76 \pm 0.73	2.42 \pm 0.01
Glucose (mmol/L)	3.36 \pm 0.16	2.58 \pm 0.47
Urea (mmol/L)	5.21 \pm 0.17	4.36 \pm 0.5
Cholesterol (mmol/L)	2.94 \pm 0.61	2.19 \pm 0.31
Creatinine (μ mol/L)	101.22 \pm 3.09	80.67 \pm 0.66
Calcium (mmol/L)	2.937 \pm 0.03	3.224 \pm 0.02
Triglycerides (mmol/L)	0.039 \pm 0.01	0.186 \pm 0.003
Sodium (mmol/L)	111.35 \pm 5.45	118.09 \pm 0.54
Potassium (mmol/L)	13.92 \pm 7.41	10.05 \pm 0.05
Magnesium (mmol/L)	1.113 \pm 0.33	1.019 \pm 0.02
Copper (ppm)	0.42 \pm 0.11	0.338 \pm 0.016

Additionally, the fully calcified cysts were observed in the liver with estimated fertility percentage of 8.6 % (3/35) and the rest cysts was found sterile (5.7 %, 2/35) as depicted in Fig. 1. Concerning the cyst viability, the current study displayed that the viability rate of protoscoleces was 60.7 % from fertile lung cysts.

Additionally, the current finding showed that the size of hydatid cysts collected from lungs and liver ranged from 1 – 9 and 1 – 2 cm in diameter, respectively. Furthermore, the average weight of the recovered cysts from lungs and livers were 70.9 g (7 – 135 g) and 25g (15 – 35g), respectively.

Seasonal dynamics of hydatid cysts was also taken under con-

sideration. Data analysis revealed that camels had a distinct pattern of hydatidosis in different seasons: winter (45.5 %) > summer (27.3 %) > autumn (18.2 %) > spring (9.1 %) signifying the infection throughout year. The statistical analysis, however, confirmed that these differences were not statistically significant ($\chi^2=4.2424$, $P=0.236$).

Biochemical results

The comparison between the biochemical profile of HCF isolated from intermediate hosts including camel and cattle was depicted in Table 2. From this table, it was observed that, the mean value of

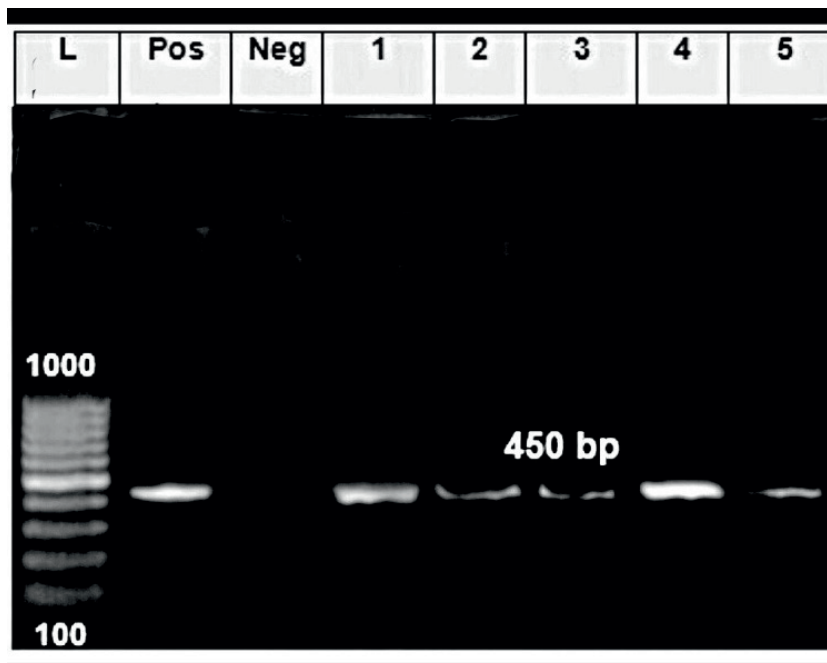


Fig. 2. Agarose (1.5%) gel electrophoresis of PCR-products of *cox1* gene (450bp) of *E. granulosus* isolates in camel samples. L: ladder, Pos.: positive control, Neg.: negative control, 1, 2, 3, 4 and 5 samples (all +ve).

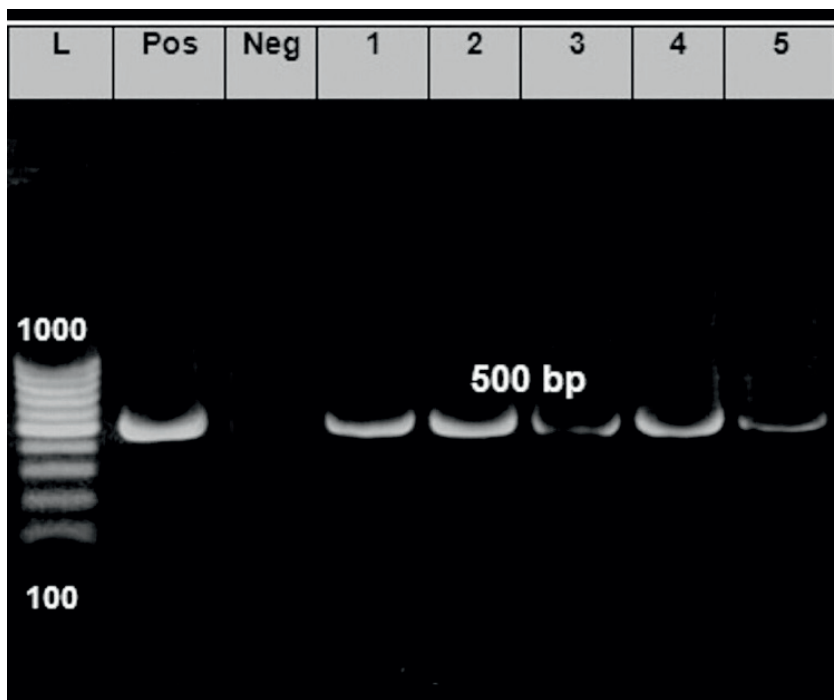


Fig. 3. Agarose (1.5%) gel electrophoresis of PCR-products of *Nad1* gene (500bp) of *E. granulosus* isolates in camel samples. L: ladder, Pos.: positive control, Neg.: negative control, 1, 2, 3,4and 5 samples (all +ve).

total protein, glucose, urea, cholesterol, magnesium, potassium, copper and creatinine was higher in HCF collected from camels as compared to the cystic fluid collected from cattle, while the mean value of calcium, triglyceride, sodium was found lower in the camel HCF as compared to cattle HCF.

Molecular and Phylogenetic analysis

As far as our knowledge, the present study genetically showed the presence of *E. granulosus* (G7, pig strain) in camel for the first time in Egypt.

PCR amplification with specific primers generated two different bands of 450-bp and 500-bp in regard to PCR amplification of *cox1* and *nad1* genes, respectively (Figs. 2 & 3).

The Blast analysis results of the sequenced data indicated the existence of one isolate belonging to G7 genotype (pig strain, *E. granulosus*) from the examined fertile cysts using GenBank database. It should be considered that the detected G7 genotype, using

cox1 gene, subsequently, was also confirmed by amplification and sequencing of *nad1* gene. Sequences relevant to *cox1* and *nad1* genes were deposited in the GenBank with accession numbers (MW173484, MW173485) and (MW183239, MW 183240), respectively. In two G7 samples detected by *cox1* sequence in the current study, 100 % homology was observed with reference sequences used in the phylogenetic trees from pigs and different countries (Italy and France). Moreover, the current G7 genotype isolate generated a sequence identical to G7 sequence MH301013 from pig cysts in France, while showed a 99.18 % identity to sequences MH301016 and KX510135 from pig cysts in France and Serbia based on *nad1* analysis.

Sequencing of our samples revealed mutations in three nucleotides generating a change at the level of 180, 381 and 387 nucleotide, where a G replaced by A for G7 isolate from France. In addition, our isolate revealed three other mutations at the levels of 180, 381 and 387 nucleotides, where G substituted A and further

Table 3. Difference in bases at the *nad1* locus of *E. granulosus* s.l.

Accession No.	Genotype	Nucleotide Substitution No.	Substitution/ Position	Substitution/ Position	Substitution/ Position
(MH301013)	G7 (Pig, France)	Three	G to A 180/381/387	-	-
(KX010880)	G6/7 (Cattle, Ethiopia)	Four	G to A 180 / 381	C to T 336	A to G 432
(MT166290)	G6 (Camel, Nigeria)	Five	G to A 180 /381/387	C to T 336	A to G 432

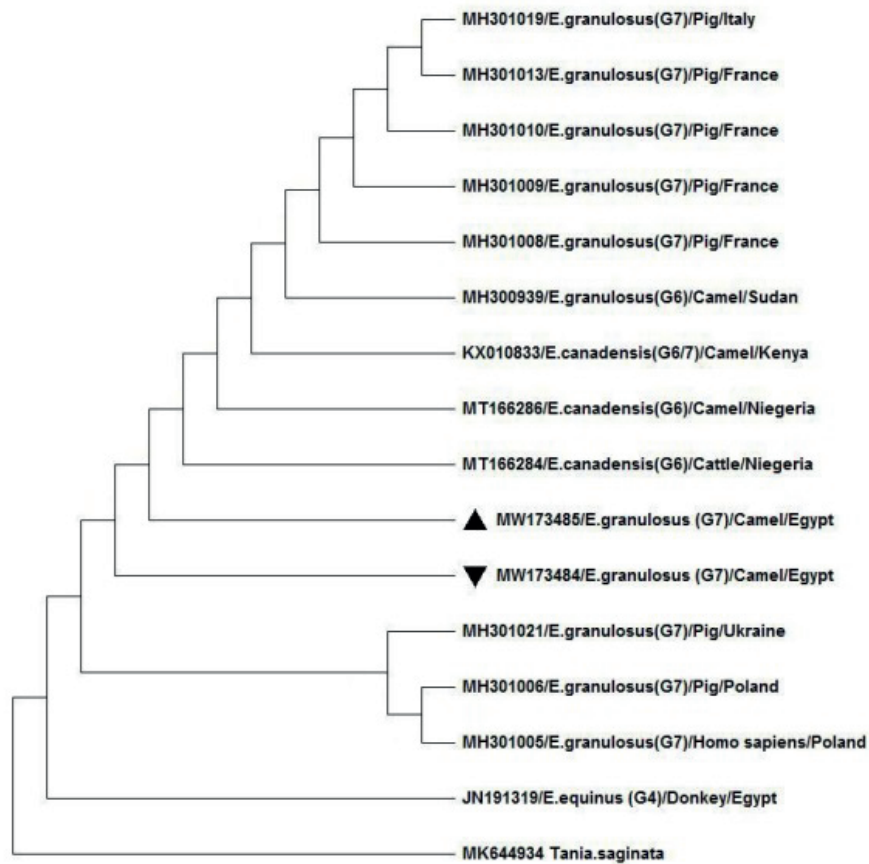


Fig. 4. Genetic relationships of the obtained genotypes from camel in the present study and reference sequences related genotypes of *E. granulosus* as well as *Taenia saginata* as the outgroup. The relationships were inferred based on phylogenetic tree (*cox1* gene). The phylogenetic tree was constructed using Maximum Likelihood Tree implemented in MEGA software version 6.

one mutation was occurred at level 336, where C replaced by T. while, the fifth mutation at position 432, where A substituted with G for G6 isolate from Nigeria as shown in Table 3.

Phylogenetic analysis revealed a robust tree associating our isolate of G7 genotype with a variety of G7 genotype (common pig strain) sequences from different geographical regions of the world, although it was more genetically related to the France isolate.

Discussion

Echinococcus granulosus (s.l.), one of the smallest cestodes, displays a global public health and veterinary interest because its metacystode causes the life threatening cyclo-zoonotic disease (Eckert & Deplazes, 2004).

The current survey declared that the overall prevalence of HC was 10 % among slaughtered camels. The current findings were nearly close to the previous reports carried out in Assiut, Egypt (9 %) (Khalifa *et al.*, 2014). Other reports in several Egyptian governorates reported relatively higher prevalence rates among slaughtered camels e.g. 16.25 % in Qalyubia (Mahmoud, 2012)

and 39.5 % Beni-Suef (El-Dakhly *et al.*, 2019). However, the infection rates of 2.5 %, 7.7 %, 5.6 % and 5 % and 8.32 % has been reported for camel hydatidosis from Mansura city (Haridy *et al.*, 2006), Assiut and Aswan (Dyab *et al.*, 2005), Giza (El-Dakhly *et al.*, 2019) and Aswan (Omar *et al.*, 2013 & Dyab *et al.*, 2018), respectively. The infection rate of 59 %, 29.7 %, 32.8 %, 30.1 % and 61.4 % has been reported from Sudan (Omer *et al.*, 2010; Ibrahim *et al.*, 2011), Saudi Arabia (Ibrahim, 2010), Mauritania, and Kenya (Njoroge *et al.*, 2002), respectively. Additionally the present finding was also greater than worldwide reports done in Libya (3.6 %) and Tunisia (6.5 %), respectively (Kassem & Gdoura, 2006; Lahmar *et al.*, 2004). This variation in prevalence could be due to several factors including differences in environmental conditions that are conducive for the perpetuation of the parasite, social activities and culture, approach towards dogs and difference in husbandry and hygienic system. Other reasons like the personal behavior of workers about the elimination of infected offal in abattoirs and the nature of the pasture may contribute to this variation (Salih *et al.*, 2011; Abdelbaset *et al.*, 2021).

The present study revealed that the prevalence of HCs varies

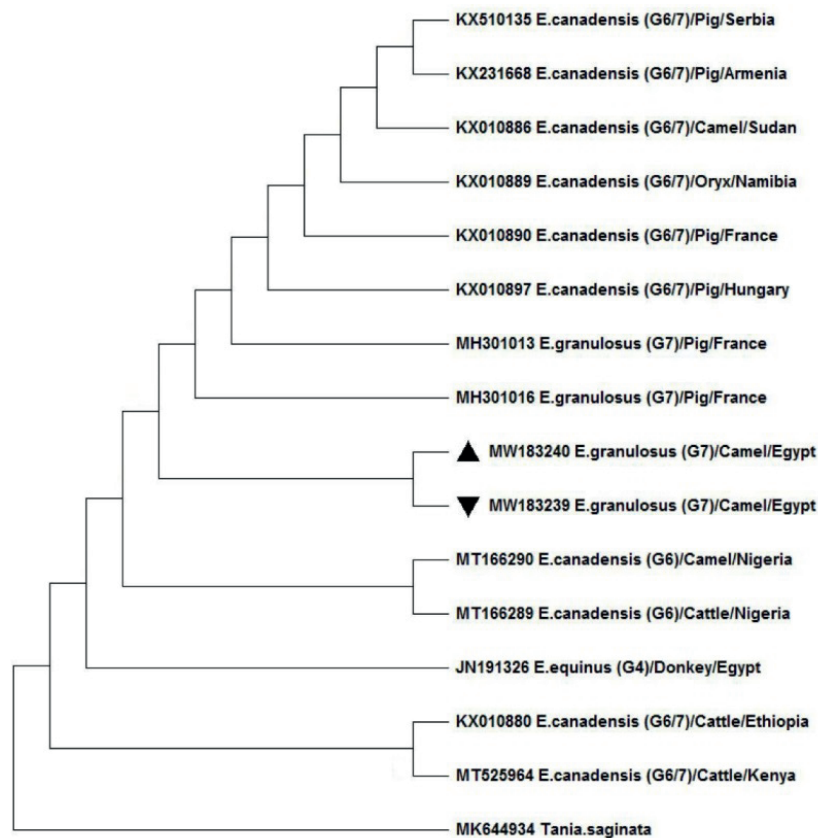


Fig. 5. Genetic relationships of the obtained genotypes from camel in the present study and reference sequences related genotypes of *E. granulosus* as well as *Taenia saginata* as the out-group. The relationships were inferred based on phylogenetic tree (*nad1* gene). The phylogenetic tree was constructed using Maximum Likelihood Tree implemented in MEGA software version 6.

greatly among screened age groups; those with age of 3 years and over (>3) were highly infected (12.3 %) with cysts as compared to the age group of 1 – 2 years old (6.7 %). These findings were in line with the previous studies (Azlaf & Dakkak, 2006; Regassa *et al.*, 2010). This could be mainly due to the fact that aged animals have longer exposure time to eggs of *E. granulosus* in addition to weaker immunity to combat against the infection (Regassa *et al.*, 2014). Furthermore, the prevalence of hydatid cysts was recorded slightly higher in male animals (10.1 %) as compared to females (9.1 %), in contrast to the results observed by previous reports in Zambia and eastern Ethiopia (Banda *et al.*, 2013; Lemma *et al.*, 2014). This might be due to few number of tested female. Differences in livestock management for males and females in Egyptian community could be another issue.

The current figures indicated that the rate of infection in lungs (72.7 %) was higher than that of the liver (27.3 %). Our observation was in accordance with those noticed in camels in several African countries, such as Mauritania (Bardonnet *et al.*, 2003), Sudan (Ibrahim *et al.*, 2011), and Ethiopia (Salih *et al.*, 2011). This might be due to the fact that camels are slaughtered at an older age, during which period the liver capillaries are dilated and most oncospheres pass directly to the lungs; additionally, it is possible

for the *Echinococcus* oncosphere to enter the lymphatic circulation and be carried via the thoracic duct to the heart and lungs in such a way that the lung may be infected before or instead of the liver (Arene, 1985).

The fertility of cysts is an important factor affecting the spread of *E. granulosus*. The current finding showed that the fertility rate among the screened organs was found higher in lungs (54.3 %) compared to liver which was Zero. It has been stated that the fairly softer consistency of lung tissue allows the easier development of the cyst (Himonas *et al.*, 1987). Our result was also in consistent with earlier research which demonstrated that the fertility of the cyst from lungs was 69.7 % as compared to liver in slaughtered camel in five different abattoirs from Iran (Ahmadi, 2005). The peak prevalence and high fertility rate of lung cyst over hepatic one of camel specify the importance of viscera as a potential source of infection for dogs. Additionally, the higher yield of calcified cysts in the liver could be attributed to the relatively higher reticuloendothelial cells and abundant connective tissue reaction of the organ. Similarly, the high percentage of small cysts may be due to the immunological response of the host which might prevent their development (Lahmar *et al.*, 1999). In the present study, viable protoscoleces in lung cysts were higher than the previous report in

Saudi Arabia (Mohamed, 2010). Moreover, previous report on the viability of cysts, recovered from the camel lungs, have shown a higher viability in comparison with the results of the present study (Lahmar *et al.*, 2013). The difference in viability of protoscolecemes could be due to the numerous factors, such as temperature and humidity, and genetic variations of *E. granulosus* (Diker *et al.*, 2007).

The current data revealed a vital seasonal fluctuations for hydatid cyst in camel among the various seasons. The peak infection rate was observed in winter and summer seasons. Likewise, several previous reports found a seasonal variation in infection prevalence, with the highest trend of occurrence in winter (Daryani *et al.*, 2007; Almalki *et al.*, 2017). The seasonal variation in infection prevalence maybe related to the differences in the environmental conditions that are responsible for the dissemination of the parasite, the availability of infected final hosts, and the nature of the pasture among seasons (Ernest *et al.*, 2009).

In this study, the existence of *E. granulosus* can be explained by different hypotheses. One possibility is that some uncontrollable slaughtering (i.e. done outside the slaughterhouse without veterinary supervision), without removing all the infected organs. Second, infected dogs can contaminate pastures. Finally, the dogs that die near the lakes, rivers or on roads and their bodies were not collected and processed properly were considered as a main source of infection. Research on camel-dog cycle will assess the persistence of *E. granulosus* in Egypt.

The chemical composition of hydatid cyst fluid (HCF) plays a significant role in the immunology, metabolism, physiology and existence of the cyst (Muhsin *et al.*, 2015). Mover, the biochemical analysis of HCF collected from different intermediate hosts can provide evidence as an additional diagnostic tool to distinguish different circulating isolates in the area, and can help to identify common isolates that circulate in different hosts (Muhsin *et al.*, 2015). The current study declared a quantitative variation in the biochemical profiles of hydatid fluids of camels from cattle, proposing the existence of camels strains of *E. granulosus* in the study area. Similar trend was reported in Iran, that found the higher values of magnesium, total protein, glucose, copper and creatinine in the camel hydatid cyst fluids as compared to cattle (Radfar & Iranyar, 2004). The level of urea was higher in the HCF collected from camels as compared with cattle. In contrast, a study in Iran has shown the same value of urea in HCF of both animals (Radfar & Iranyar, 2004). The values of calcium, triglyceride and sodium in camel HCF was lower in comparison with cattle HCF. On the contrary, compared to Iranian camels, these values were found to be higher for cattle cysts (Radfar & Iranyar, 2004). This variability could be attributed to the differences in metabolic activities, cyst status (degree of growth, localization/ fertility) and isolates of *E. granulosus sensu lato* prevailing.

The current sequence analysis of two camel hydatid cysts revealed the presence of one genotype of *E. granulosus*, pig strain (G7 genotype) in camels for the first time in Egypt. This genotype

has been previously described in European beaver, pig, wild boar, cattle, goats and sheep worldwide, notably in Poland, Slovakia, Ukraine, Spain and Greece (Kedra *et al.*, 1999, Kedra *et al.*, 2000; Turcekova *et al.*, 2003; Mwambete *et al.*, 2004; Roiniotti *et al.*, 2016).

Globally, several studies have been conducted on camel isolates from different countries, mainly in Africa. The previous report carried out in Mauritania found that G6 genotype in all twenty camel isolates (Bardonnet *et al.*, 2002). In another DNA sequence-based revision in North Africa, all camel isolates belonged to G6 genotype (Bart *et al.*, 2004). Earlier investigation, revealed that all camel isolates belonged to G1 and G6 genotypes in Libya and Algeria, respectively (Tashani *et al.*, 2002; Bardonnet *et al.*, 2003). Furthermore, *E. granulosus* s.s. (G1- G3 complex) is also the principal genotype in humans, cattle, sheep, camel and goats in various European and Latin American countries (Beato *et al.*, 2010; Piccoli *et al.*, 2013).

In Egypt, previous studies upon nuclear (Khalifa *et al.*, 2014) and mitochondrial genes (Abdel-Aaty *et al.*, 2012) indicated the presence of G6 genotype as the only predominant genotype in slaughtered camels in Qalyubia and Sohag Governorate, respectively. Another investigation demonstrated the presence of *E. canadensis* (G6) in 26 out of 28 Egyptian camel HCs (Amer *et al.*, 2015), while the 2 remaining cysts belonged to *E. granulosus* s. s. (G1) and *E. ortleppi* (G5). Furthermore, the sequencing analysis of 40 Egyptian camels samples revealed the predominance of G6 genotype (camel strain) in camel isolates (Alam-Eldin *et al.*, 2015) and more recent study revealed that G6 genotype was the predominant strain in Egyptian camels (Mousa *et al.*, 2020).

The association with distinct host species, largely separate geographical distribution and limited rate of cross-fertilization are the main factors that have limited the gene flow between genotypic groups. G6 is commonly involved in a cycle between goats/camels–dogs and G7 mainly pigs–dogs, these two also share some overlap in their life cycles as both can infect the same intermediate hosts. Furthermore, G6/G7 share the same final host (dog) cross-fertilization has apparently been frequent enough to guarantee that G6/G7 have not diverged (Laurimäe *et al.*, 2018).

In conclusion, the current findings report for the first time the dominance of *E. granulosus* (G7, pig strain) in Egyptian camels. The existence of this strain and especially the fact that the zoonotic concern, makes *E. granulosus* a major public health disquiet in Egypt. Additionally, the present study indicates that camel can be considered a suitable new host for the G7 genotype. For this reason, the role of camels in maintaining the transmission cycle of different *E. granulosus* genotypes warrants more investigations, with further epidemiological studies on human CE and the strains of the parasite infecting humans.

Conflict of Interest

Authors state no conflict of interest.

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