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Prevalence of Hydatid Cysts in Slaughtered Animals from Elbehera Governorate, Egypt, with a Focus on Histopathology and Molecular Characterization of Camel Cysts

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Abstract

Cystic echinococcosis is a worldwide zoonotic infection that triggers significant economic losses in animals. The study's goal was to reveal the infection with hydatid cysts in animals slaughtered in Elbehera Governorate, Egypt, as well as perform histopathology and molecular characterization of camel cysts using the *cox-1* and *G1Y162* genes. Elbehera governorate had a total prevalence of 0.35%. Kom-Hamada abattoir had the highest prevalence of 1.7%. Camels had the highest prevalence of 2.17% among the examined animals. Summer had the highest prevalence, at 0.55%. Females had a higher prevalence than males, except for camels. The older animals were infected at a higher rate than the younger ones, except for the buffalo. Location and season had a significant impact on the prevalence, while sex only impacted the prevalence in cattle and buffaloes. The lung had a higher infection than the liver. The *cox-1* and *G1Y162* genes PCR reactions provided specific DNA bands, and the sequences were for *Echinococcus canadensis*. The phylogenetic tree of *cox-1* indicated that the Egyptian sequence of Elbehera governorate belongs to genotype 6 (G6) of the *Echinococcus* complex. The sequence shared an identity percentage of up to 99% with previous Egyptian sequences and other *E. canadensis* in camels from Iran, Mauritania, Algeria, and Sudan. The phylogenetic tree of the *G1Y162* protein sequences confirmed that the Egyptian sequence is *E. canadensis*. Due to the presence of cysts of zoonotic *Echinococcus* species in slaughtered animals, stringent health regulations are required to prevent infection in animals and humans.

KEYWORDS
Hydatid cyst, *Echinococcus canadensis*, *Cox-1* and *G1Y162* PCR, Camel, Elbehera, Egypt

INTRODUCTION

Cystic echinococcosis is an international zoonotic infection in animals and human intermediate hosts. Infection with *Echinococcus granulosus* is disseminated around the world (Eckert and Deplazes, 2004). Dogs are the main definitive hosts (Tamarozzi *et al.*, 2020). The infection causes severe economic losses in intermediate animal hosts due to low production, organ condemnations in slaughterhouses, or deaths (Cardona and Carmena, 2013). In humans, the costs of surgical treatment of intact cysts that affect vital organs result in significant economic losses (Tamarozzi *et al.*, 2020).

The egg is mature, containing a single onchosphere. Infection occurs when eggs are consumed through contaminated food or water. The embryo of *E. granulosus* develops into one hydatid cyst which is a unilocular cyst filled with fluids. The size of the cyst increases with time and results in lesions characterized by space in different organs. The cyst causes pressure atrophy of the organs and may lead to death (Kniepeiss and Schemmer, 2023). Cyst occurs in several organs and tissues of the intermediate host, mainly including the liver and lungs, and other organs (Hajimohammadi *et al.*, 2022). The cyst was detected in several animal species, including ruminant animals (Hajimohammadi *et al.*, 2022). The final host is infected after consuming the hydatid cyst. Cysts were detected in slaughtered animals in several countries worldwide, such as Sudan (Ahmed *et al.*, 2013), Kenya (Mbaya *et al.*, 2014), Libya (El-Salem *et al.*, 2021), the Kingdom of Saudi Arabia (Ibrahim, 2010), the Sultanate of Oman (Al Kitani *et al.*, 2015), Iran (Hajimohammadi *et al.*, 2022), Brazil (de La Rue, 2008), and Australia (Wilson *et al.*, 2019).

There are four assigned species of *E. granulosus* with ten genotypes and the fifth species infects the lion with an unassigned genotype; all but genotype 4 are infective to humans (Thompson, 2020). Molecular characterization of *E. granulosus* cysts was

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achieved utilizing polymerase chain reaction (PCR) and sequencing of several genetic markers, such as NADH dehydrogenase subunit 1 (nad-1), cytochrome c oxidase subunit 1 (*cox-1*), and internal transcribed spacer 1 (ITS-1) (Varcasia *et al*., 2007; Alam-Eldin *et al*., 2015; Amer *et al*., 2015; Sharbatkhori *et al*., 2016; Hajimohammadi *et al*., 2022). The Eg*G1Y162* gene encodes a surface protein found in all stages of *E. granulosus* and it was evaluated as a vaccine candidate for echinococcosis (Zhang *et al*., 1998). In Egypt, surveys and characterization of the cysts of *E. granulosus* were conducted in some districts (Aaty *et al*., 2012; Alam-Eldin *et al*., 2015; Amer *et al*., 2015; Mahdy *et al*., 2014). There was no available literature on the genetic characterization of hydatid cysts in the Elbehera governorate. Therefore, the current study aimed to investigate the prevalence of the *E. granulosus* cyst from slaughtered animals in the abattoirs of Elbehera governorate, Egypt with special reference to pathology and molecular characterization of camel cysts.

MATERIALS AND METHODS

Animals and study area

Cysts were collected from various infected slaughtered animals in the abattoirs of the Egyptian governorate of Elbehera between October 2015 and September 2016. The total number inspected was 79972. The abattoirs investigated were Kom-Hamada, HoshEssa, El-Mahmoudia, AbouElmattamer, Damanhour, KafrEldawar, Rashid, Edko, Eldelingat, AbouHomous, Shibrakhit, Wadi Elnatroon, and Itaielbarowd. Camels, cattle, buffaloes, sheep, and goats were among the examined animals. The age range was > 2 years (6760) and < 2 years (100) in camels, > 3 years (773) and < 3 years (56748) in cattle, > 5 years (735), < 2 years (7555), and < 1 year (96) in buffaloes, > 2 years (5432) and < 2 years (480) in sheep, and > 2 years (1123) and < 2 years (170) in goats. The sex groups were camel males 6810 and females 50; cattle males 56824 and females 697; buffalo males 7651 and females 735; sheep males 591 and females 5321; and goat males 129 and females 1164.

Cyst sampling

Cysts were placed on ice until reaching the laboratory of parasitology. The cyst fluid was aspirated. Cysts were opened, and part of the germinal layer was kept in 70% ethanol for DNA extraction. The cyst's remaining portion was preserved in 10% neutral buffered formalin and transmitted to histopathology. Most of the cysts were calcified, and the fertile ones were processed.

Histopathological examination

Thirty cysts from the camel lung were subjected to histopathological examination. After 72 hours of fixation, the samples were dehydrated, coated in paraffin wax for subsequent staining, and sectioned (3µm). The tissues were stained with hematoxylin and eosin (HE) staining (Bancroft and Gamble, 2008) and modified Giemsa stain (Sigma-Aldrich). Histological photographs were taken utilizing a Leica ec3 camera.

DNA extraction

The cyst's inner layer was collected and macerated. The QIAamp® DNA mini kit was then used to extract the DNA (Qiagen, USA). DNA was quantified utilizing a spectrophotometer and kept at -20 °C.

PCR Assay

PCR for the *cox-1* and *G1Y162* genes was conducted on the GenAmp® PCR system 9700 (Applied Biosystems, USA). The reaction comprised 0.25 µL (1.25 units) of Taq DNA polymerase, 5 µL 10X buffer, 1µL of both primers, 2 µL of DNA, 5 µL dNTP, and up to 25 µL autoclaved double-distilled water. *Cox-1* PCR primers Forward F 5´-GTGTTGATTTTGCCTGGATT-3´ and reverse R 5´-AGCCACCACAAACCAAGTATC-3´ primers were used in the reaction. They were created using GenBank sequences, including EF367287, Ab271912, KF731903, KF731905, and KF731907. The eg*G1Y162* primers were used in the reaction, forward F 5´-CAGAGCTAATGGCAAAGTTG-3´ and reverse R 5´-GAGTAAGTAATAGGAGCCCAGCC-3´. The primers were prepared using the sequences of *E. granulosus* Eg*G1Y162* (AB458258, AB458259, and AB462014) and *E. multilocularis* EmY162 (AB303297). The PCR reaction started with 5 min of denaturation at 95 °C and was followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 55.9 °C for *cox-1* and 55 °C for *G1Y162*, and extension for 1 min at 72 °C. The final extension lasted 7 min at 72 °C. Five microliters of the PCR products were run on a 1% ethidium bromide-tainted agarose gel.

DNA Sequencing

A PCR purification kit was used to purify the PCR products from 100 µL reactions. The purified DNA was submitted for sequencing on an ABI Prism 3100 genetic analyzer from Applied Biosystems, the United States. The obtained sequences were searched on the BLAST (Basic Local Alignment Search Tool) website. The sequences of camel origin were *E. canadensis*. The sequences were submitted to get accession numbers from GenBank.

Phylogenetic Analysis

Using the neighbor-joining method, the phylogenetic trees were constructed by CLUSTALX 1.8 and N-J Plot. The *cox-1* gene sequence (LC160333) was used for phylogenetic analysis with other sequences in the GenBank. The sequences were *E. granulosus* (G1) (AJ508011, KF612390, Ab921094, HM563002, KU756225, KT074947, HM4563013, HM4563021, EF367287, KU006779, M84661, KM401619, and KF731903), *E. granulosus* (G2) (DQ341576, M84662, and LC068958), *E. granulosus* (G3) (M84663, KT074949, HM563017, KF731905, and KF731907), *E. equinus* G4 (M84664), *E. ortleppi* (G5) (M84665 and AB921055), *E. canadensis* (G6) (M84667, KM563019, KF349037, Ab271912, AB921086, AB921085, AB921084, AB921083, EU048813, LC160333), *E. canadensis* G8 (DQ144021), and *E. multilocularis* (KC582526 and KC133277). *T. solium* (AB524780) was used as an out-group. The *G1Y162* sequence (LC160334) was used for phylogenetic analysis with other sequences in the GenBank. *E. granulosus* Eg*G1Y162* (AB458258, AB458259, and AB462014) and *E. multilocularis* EmY162 (AB303298 and AB303297) sequences were used in the study. *T. solium* (AB524780) was used as an out-group. The amino acid sequence of the *G1Y162* protein from this study and those of genes used in the phylogenetic tree of *G1Y162* nucleotide sequences were used to generate the amino acid phylogenetic tree with the same parameters.

Statistical analysis

The Chi-square test was utilized to investigate the effect of locality, age, sex, and seasonal variations on the infection. A value

of P< 0.05 was considered significant. The test was achieved by GraphPad Prism 5 (GraphPad Software, San Diego, CA).

RESULTS

Prevalence

The prevalence of hydatid cysts was 0.35% (28/79972) in the Elbehera governorate, Egypt. In the abattoirs, the prevalence was 1.7% (196/11709) in Kom-Hamada, 0.17% (23/13414) in Damanhour, 0.1% (7/7030) in KafrEldawar, 0.13% (7/5602) in Rashid, 0.17% (4/2330) in Edko, 0.095% (9/9401) in AbouHomous, 0.062% (6/9586) in AbouElmattamer, 0.069% (5/7168) in HoshEssa, 0.16% (3/1889) in Eldelingat, 0.165% (7/4239) in Shibrakhit, 0.233% (6/2571) in Wadi Elnatroon, 0.173% (7/4053) in Itaielbarowd, and 0% (0/980) in El-Mahmoudia (Table 1). The locality had a significant (X^2 = 681.7, P < 0.0001) effect on the prevalence of infection. The prevalence was 0.55% (116/21121) in summer, 0.31% (55/17484) in autumn, 0.3% (64/21262) in winter, and 0.22% (45/20105) in spring. The season had a significant (P < 0.0001, X^2 = 35.01) effect on the hydatid cyst prevalence (Table 2). The prevalence was 2.17% (149/6860) in camels, 0.17% (97/57521) in cattle, 0.20% (17/8386) in buffalo, 0.27% (16/5912) in sheep, and 0.08% (1/1293) in goats. The type of animal had a significant (P < 0.0001, X^2 = 699.6) effect on the prevalence (Table 3). The prevalence was 2.2% (149/6760) in camels > 2 years and 0% (0/100) in camels < 2 years (Table 4). The prevalence was 3.75% (29/773) in > 3 years cattle and 0.12% (68/56748) in the < 3 years (Table 4). The prevalence was 1.09% (8/735) in > 5 years, 0.08% (6/7555) in > 2 years, and 3.13% (3/96) in < 1-year buffalo (Table 4). The prevalence was 0.29% (16/5432) in > 2 years and 0% (0/480) in the < 2 years old sheep (Table 4). The prevalence was 0.08% (1/1123) in > 2 years and 0% (0/170) in < 2 years goat age groups (Table 4). The age significantly (P < 0.0001) in-

Table 1. The prevalence of hydatid cysts in slaughterhouses in different localities at Elbehera governorate, Egypt

City	Number examined	Number infected	%	X^2	P-value*
Damanhour	13414	23	0.17	681.7	< 0.0001****
KafrEldawar	7030	7	0.1		
Rashid	5602	7	0.13		
Edko	2330	4	0.17		
El-Mahmoudia	980	0	0		
KomHamada	11709	196	1.7		
AbouHomous	9401	9	0.10		
AbouAlmatamir	9586	6	0.06		
HoshEissa	7168	5	0.07		
Eldelingat	1889	3	0.16		
Shibrakhit	4239	7	0.17		
Wadi Elnatroon	2571	6	0.23		
Itaielbarowd	4053	7	0.17		
Total	79972	280	0.35		

* Significant at P < 0.05 **** Highly significant

Table 2. The prevalence of hydatid cysts according to the season in slaughtered animals at Elbehera governorate, Egypt

Season	Number examined	Number infected	%	X^2	P-value*
Spring	20105	45	0.22	35.01	< 0.0001****
Summer	21121	116	0.55		
Autumn	17484	55	0.31		
Winter	21262	64	0.3		

* Significant at P < 0.05 **** Highly significant

Table 3. The prevalence of hydatid cysts in different animals in abattoirs at Elbehera governorate, Egypt

Animals	Number examined	Number infected	%	X^2	P-value*
Camel	6860	149	2.17	699.6	< 0.0001****
Cattle	57521	97	0.17		
Buffalo	8386	17	0.2		
Sheep	5912	16	0.27		
Goat	1293	1	0.08		

* Significant at P < 0.05 **** Highly significant

fluenced the prevalence in cattle (X^2 = 35.01) and buffalo (X^2 = 73.01) (Table 4). The prevalence in camels was 2.19% (149/6810) in males and 0% (0/50) in females (Table 5). The prevalence in cattle was 0.12% (68/56824) in males and 4.16% (29/697) in females (Table 5). The prevalence in buffalo was 0.12 (9/7651) in males and 1.08% (8/735) in females (Table 5). The prevalence in sheep was 0.12% (0/591) in males and 0.03% (16/5321) in female animal groups (Table 5). The prevalence in goats was 0% (9/129) in males and 0.09% (1/1164) in female animals (Table 5). The sex only significantly (P < 0.0001) impacted the infection in cattle (X^2 = 640.4) and buffalo (X^2 = 30.87) (Table 5). Regarding organ distribution of cysts, the only organs infected were the liver and lungs. The lung had a higher prevalence of hydatid cysts at 77.41% (305/394) than the liver, which had a prevalence of 22.59% (89/394) (Table 6). Organ prevalence was 85.2% (127/149) for the lung and 14.8% (22/149) for the liver of the camel (Table 6). It was 85.2% (73/97) for the lung and 14.8% (24/97) for the liver of the cattle (Table 6). The prevalence was 64.7% (11/17) for the lung and 35.3% (6/17) for the liver of the buffalo (Table 6). The prevalence was 56.25% (9/16) for the lung and 43.75% (7/16) for the liver of the sheep

Table 4. The prevalence of hydatid cysts according to the age in slaughtered animals at Elbehera governorate, Egypt.

Animal	Age	No. examined	No. infected	(%)	X^2	P-value*
Camel	> 2 years	6760	149	2.2	2.21	0.14
	<2years	100	0	0		
Cattle	>3 years	773	29	3.75	35.01	< 0.0001****
	<3 years	56748	68	0.12		
Buffalo	>5 years	735	8	1.09	73.01	< 0.0001****
	<2 years	7555	6	0.08		
	< 1 year	96	3	3.13		
Sheep	> 2 years	5432	16	0.29	1.414	0.2345
	<2 years	480	0	0		
Goat	> 2 years	1123	1	0.08	0.15	0.70
	<2 years	170	0	0		

* Significant at P < 0.05 **** Highly significant

Table 5. The prevalence of hydatid cysts according to the sex in slaughtered animals at Elbehera governorate, Egypt

Animal	Sex	No. examined	No. infected	%	X^2	P-value*
Camel	Male	6810	149	2.19	1.09	0.30
	Female	50	0	0		
Cattle	Male	56824	68	0.12	640.4	< 0.0001****
	Female	697	29	4.16		
Buffalo	Male	7651	9	0.12	30.87	< 0.0001****
	Female	735	8	1.08		
Sheep	Male	591	0	0	1.78	0.18
	Female	5321	16	0.3		
Goat	Male	129	0	0	0.11	0.74
	Female	1164	1	0.09		

* Significant at P < 0.05 **** Highly significant

Table 6. Organ distribution of Hydatid cyst in slaughtered animals at Elbehera governorate, Egypt.

Animals		Total Number Infected	Organ infected cases			
			Lung		Liver	
			Frequency	%	Frequency	%
Camel	Bull	149	127	85.2	22	14.8
Cattle	Cow	29	29	100	0	0
	Bull	68	44	65.22	24	34.78
	Total	97	73	75.26	24	24.74
Buffalo	Cow	8	5	62.5	3	37.5
	Bull	6	4	66.67	2	33.33
	Veal	3	2	66.67	1	33.33
	Total	17	11	64.7	6	35.3
Sheep	Ewe	16	9	56.25	7	43.75
Goat	Doe	1	1	100	0	0

(Table 6). The prevalence was 100% (1/1) for the lungs and 0% (0/1) for the liver of the goat (Table 6).

Histopathology of the camel cysts

Grossly, several hydatid cysts of various sizes were impeded in the lung tissues of the camel (Fig. 1a). These hydatid cysts contain a transparent fluid in their cavities, which contain the hydatid sand (Fig. 1b). Microscopically, the wall of the hydatid cyst comprises five layers and is surrounded by lung tissue (Fig. 2a and b). The internal layer of the cyst wall, or germinal membrane (I), had the germinal cells, which were surrounded by the laminated layer (II), the band of foreign body giant cells (III), and finally by the fibrous connective tissue layer (IV) (Fig. 3 a, b, and c). Lung tissues surrounding the cyst wall showed a band of mononuclear cell aggregates, connective tissue proliferation, and engorgement of interalveolar venules along with macrophage accumulation in interalveolar tissues (Fig. 4a and b). The sand of the hydatid cyst had the scolex and hooks of *E. granulosus*, which form the rostellar pad (Fig. 5).

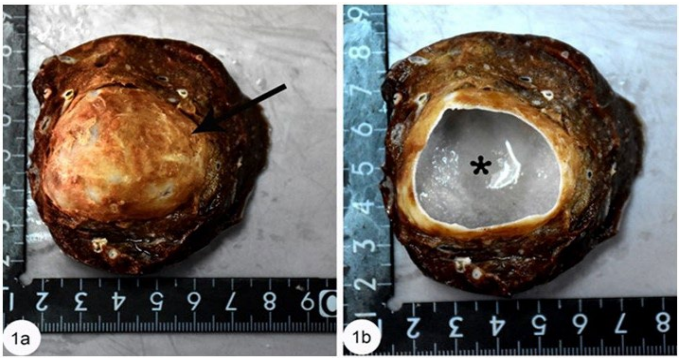

Fig. 1. Gross photographs of hydatid cysts in camel lung tissues. 1a, showing an intact hydatid cyst (arrow). 1b, showing an opened hydatid cyst (asterisk).

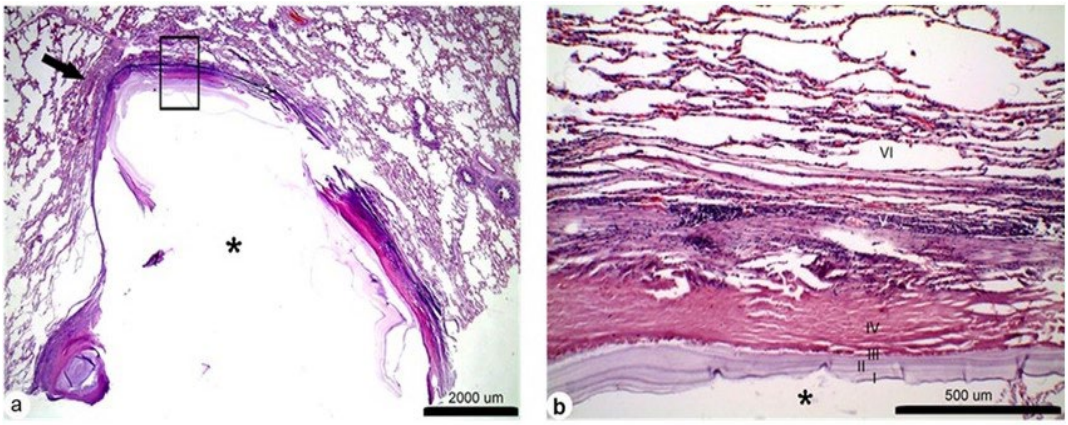

Fig. 2. Panoramic view of hydatid cysts in camel lung tissues. a) The rectangular bordering parts of the cyst cavity (asterisk), cyst wall, and lung tissue (arrow). H and E stain; scale bar is 2000 µm. b) Higher magnification of the hydatid cyst wall in panel a, demonstrating the cyst cavity (asterisk), layers of the cyst wall (I, II, III, IV, and V), and lung tissue (VI). H and E stain; scale bar is 500 µm.

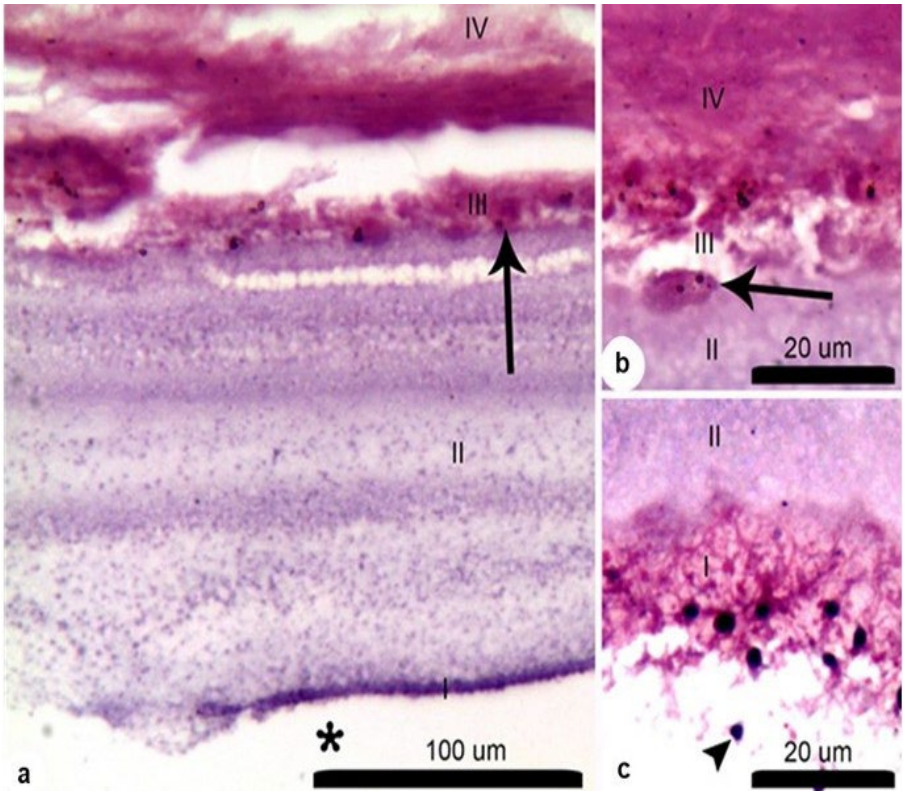

Fig. 3. Higher magnification of the hydatid cyst wall of Fig. 2b in panels a, b, and c. I is the inner surface of the cyst wall or the germinal membrane, which has the germinal cells (arrowhead); II, the laminated layer; III, the layer of foreign body giant cells (arrows); and IV, the fibrous connective tissue layer. Asterisk is the cyst cavity. H&E stain, scale bars, 4a 100 µm; 4b & 4c 20 µm.

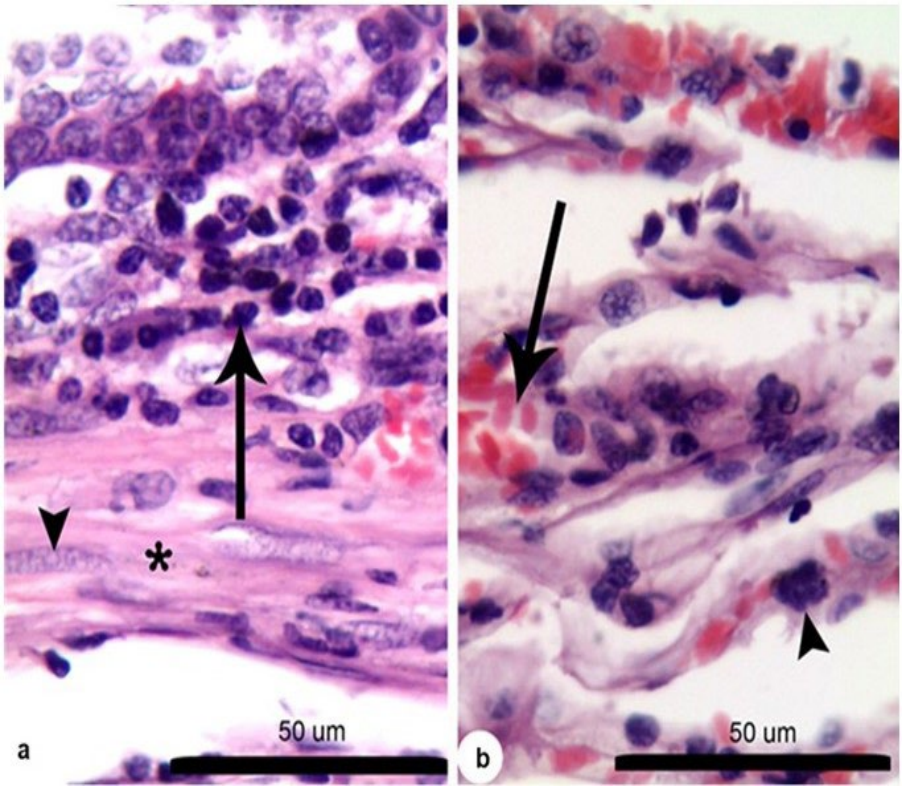

Fig. 4. Higher magnification of Fig. 2b. a) Layer (V) shows a band of mononuclear cell aggregates (arrow) surrounding the cyst wall. Arrowhead, the nucleus of fibrocyte; asterisk, connective tissue bundles. Scale bar 50 µm. b) Layer (VI) shows the congestion of interalveolar venules (arrow) and aggregation of macrophages in interalveolar tissues (arrowhead). Staining in H and E; scale bar 50 µm.

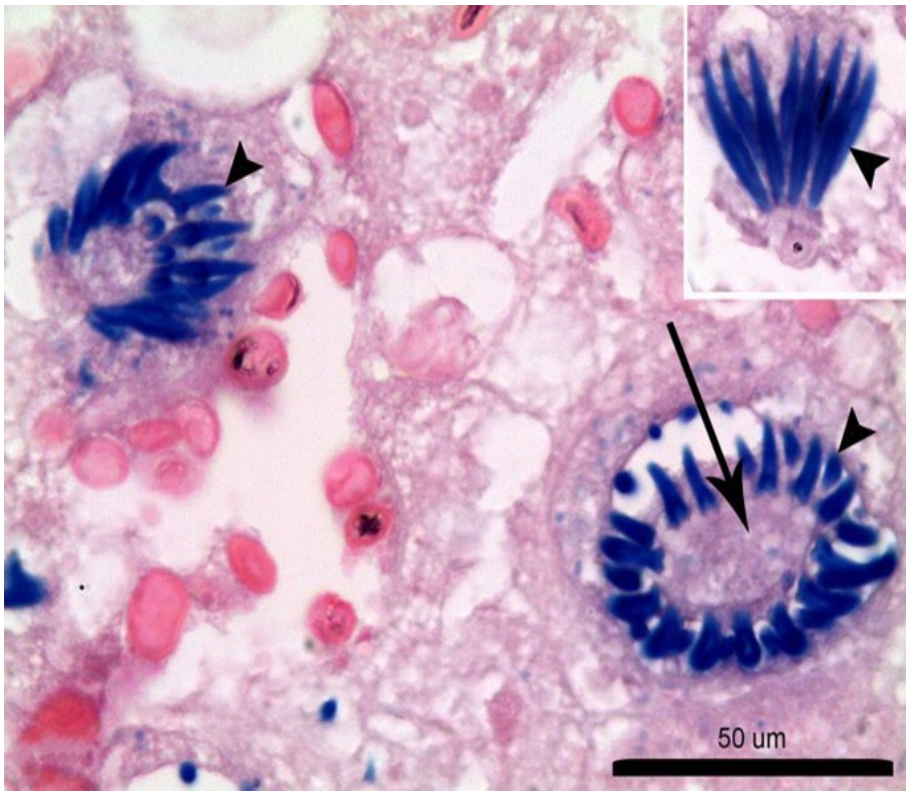

Fig. 5. Giemsa staining of the cyst hydatid sand. The micrograph shows the scolex and hooks of *Echinococcus canadensis*. Rostellar pad, arrow; Rostellar hooks, arrowhead; scale bar 50 µm.

PCR and sequence analysis

The *cox-1* PCR amplified a specific band of 395 bp. The blast through NCBI confirmed the *cox-1* sequence as *E. canadensis*. The sequence was similar to *cox-1* and had an identity percent of 98.68% with *E. canadensis* genotype 6 (G6) from Egyptian camels (AB921083, AB921084, AB921085, and AB921086), 98.63% with *E. canadensis* in cattle (G6) from Mauritania (EU048813), 98.62% *E. canadensis* (G6) from an Iranian camel (KM513633 and HM563019), 98.60% in an Iranian camel (G6) (HM626406), and 98.36% *E. canadensis* (G6) in the lung of an Iranian camel (KF731904 and KF731906). It had an identity percent of 98.22% with *E. canadensis* in camels from Algeria (KR349037) and Sudan (AB271912), 97.80% with *E. canadensis* (G6) in a man from

Iran (HM563020), 91.59% in a man from Algeria (DQ341576), and 90.98% with the Iranian *E. granulosus* (G3) in camel lung (KF731907) and liver (KF731905). The sequence had an identity percent of 90.59% with *E. granulosus* (G1) in camels from Morocco (EF367287 and EF367293), 90.33% with *E. granulosus* sensu stricto in Tunisian camels (KM014633 and KM014619), and 90.08% with *E. granulosus* in Australian cattle (AJ508030). The *cox-1* sequence had an identity percent of 89.81% with Iranian *E. granulosus* (G1) in camel (HM563021, HM563013, and KT074947), sheep (KT074944), and cattle (KT074945), 88.62% with *E. granulosus* (G1) in camel (KU756225) from Iran, and 87.43% with a cyst in an Iranian camel (LC068914). The sequence of *E. canadensis* from Egypt (LC160333) occurred in the same genetic clade as camel *E. canadensis* genotype 6 (G6) from Mauritania (EU048813), Egypt (AB921083, AB921084, AB921085, and AB921086), Sudan (AB271912 and M84667), Algeria (KR349037), and Iran (HM563019) (Fig. 6).

The *G1Y162* PCR amplified a specific band of 594 bp from *E. canadensis*. The *EcG1Y162* (LC160334) sequence from Elbehera, Egypt has identity percents of 93.84%, 93.65%, and 92.12% and 92.06% and 90.37% with the *G1Y162* from *E. granulosus* cysts from the liver (AB462014), forebrain (AB458259), and liver (AB458258) of sheep from China and *E. multilocularis* adult worms in Japan (AB303298 and AB303297), respectively. The Egyptian sequence is in the same genetic clade as other strains of *Echinococcus*, away from the control *T. solium* out-group (Fig. 7A). While the phylogenetic tree of the amino acid sequence demonstrated that the amino acid sequence of *E. canadensis G1Y162* from Egypt was present in a separate taxon from *E. granulosus*, *E. multilocularis*, and *T. solium* (Fig. 7B).

DISCUSSION

The cyst of *Echinococcus* is widely prevalent in different animals. It causes serious losses in animals due to the condemnation of organs or carcasses; therefore, we tried to investigate the prevalence among butchered animals in Elbehera governorate, Egypt. Egypt's Elbehera governorate demonstrated a lower prevalence of *E. granulosus* compared to Spain (Carmena *et al*., 2008), Greece (Chaligiannis *et al*., 2015), Italy (Bosco *et al*., 2021), Brazil (de La Rue, 2008), Iran (Azami *et al*., 2013), Kingdom Saudi Arabia (Ibrahim, 2010), Australia (Wilson *et al*., 2019), Libya (El-Salem *et al*., 2021; Elmajdoub and Rahman, 2015), and Egypt (Barghash *et*

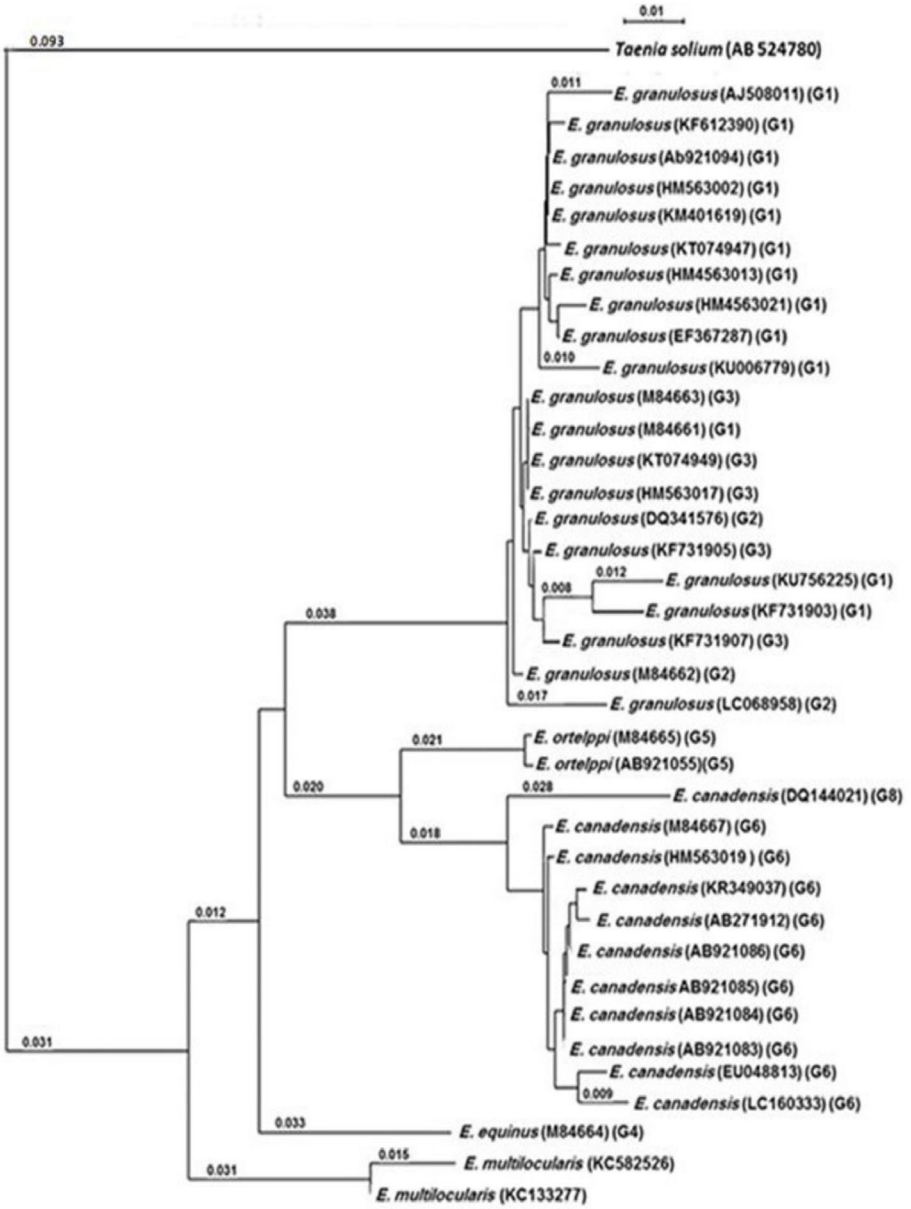

Fig. 6. Phylogenetic tree of the *cox-1* gene of the *Echinococcus canadensis* hydatid cyst from Elbehera governorate. The neighbor-joining method was used to construct the tree. The sequences of *cox-1* from Elbehera (LC160333) and reference sequences from GenBank were used. The tree showed the scale bar and branch lengths.

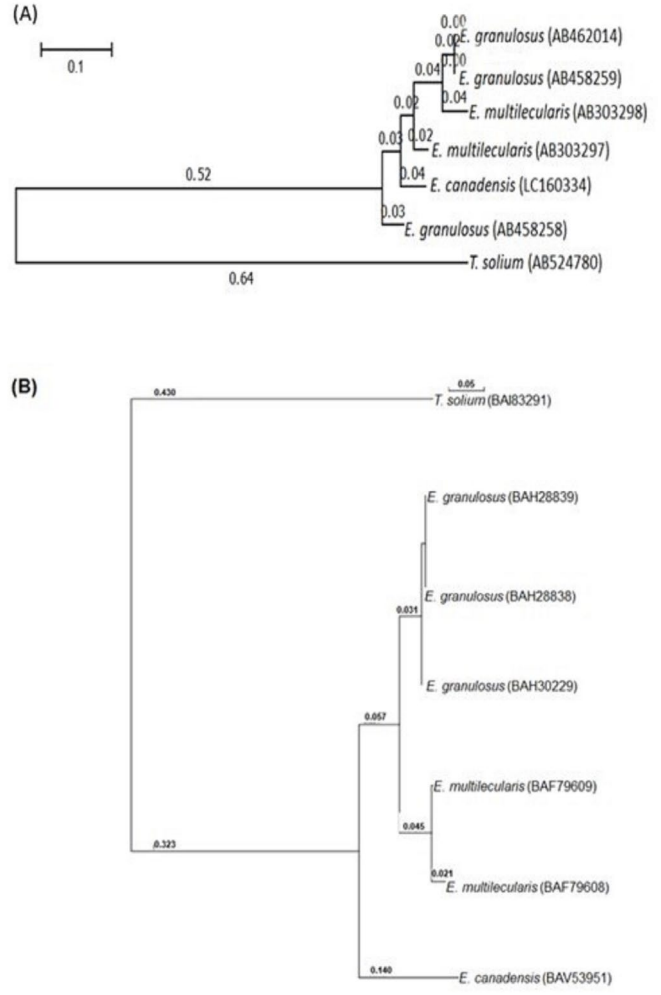

Fig. 7. Phylogenetic trees of the *G1Y162* of *Echinococcus canadensis* from Elbehera, Egypt. The neighbor-joining method was used to construct the trees. a) The phylogenetic tree of the *EcG1Y162* gene (LC160334). The nucleotide sequences from GenBank were used, including *Echinococcus granulosus* Eg*G1Y162* (AB458258, AB458259, and AB462014) and *Echinococcus multilocularis* EmY162 (AB303298 and AB303297) sequences. b) The phylogenetic tree of amino acid sequences of the *G1Y162* protein and those of the proteins used in panel a. The trees showed the scale bar and branch lengths.

da had the highest prevalence of cysts, especially in camels, among the cities of Elbehera governorate, while the infection was not revealed in El-Mahmoudia. This may be due to the high level of camel rearing in Kom-Hamada, which is a desert area, and the preference of people to eat camel meat, leading to a high level of slaughter and the subsequent detection of cysts in camels. Also, the high infection rate of camels led to an increased prevalence of cysts in the abattoir of this district. The infection rate in camels from the Elbehera governorate (2.17%) was greater than that recorded in Nigeria (1.73%) (Igwenagu *et al*., 2018). While prevalence was lower than that stated in Libya (12.54%) (Elmajdoub and Rahman, 2015), Oman (5.3%) (Al Kitani *et al*., 2015), Kenya (6.94%) (Mbaya *et al*., 2014), Sudan (35%) (Ahmed *et al*., 2018), the Kingdom of Saudi Arabia (32.85%) (Ibrahim, 2010), and Iran (14.64% and 38.9%) (Hajimohammadi *et al*., 2022; Mirzaei *et al*., 2016). Also, it is lower than that reported in other governorates in Egypt, such as Sharkia (3.7%) (Ahmed *et al*., 2021), Aswan (8.3%) (Dyab *et al*., 2018), Matrouh (13.7%) (Barghash *et al*., 2017), Cairo (18.9%) (Mahdy *et al*., 2014), and Minoufyia (29.3%) (El-Bahy *et al*., 2019). This may be attributed to the differences in environmental conditions and contact with dogs. The prevalence was highest in the summer while Azami *et al*. (2013) and Dyab *et al*. (2018) reported the highest prevalence in winter and autumn. The older animals were infected at a higher rate than the younger ones; this may be due to the higher chance of old animals being exposed to eggs in dog feces. This is consistent with former research (Ibrahim *et al*., 2011; Ibrahim, 2010; Dyab *et al*., 2018; Mirzaei *et al*., 2016). The females had higher infection rates than males; this is agreed upon by Mirzaei *et al*. (2016), Dyab *et al*. (2018), and Azami *et al*. (2013). This may be because females are kept for breeding and milking for longer periods than males and are in more contact with dogs in contrast to males confined to fattening lots. Except male camels had a higher prevalence than females, this could be because Komhamada City residents prefer the soft meat of young male camels, which leads to an increase in the slaughter of young male camels and the detection of cysts. Lung infection was most prevalent, trailed by liver infection; this agrees with Hajimohammadi *et al*. (2022), Dyab *et al*. (2018), and Ibrahim *et al*. (2011) but disagrees with Ibrahim (2010). This could be due to the soft nature of the lung tissues, making them easily infected, as well as the fact that the high blood circulation in the lung provides oxygen for the cyst to develop.

Microscopically, the wall of the hydatid cyst comprises five layers and is surrounded by lung tissue. The inner surface of the cyst wall, or germinal membrane, had the germinal cells, surrounded by the laminated layer, the layer of foreign body giant cells, and the fibrous connective tissue layer. The lung tissues surrounding the cyst wall showed a band of mononuclear cell aggregates, connective tissue proliferation, congestion of interalveolar venules, and accumulation of macrophages in interalveolar tissues, consistent with previous studies from Egypt in Aswan (Dyab *et al*., 2019), Minoufyia (El-Bahy *et al*., 2019), and Sharkyia (Alhoot *et al*., 2019).

In the PCR, a specific band of the *cox-1* gene of 400 bp was amplified from the cysts. The sequence was for the *E. canadensis cox-1* gene by blast search. The sequence had a high identity percentage of up to 99% with genotype 6 (G6) of *E. canadensis* from Egyptian camels (Amer *et al*., 2015). Egypt's sequence (LC160333) occurred in the same genetic clade as the camel *E. canadensis* genotype 6 (G6) of Mauritania (EU048813), Egypt (AB921083, AB921084, AB921085, and AB921086), Sudan (AB271912 and M84667), Algeria (KR349037), and Iran (HM563019). Therefore, phylogenetic analysis confirmed that the sequence is *E. canadensis* genotype 6 (G 6). This agrees with the genotype 6 sequences reported from camels in Iran (Sharbatkhori *et al*., 2016), Algeria (Zait *et al*., 2016), China (Zhang *et al*., 1998), and Mauritania (Maillard *et al*., 2009). Other *Echinococcus* complex genotypes in camels were reported, including genotype 7 in Upper Egypt (Elshahawy *et al*., 2022), genotype 3 (G3) in Iran (Sharbatkhori *et al*., 2016), and genotype 1 (G1) in Libya (Tashani *et al*., 2002) and Iran (Sharbatkhori *et al*., 2016). Therefore, camels are considered a source of infection for dogs and consequently zoonotic infection for humans. The *EcG1Y162* PCR amplified a specific band of 594 bp. The *EcG1Y162* (LC160334) gene sequence from this study shared a high identity percentage with the *G1Y162* of *E. granulosus* cysts from GenBank, and it was found in the same taxon as *E. granulosus* and *E. multilocularis* but not with *T. solium*. In the phylogenetic tree of the amino acid sequence, the *E. canadensis G1Y162* sequence from Egypt occurred in a separate genetic clade from *E. granulosus* and *E. multilocularis*, therefore, *G1Y162* may be utilized as a confirmatory genetic marker for *E. canadensis*.

CONCLUSION

This study presented the prevalence of *E. granulosus* in slaughtered ruminants from Elbehera Governorate, Egypt, as well as the pathology and molecular characterization of the *E. canadensis* cysts in camels using the *cox-1* and *EcG1Y162* genes. The amino acid sequence of *EcG1Y162* might aid in the parasite's phylogenetic identification. Due to the presence of these zoonotic cysts in slaughtered animals, stringent healthcare regulations are required in this area to prevent infection in both animals and humans.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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