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Prevalence, Molecular Characterization, and Economic Impact of Hydatid Cysts in the Slaughtered Animals in Abattoirs of Minoufyia Governorate, Egypt

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Abstract

The hydatid cyst is considered a hazardous obstacle for public health and the livestock industry. The present investigation sought to ascertain the prevalence of hydatid cysts, histopathology, and economic losses in slaughtered food animals in Egypt's Minoufyia governorate. Furthermore, the molecular characterization of the cysts was performed using an analysis of the NADH dehydrogenase 1 sequence. Visual meat inspection of 6417 slaughtered animal carcasses in Minoufyia Governorate abattoirs was conducted over two years, from March 2019 to February 2021. The prevalence of hydatid cysts in slaughtered animals was 1.48%. The prevalence in different animal carcasses was 1.5% in sheep, 1.33% in cattle, 0.71% in buffaloes, and 9.5% in camels while cyst was not detected in goats. Females had a higher prevalence than males. Autumn had the highest prevalence of hydatid cysts at 0.44%, followed by winter at 0.40%, spring at 0.39%, and summer at 0.25%. The liver and lungs were the most infected organs, with infection rates of 57.9% and 42.1%, respectively. In addition, the histopathology of recovered hydatid cysts was recorded. The sequence analysis of NADH dehydrogenase revealed that the sequence of camel and cattle is *E. canadensis* and the buffalo sequence is *E. ortleppi*. The phylogenetic tree revealed that *Echinococcus canadensis* from Egyptian camels and cattle belonged to the same taxon as genotypes 6-10 of the *E. granulosus* complex. The *E. ortleppi* sequence from Egyptian buffalo was found in the same clade as genotype 5 of the *E. granulosus* complex. The economic costs of organ condemnation amounted to 47800 EGP. The results of this survey present the prevalence, economic impact, and molecular characterization of hydatid cysts from animals in Minoufyia governorate, Egypt. Strict hygienic measures are needed to control this infection in food animals and humans.

KEYWORDS
Echinococcus canadensis; *Echinococcus* ortleppi; Hydatid cyst; Minoufyia Governorate; NADH-1

INTRODUCTION

Taeniasis is a parasitic disease spread through food that has serious public health implications. The hydatid cyst, the metacestode stage, develops in the viscera of intermediate hosts (primarily the lungs and liver) and causes cystic echinococcosis (CE; hydatidosis) (Agudelo Higuita *et al.*, 2016). CE is a zoonotic parasitic disease caused by the consumption of eggs from various *Echinococcus* spp. (*E. granulosus* and *E. multilocularis*) live in the small intestines of dogs and other carnivores. Intermediate hosts include humans and herbivorous animals (sheep, cattle, and camels) (Macpherson 1985; Eckert *et al.*, 2000; Brunetti *et al.*, 2010; Samorek-Pieróg *et al.*, 2016). The main causes influencing the continuity of *E. granulosus* in the Mediterranean area are the suitable climatic and ecological characteristics, as well as the large population of stray dogs. It is found in nearly every community in both the developing and advanced worlds (Craig and Larrieu, 2006). Hydatid cysts are economically significant because the disease not only reduces yield from offal and other products such as milk and meat, but it can also reduce overall productivity and even cause death (Lahmar *et al.*, 1999; Mohey *et al.*, 2016).

Cystic echinococcosis is a pathological condition caused by *E. granulosus*, which infects the internal viscera of herbivore animals and causes a unilocular fluid cyst (Rinaldi *et al.*, 2008; Jiménez *et al.*, 2020). In the final host, the adult worm of *E. granulosus* resides in the carnivores' small intestine, where it produces its eggs (Rinaldi *et al.*, 2008; Jiménez *et al.*, 2020). Infection in herbivores (the intermediate host) occurs through the ingestion of eggs enclosing oncospheres, which grow into cysts primarily in the liver and lungs (Seimenis, 2003; Rinaldi *et al.*, 2008). The cyst is surrounded by the host's fibrous connective tissue and has a wall consisting of an outer cellular laminated carbohydrate-protein and inner germinal layers. Within the cyst, there are brood capsules with protoscolices attached to the germinal layer (Jiménez *et al.*, 2020; Reinehr *et al.*, 2020). The fertility of a cyst depends on the existence or lack of protoscolices (Stoore *et al.*, 2018; Jiménez *et al.*, 2020).

Currently, the *E. granulosus* complex comprises four species

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and ten distinct strains (genotypes G1-11) (Sharbatkhori *et al*., 2010). All other strains, except for the G4 genotype, have been found to infect humans (Moro and Schantz, 2009; Nakao *et al*., 2013). NADH dehydrogenase subunit 1 (NADH-1) was used for the molecular characterization of cysts from different animals and humans in several countries. The study of the prevalence of hydatid cysts was previously conducted in the Minoufyia governorate without molecular characterization or evaluation of the economic impact of the cyst (El-Bahy *et al*., 2019). Therefore, this study aimed to verify the prevalence, molecular characterization of unilocular cysts from slaughtered animals in the Minoufyia governorate of Egypt, and their economic impact.

MATERIALS AND METHODS

Ethical approval

The study was approved ethically by the University of Sadat City (Ethical approval number: VUSC-034-1-22). No experiments were conducted on animals. The samples were collected from the carcasses after the animals were slaughtered.

Samples and study area

This survey was performed from March 2019 to February 2021 to verify the prevalence of hydatid cysts in slaughtered food animals. An overall of 6550 carcasses were examined at El-Shohada, El-Bagour, and Menof abattoirs of the Minoufyia governorate, Egypt. The slaughtered animals examined were cattle (4670), buffaloes (989), camels (221), goats (133), and sheep (537). The sex groups of the animals were: 2500 males and 2170 females of cattle; 503 males and 486 females of buffalo; 215 males and 6 females of camels; 80 males and 53 females of the goat; and 341 males and 196 females of sheep. Except for the sheep and goat age groups, which were 1.5–3 years and >3 years old, all the inspected animals' age groups were 1.5–3 years and 5–15 years old.

Sampling

The collected cysts were transferred on ice to the Parasitology Research Laboratory in the Faculty of Veterinary Medicine at the University of Sadat City. For histopathological examination, parts of the cyst walls were placed in 10% neutral buffered formalin, while parts of the germinal layers were placed in 70% alcohol for DNA extraction.

Histopathological examination of hydatid cyst

Eighteen (9 cattle, 9 buffalo) liver and lung samples from infected animals were preserved for 3 days in 10% neutral buffered formalin. After fixation, tissues were cut, processed in chemicals, and embedded into paraffin blocks. Paraffin blocks were trimmed into 3-µm sections that were deparaffinized and stained with hematoxylin and eosin stain (H&E) for light microscopic investigation (Bancroft and Layton, 2013).

DNA extraction and PCR

DNA was extracted by a DNA extraction kit (Intron, Seoul, Korea). The extracted DNA was measured using a spectrophotometer (Nanodrop 2000, Thermo Scientific, USA). The primers used in the reactions were forward JB11 (5´-AGATTCGTAAGGGGCCTAATA-3´) and reverse JB12 (5´-ACCACTAACTAATTCACTTTC-3´) primers of NADH dehydrogenase subunit 1 (NADH-1) (Bowles and McManus 1993). The PCR reaction includes 2 µL DNA, 10 µL Green Master mix (Intron, Seoul, Korea), 1 µL of primer pairs, and double-distilled water up to 25 µL. The reaction was carried out on a thermocycler (Applied Biosystems, The USA). The reaction conditions used in this study were described by Bowles and McManus (1993). The amplified DNA was analyzed on 1% agarose gel after staining with ethidium bromide.

Sequencing and phylogenetic analysis

The amplified DNA was purified using a gel extraction kit (Qiagen, USA). The purified DNA was sent for sequencing in Korea (Intron, Seoul, Korea). The resulting sequences were blasted on the BLAST site (Basic Local Alignment Search Tool). The sequences were used to obtain accession numbers from GenBank. The NADH-1 sequences of camel (LC757643), buffalo (LC758545), and cattle (LC758546) from Minoufyia governorate and GenBank (AB921124, AB921091, AJ508069, AJ237975, AJ241204, EU151429, HQ231402, HQ231403, GQ168807, JX067637, JX067638, KT074939, KU842044, KX010904, KY766906 KY766907, KY766908, MH300950, MH300951, MH300952, MH300953 MH300954, MH300971, MH686293, MN340038, MN696571, MW660886NC_011122, OK489951, OP471630, OP471631, OP471632, OP471633, and OP471634) were used to build the phylogenetic tree with the neighbor-joining method. As an outgroup, the sequence of NADH-1 from Taenia saginata (AY684274) was used.

Estimation of economic losses

The economic loss (EL) caused by organ disapproval was calculated using the following equation: Ogunrinade and Ogunrinade (1980) adopted EL=NPW, where N represents the number of rejected organs, P represents the average organ cost (EGP/kg), and W represents the average organ weight (kg).

Statistical analysis

The relationship between risk factors including animal type, locality, season, age, and sex were analyzed using the Chi-square test. The test result was significant at $P < 0.05$. All analysis was achieved using the SPSS program version 20.

RESULTS

Prevalence

The total prevalence of hydatid cysts in slaughtered animals was 1.45%. Concerning the type of animal carcasses, the prevalence was 9.50% (21/221) in camels, 1.50% (5/537) in sheep, 1.33% (62/4670) in cattle, and 0.71% (7/989) in buffaloes (Table 1). The type of animal significantly impacted the prevalence of the hydatid cyst (X^2 =103.4114 and $P < 0.00001$) (Table 1). Concerning the locality, the prevalence of hydatid cysts in cattle was 1.32%, 1.9%, and 0.93% in the abattoirs of El-Shohada, El-Bagour, and Menof, respectively. The prevalence of the cyst in buffalo was 0.8%, 0.45%, and 0.7% in El-Shohada, El-Bagour, and Menof abattoirs, respectively. The prevalence of cysts in camels was 7.84% for El-Shohada and El-Bagour and 10% for Menof abattoirs. The prevalence in sheep was 1.98%, 0.87%, and 0% in El-Shohada, El-Bagour, and Menof abattoirs, respectively. The locality had no significant impact on the prevalence of all the inspected animals' carcasses (Table 2). In terms of season, the prevalence of cysts in

cattle was 1.08% in winter, 0.92% in spring, 1.51% in summer, and 2.37% in autumn. The prevalence of cysts in buffalo was 1.22% in winter, 0.30% in spring, 0.55% in summer, and 0.87% in autumn. The prevalence of cysts in camels was 11.39% in winter, 8.57% in spring, 9.30% in summer, and 7.69% in autumn. The prevalence of cysts in sheep was 7.40% in winter, 1.78% in spring, and 0% in summer and autumn. The season only significantly impacted the prevalence in cattle (X^2 = 10.45, P < 0.015) (Table 2). Concerning gender, the prevalence was 0.047% males and 0.92% females in cattle, 0.016% males and 0.094% females in buffalo, 0.327% males and 0% females in camel, and 0.031% males and 0.047% females in sheep. Gender only had a significant impact on the prevalence in cattle (X^2 = 59.8949, P < 0.00001) (Table 2). Concerning the age of inspected animal carcasses, the age range was 1.5–3 years in males of all animals and > 3 years in sheep females, while 5–10 years in cattle, buffalo, and camel females. Gender was associated with the prevalence of cysts in various age groups. The prevalence was 0.047% 1.5–3 years and 0.92% 5–10 years cattle, 0.016% 1.5–3 years and 0.094% 5–10 years buffalo, 0.327% 1.5–3 years and 0% 5–10 years camel, and 0.031% 1.5–3 years and 0.047% > 3 years sheep. Age had the only significant impact on prevalence in cattle (X^2 = 59.8949, P < 0.00001) (Table 2). In terms of cyst organ distribution, the liver was the most infected organ with a frequency of 58.33%, followed by the lung with a frequency of 41.67%. (Table 3). The frequency of infection was 68.25% for the liver and 31.75% for the lungs of cattle, 71.43% for the liver and 28.57% for the lungs of buffalo, 9.52% for the liver and 90.48% for the lungs of camels, and 100% for the liver and 0% for the lungs of sheep (Table 3). Coinfection of the liver and lungs was only detected in one cattle. The inspected goats (n=133) were free from hydatid cysts.

Table 1. Prevalence of the hydatid cyst in slaughtered animals in abattoirs of Minoufyia Governorate, Egypt.

Animal	Inspected	Infected (%)	X^2	P
Cow	4670	62 (1.33)	103.41	< 0.00001****
Buffaloes	989	7 (0.71)		
Camels	221	21 (9.5)		
Sheep	537	5 (0.93)		
Goat	133	0 (0)		
Total	6417	95 (1.48)		

*Statistically significant at P < 0.05

Table 2. Risk factors affecting prevalence of hydatid cyst at Minoufyia Governorate abattoirs

Risk Factor	Animal	Cattle		Buffalo		Camel		Sheep	
		Inspected	Infected (%)	Inspected	Infected (%)	Inspected	Infected (%)	Inspected	Infected (%)
Locality	El-Shohada	3023	40 (1.32)	622	5 (0.8)	110	11 (10)	334	4 (1.98)
	El-Bagour	999	16 (1.9)	223	1(0.45)	51	4 (7.84)	115	1 (0.87)
	Menof	648	6 (0.93)	144	1 (0.7)	60	6 (10)	88	0 (0)
	X^2	1.37		0.30		0.21		1.09	
	P	0.50		0.86		0.90		0.58	
	Total	4670	62 (1.33)	989	7 (0.71)	221	21 (9.5)	537	5 (0.93)
Season	Winter	1204	13 (1.08)	245	3 (1.22)	78	9 (11.39)	136	1 (7.40)
	Spring	1851	17 (0.92)	330	1 (0.30)	35	3 (8.57)	225	4 (1.78)
	Summer	729	11 (1.51)	183	1 (0.55)	43	4 (9.30)	116	0 (0)
	Autumn	886	21 (2.37)	231	2 (0.87)	65	5 (7.69)	60	0 (0)
	X^2	10.45		1.85		0.66		3.46	
	P	0.015**		0.60		0.88		0.33	
	Total	4670	62 (1.33)	989	7 (0.71)	221	21 (9.5)	537	5 (0.93)
Sex and Age (years)	Male (1.5-3)	2500	3 (0.047)	503	1 (0.016)	215	21(0.327)	341	2 (0.031)
	Female (5-15 & >3 for sheep)	2170	59 (0.92)	486	6 (0.094)	6	0 (0)	196	3 (0.047)
	X^2	59.89		3.77		0.65		1.20	
	P	< 0.00001****		0.05		0.42		0.27	

*Statistically significant at P < 0.05

Table 3. Organ distribution of the hydatid cyst in slaughtered animals in Minoufyia governorate, Egypt

Organ	Cattle†		Buffaloes		Camels		Sheep	
	Liver	Lung	Liver	Lung	Liver	Lung	Liver	Lung
Infected	43	20	5	2	2	19	5	0
Infection percent	68.25%	31.75%	71.43%	28.57%	9.52%	90.48%	100%	0%
Total	63		7		21		5	

†There was coinfection of the liver and lungs in one animal.

Histopathological examination of hydatid cyst

Macroscopically, the liver and lungs had single or multiple fluid-filled cysts of *E. granulosus*. The size was different and ranged from small to large cysts. The cysts were surrounded by the fibrous connective tissue of the host. In severe cases, conglomerates of several small cysts were observed with a spongy-like appearance that replaced most of the involved tissue's parenchyma. The cross-section of the cysts showed the inside with its laminated wall layer and fluid (Fig. 1).

Microscopically, the cysts occupied and replaced a large portion of tissue parenchyma. The cyst has a wall consisting of an outer acellular laminated layer and an inner germinated epithelial layer. Fertile cysts showed daughter cysts or brood capsules with protoscolices attached to the germinal epithelium. The protoscolices had central suckers (refractile hooklets) and bilateral suckers. Other cysts were infertile and without protoscolices. An inflammatory reaction including lymphocytes, eosinophils, and giant cells was observed that was more severe around ruptured cysts than intact cysts (Fig. 2).

PCR and phylogenetic analysis

Specific PCR amplification of NADH-1 was revealed from the DNA of cysts from cattle, buffalo, and camels. The BLAST search revealed that the sequence of the camel and the cattle from Minoufyia, Egypt, was *E. canadensis*, genotype 6 (G6). The buffalo sequence of NADH-1 was *E. ortleppi*, genotype 5 (G5). *E. canadensis* NADH-1 from the cattle cyst had an identity percent of 99.12% with the sequence from the camel cyst in this study. *E. canadensis* NADH-1 from the cattle had identity percentages with sequences from cysts from different animals in different countries, including 99.37% identity percent with *E. canadensis* NADH-1 of *E. canadensis* (G6) in Egypt from a buffalo (AB921109) and a camel (AB921093–AB921118, except AB21117) and Iran from a man (MZ927665). It had an identity percent of 99.17% with *E. canadensis* (G6) in yak (MN340038) and sheep (MN340039) of China, in camels of Mauritanian (MH300953 and MH300954), Sudan (MH300939, MH300950, MH300952, KX010882, KX010885, and KX010888), Kenya (KX010873, KX010874, KX010876, and MT525967), Nigeria (MT166286, MT166287, MT166288, and MT166290), Kazakhstan (AB208063 and NC_011121), and Iran (HM749615, HM749616, HM749618, MH300932, and NC_038227), donkeys in Kenya (OK489953), goat in Sudan (MH300943 and MH300951) and Argentina (MH300933, MH300934, and MH300935), sheep in Sudan (MH300941, MH300942, MH300947, MH300948) and Nigeria (MT166289), cattle in Sudan (MH300946, MH300945, MH300944, and MH300940) and Nigeria (MT166289), and man of Mongolia (MH300971) and Kenya (MH300936, MH300937, and MH300938). The identity was 99.17% with *E. canadensis* Nadh-1 in a camel from Sudan (KX010881). It had an identity of 99.16% with *E. canadensis* (G6) of camel in Egypt (AB921117) and man in Iran (KT316343) and Algeria (KR349048). It had an identity of 98.96% with *E. canadensis* (G6) in cattle of Nigeria (MN025266 and MT166284), Ethiopia (KX010880), and Kenya (MT525964), in camels of Sudan (KX010887 and KX010884), Kenya (MT525965, KX010877, and KX010879), Nigeria (MT166285), and Iran (MH300930 and MH300931), the goat of Kenya (KX010878), and a man in Iran (KX893481). It had an identity percentage of 98.75% with *E. canadensis* of Kenya from a donkey (OK489954) and a

Table 4. Economic losses of hydatid cyst in slaughtered animals.

Condemned part	No. infected organs	Weight (kg)	Total condemned (kg)	Price/kg (EGP)	Total loss (EGP)
Liver	55	6	330	120	39600
Lung	41	5	200	35	8200
Total	96	-----	530	---	47800

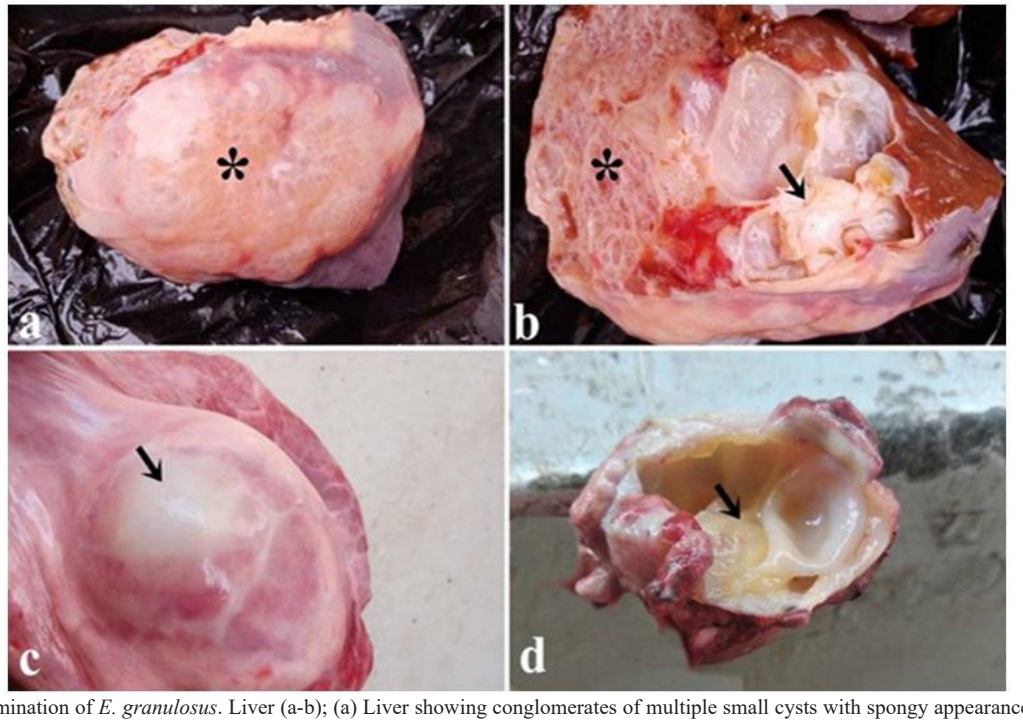

Fig. 1. The Gross examination of *E. granulosus*. Liver (a-b); (a) Liver showing conglomerates of multiple small cysts with spongy appearance (asterisk) which replaced hepatic parenchyma. (b) The cross-section of the cysts showed its content, laminated layer (arrow), and spongy appearance of multiple cysts (asterisk). Lung (c-d); (c) showing the presence of cyst in the lung tissue (arrow). (d) The cross-section of the cysts showed its content (arrow).

camel (MT525966) and *E. granulosus* genotype 7 from pigs in Lithuania (MH301020) and Italy (MH301018 and MH301019).

The camel *E. canadensis* NADH-1 had a sequence identity percent of 98.94% with sequences from Egypt (MK492611, MK492612, MK492616, and MK492618), 98.74% with *E. canadensis* (G6) in yak (MN340038) and sheep (MN340039) from China, a man from Mongolia (MH300971), camels of Egypt (AB921093–AB921118), Mauritania (MH300953, MH300954, and KT363811), Nigeria (MT166286–MT166290), Sudan (MH300950–MH300952, KX010881, KX010882, KX010885, KX010888, and JN637176), Kenya (KX010873, KX010874, KX010876, and MT525967), Iran (MH300932, NC_038227, and KT200209), and Kazakhstan (NC_011121 and AB208063), goat from Sudan (MH300939, MH300943, and MH300949) and Argentina (MH300933-MH300935), sheep of Sudan (MH300940-MH300942, MH300947, and MH300948), buffalo from Egypt (AB921109), cattle from Sudan (MH300944-MH300946) and Nigeria (MT166289), a donkey from Kenya (OK489953), a man in Iran (MZ927665) and Kenya (MH300936-MH300938). It had an identity percentage of 98.72% with *E. canadensis* from a man in Iran (MT793712). It had an identity percentage of 98.54% with *E. canadensis* in camels of Egypt (AB921117), Sudan (KX010884 and KX010887), Algeria (KT316343), Kenya (KX010877, KX010879, and MT525965), and Iran (MH300930, MH300931, HM749615, HM749616, and HM749618). It had an identity percentage of 98.53% with *E. canadensis* from cattle in Nigeria (MN025266, MT525964, and MT166284) and Ethiopia (KX010880), a camel in Nigeria (MT166285), a goat in Kenya (KX010878), and humans from Iran (KX893481 and KR349048). It had an identity percentage of 98.32% with *E. canadensis* from Kenya, from a donkey (OK489954) and a camel (MT525966), and from Slovenia, from a pig (MT239145) and a man (MT239141).

The *E. ortleppi* NADH-1 from buffalo had a sequence identity of 97.67% with *E. ortleppi* from Mozambique (MZ254630), 97.46% with *E. ortleppi* sequences from Mozambique (MZ254631, MZ254632, MZ254636, and MZ254638-MZ254641), 97.31% with a liver cyst in a man from Poland (MH492788), and 97.25% with sequences from Mozambique (MZ254633-MZ254635, MZ254637, and MZ254642). It has an identity percent of 97.13% with sequences from Mozambique (MZ190836 and MZ322609), 97.13% with *E. ortleppi* from Egypt (MK492617), 97.06% with *E. ortleppi* in camels from Egypt (AB921092) and Sudan (JN637177), and 97.02% with a sequence of NADH-1 in cattle from the Netherlands (DQ402037). It has an identity of 96.96%, with sequences of buffalo in India (KY766906-KY766908), cattle from Sudan (KU842045), Ethiopia (KU842044), Kenya (KX010904), Argentina (KC579444), and Pakistan (MN886293 and MN886292), a donkey from Kenya (OK489951), and pigs (OP471631, OP471634), a yak (OP471630), and humans (OP471632 and OP471633) in China. It had an identity percentage of 96.85% with a sequence in a man from Japan (AB979274). It has 96.75% identity with sequences from a pig from China (OP471635) and cattle from Brazil (KU842047), Zambia (KU842046), and Namibia (KX138069). Sequences of *E. canadensis* from cattle and camel occurred in the same taxon with *E. canadensis* or *E. granulosus* complex genotypes 6-10 (Fig. 3). The sequence of *E. ortleppi* from buffalo of Egypt occurred with the *E. ortleppi* sequences of *E. granulosus* complex genotype 5 (Fig. 3).

Economic losses

The financial losses after condemning portions affected by

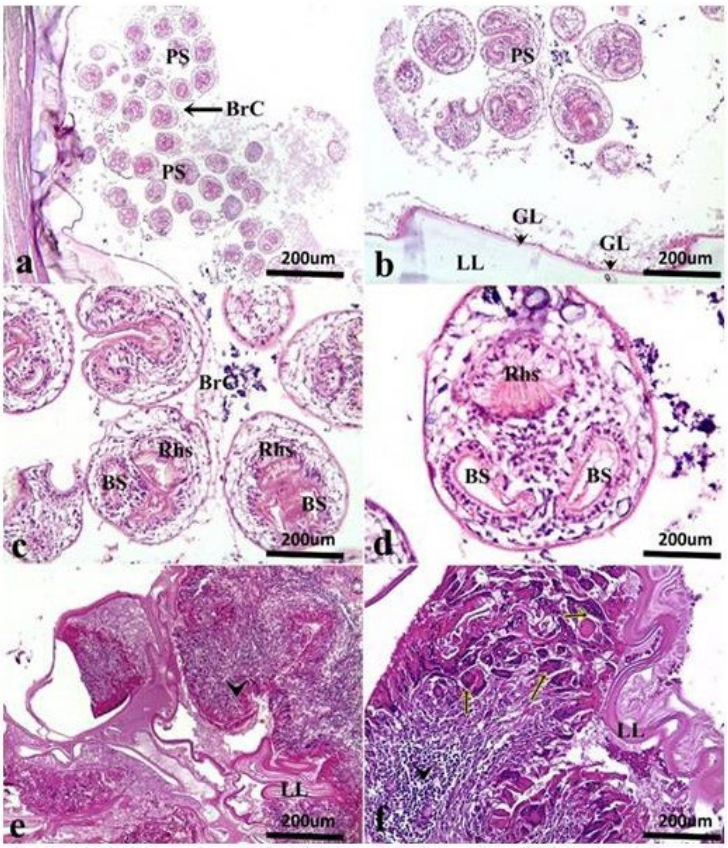

Fig. 2. Microscopical Examination of the hydatid cyst. Lung tissue; fertile *E. granulosus* (a-d); (a) daughter cysts (Brood capsule; BrC) with several protoscolices (PS). (b) E. granulosa cyst wall showing laminated acellular layer (LL) that is lined by germinal layer (GL) and protoscolices (PS). (c) Protoscolices attached to the germinal layer had central suckers with refractile hooklets (Rhs) and bilateral suckers (BS). (d) Higher magnification of PS. (e-f) infertile E. granulosa; (e) is surrounded by severe inflammatory cell infiltration (arrowhead). (f) cyst LL is surrounded by multiple giant cells (yellow arrows) and lymphocytes (arrowheads). H&E stain X4,10,20,40, 10, and 20, respectively. scale bar 200µm

the hydatid cysts in slaughtered and inspected cattle in the current study were 47800 EGP. The condemnation of the liver resulted in an economic loss of 39600 EGP. While the deficits after condemning the lungs were 8200 EGP (Table 4).

DISCUSSION

Taeniasis is a parasitic disease spread through food that has serious public health implications. The hydatid cyst develops in the internal organs (primarily the lungs and liver) of intermediate hosts and causes cystic echinococcosis. The current study focused on the gross examination of hydatid cysts from slaughtered food animals in different abattoirs in the Minoufyia Governorate, Egypt, particularly the internal organs (liver, lung, and kidney). The infection rate of hydatid cysts was 1.45%. This is lower than the reported infection rates by Barghash *et al.* (2017) at 14.4%, Roostaei *et al.* (2017) at 3%, Tolosa (2009) at 31.44%, and Hailu *et al.* (2019) at 7.39% of the inspected carcasses. While Elmonir *et al.* (2015) did not report any hydatid cysts in any animal group. This could be due to differences in ecological location and animal-rearing systems, resulting in varying degrees of exposure to infective eggs in dog feces. The prevalence in different animal carcasses was 1.33% (62/4670) in cattle, 0.71% (7/989) in buffaloes, 1.5% (5/537) in sheep, 9.5% (21/221) in camels, and 0% (0/133) in goats. For cattle, our prevalence is consistent with Hassanin *et al.* (2013) at 1.83% and Banda *et al.* (2013) at 2.1%. The prevalence is lower than Elmajdoub and Rahman (2014) at 10.56%, Azlaf and Dakkak (2006) at 56%, and Roostaei *et al.* (2017) at 5.3%. While higher than the reported prevalence by Borai *et al.* (2013) at 0.61%, Omar *et al.* (2013) at 0.068%, and Barghash *et al.* (2017) at 0.4%. Our buffalo prevalence is higher than the 0.20% reported by Borai *et al.* (2013) and the 0.4% reported by Barghash *et al.* (2017). While lower than the 2.34% described by Hassanin *et al.* (2013). Although Omar *et al.* (2013) stated that buffaloes were infection-free. Our reported prevalence of 9.7% for camels is consistent with Hassanin *et al.* (2013). While lower than the 12.54% recorded by Elmajdoub and Rahman (2014). The prevalence reported in this study is higher than the 3.24% reported by Borai *et al.* (2013) and the 5% reported by Omar *et al.* (2013). While lower than the 20% documented by Azlaf and Dakkak (2006). The reported prevalence for sheep is higher than the 0.40% reported by Borai *et al.* (2013). While the prevalence is lower than that reported by Roostaei *et al.* (2017) at 3%, Hassanin *et al.* (2013) at 7.57%, and Azlaf and Dakkak (2006) at 40%. For goats, we did not report any infection while other studies show a high level of infection, such as Roostaei *et al.* (2017) at 1%, Azlaf and Dakkak (2006) at 7%, and Hassanin *et al.* (2013) at 7.36%. The variances in prevalence may be attributed to dissimilarities in environmental contamination with eggs in dog feces. According to the organ distribution of cysts in this study, cysts of echinococcosis were present in the abdominal viscera of slaughtered animals mainly in the liver or lung alone; the cyst was found in the liver and lungs of one cattle in Elshohada abattoir. The liver was the mainly infected organ, followed by the lung. This finding is consistent with Eckert *et al.* (2000), who stated that the liver is the most common site of CE at 65%, followed by the lungs at 25%; Lahmar *et al.* (2012), who found hepatic cysts at a higher rate than pulmonary cysts in all species, and Azlaf and Dakkak (2006), who found the same except in cattle. According to Hassanin *et al.* (2013), the liver is the most infected organ with hydatidosis (16.6%), followed by the lung (11.11%). In addition, Omar *et al.* (2013) found a higher percentage in the liver (39.3%) than in the lungs (32.5%), and Elmajdoub and Rahman (2014) found the liver to be the extremely infected organ in sheep and cattle, with 46.03% and 52.94%, respectively. Similarly, Haemaei *et al.* (2017) recorded that 75% of livers and 17% of lungs were infected. Our result is inconsistent with Azlaf and Dakkak (2006), who reported the pulmonary hydatid cyst was higher than the liver cyst in cattle. Also, other studies recorded higher lung infection rates, such as Elmajdoub and Rahman (2014) at 55.21%, Roostaei *et al.* (2017) at 47.2%, Hailu *et al.* (2019) at 57.1% in the lung and 36.7% at the liver, and Barghash *et al.* (2017) at 66.1% in the lung and 17.1% in the liver.

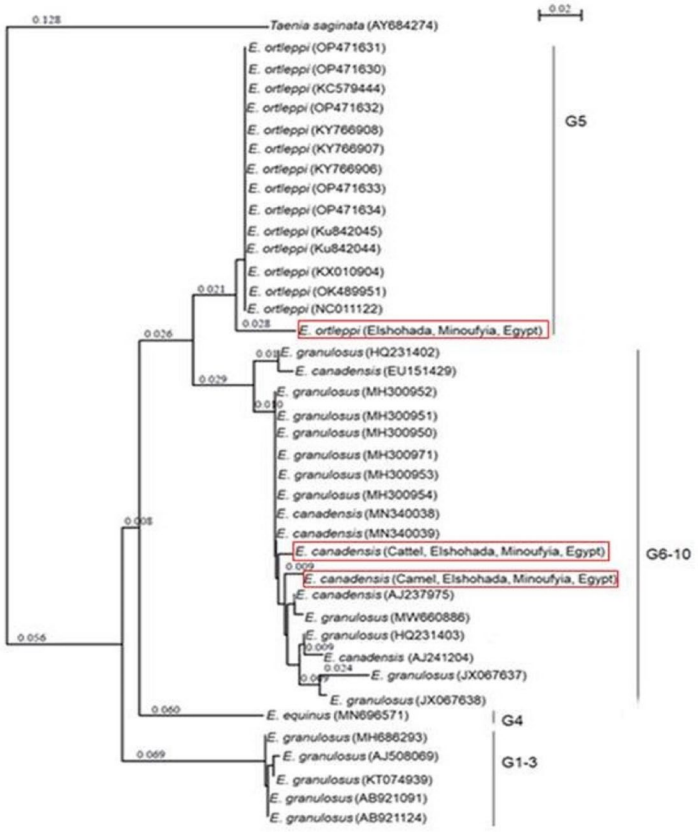

Fig. 3. Neighbor-joining phylogenetic tree of NADH-1 gene. Sequences of *E. canadensis* and *E. ortleppi* were reported from camel (LC757643) and cattle (LC758546) and buffaloes (LC758545), respectively in Minoufyia governorate, Egypt with the related ones in GenBank. The tree was drawn to scale and branch lengths were presented on the branches.

Hydatid cysts were discovered in both males and females. Female cattle over the age of 5 years had a higher prevalence of 1.061%, while male cattle had a prevalence of 0.421%. All the examined females were over the age of five, while all males were under the age of three. The prevalence of the cysts has correlated with the age of the carcass. Cattle over the age of five had the highest prevalence. This finding is consistent with those of Lahmar *et al*. (2012; Azlaf and Dakkak (2006) and Omar *et al*. (2013), who found that the prevalence was higher in older animals of different types. In this study, cysts of echinococcosis were present in the abdominal viscera of cattle and buffalo, mainly in the liver or lung alone or both organs in the same animal, as reported previously (Rinaldi *et al*. 2008; Jiménez *et al*. 2020). Winter and spring had the highest cysts prevalence in cattle at 0.195%, followed by spring at 0.17% and winter at 0.13%. Our results are coherent with those of El-Bahy *et al*. (2019), who found the greatest prevalence in winter and spring, followed by summer and autumn, at 5.56%, 5.4%, 2.16%, and 1.23%, respectively. In addition, Abd El-Aziz *et al*. (2021) found that hydatid cyst infection in sheep and cattle was greater in the winter, at 9.01% and 27.5%, respectively than in the summer, at 11.9% and 11.7%. While Shahbazi *et al*. (2016) discovered that liver and lung infections in sheep and cattle were greater in the fall, they were higher in goats in the summer. These variations could be attributed to differences in geographical distribution, environmental conditions, and farmer awareness.

The BLAST search revealed that the sequence of the camel and the cattle was *E. canadensis*, genotype 6 (G6). The buffalo sequence was *E. ortleppi*, genotype 5 (G5). *E. canadensis* NADH-1 sequence from camels had an identity percentage of 89.75% with the cattle sequence. The *E. canadensis* NADH-1 sequence from the camel of Minoufyia, Egypt had identity percentages with sequences from cysts from different animals in different countries of up to 99.37%. Also, the sequences of *E. canadensis* from camels and cattle occur in the same clade with the sequences of the genotypes 6-10 (G6-10) of *E. granulosus* complex from different countries and animals. The buffalo sequence of *E. ortleppi* occurs in the same clade with genotype 5 (G5) of the *E. granulosus* complex from different countries and animals. Therefore, the sequence and phylogenetic analysis confirm the species reported in this study are *E. canadensis* in camels and cattle and *E. ortleppi* in buffalo. Our results are consistent with the most reported species in camels, *E. canadensis*, similar to Amer *et al*. (2015). Interestingly, the cyst species found in cattle was *E. canadensis*, similar to those that were recorded in Sudan (MH300940 and MH300944–MH300946) by Laurimae *et al*. (2018) and Nigeria (MT166289) by Ohiolei *et al*. (2020). The infection of buffalo with the cattle strain is common and consistent with the reported sequences in Egypt (MK492617) and Mozambique (MZ254630–MZ254636 and MZ254638–MZ254641) by Miambo *et al*. (2022).

The cysts varied in size, with conglomerates of multiple small cysts forming large lesions that replaced tissue parenchyma (Jiménez *et al*., 2020). The host response around the cyst includes inflammatory cell infiltration and a fibrous connective tissue capsule (Jiménez *et al*. 2020; Reinehr *et al*. 2020). All examined cysts were fertile and had protoscolices as explained by Stoore *et al*. (2018) and Jiménez *et al*. (2020).

In the current study, the financial losses after condemning portions affected by hydatid cysts in slaughtered and inspected animals were 8200 EGP for the lungs and 39600 EGP for the livers, for a total of 47800 EGP, while the reported cost due to hydatid cysts condemnations in the three abattoirs was 18708300 EGP during the same period. Our findings were higher than those of Abd El-Aziz *et al*. (2021) in Egypt, who reported condemnation losses of 1188 and 3192 EGP, respectively.

CONCLUSION

This study reported the prevalence, histopathology, economic losses, and molecular characterization of *E. canadensis* from camels and cattle and *E. ortleppi* from buffaloes using nad-1 gene PCR from Minoufyia governorate, Egypt.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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