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Prevalence and Molecular Discrimination of the Neglected Hydatidosis in Camels and Humans, Egypt

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Abstract

Hydatidosis is a neglected zoonotic parasitic infestation caused by *Echinococcus granulosus*. The goal of this study was to assess the prevalence and molecular discrimination of a neglected hydatidosis from camels and humans in Egypt. The hydatid cysts macroscopically and microscopically investigated and for cyst fertility. PCR and DNA sequencing used for molecular identification. A total of slaughtered camels (1220) had an overall infestation rate of hydatidosis of 8.7%. The lung was the most often infected organ (6.9 %,) followed by the liver (1.8%). Spring and summer showed the highest infestation rate (3.03 and 2.55%) than autumn and winter (1.98 and 1.23%) seasons. The hydatid cysts' fertility rate was 65.5%. PCR using amplified *cox1* gen revealed that all human and camel hydrated cysts and only one camel sera were positive at 450 bp, The phylogenetic analysis showed that human and camel isolates exhibited high homology (95-100%) with reference sequences of *Echinococcus granulosous* G6 (camel strain) in GenBank (KU359037, KU220240, MW173484 and MW173485). The obtained results demonstrate the high prevalence of hydatid cysts in camels and reflect the spread of the infection from dogs (final host), to camels and humans (intermediate hosts) in Egypt. The strong genotyping homology between the studied camel and human hydatid cyst samples with the *E. granulosous* camel strain (G6) indicates the necessity for a bigger investigation that analyzes several hydatid cyst isolates from various geographic locations.

Keywords: Hydatid cysts**;** prevalence; genotyping; camels; humans.

1. Introduction

Hydatidosis or Cystic echinococcosis (CE), caused by the larval stage of *E. granulosus*, is an economically important global zoonotic infection that constitutes a threat to public health in many countries [1].Two hosts are necessary for the life cycle of *E. granulosus*: an ultimate (carnivore) such as dogs and an intermediate (herbivore), such as camels, sheep, and cattle [2]. Eating food contaminated with parasite eggs generated in the environment by infected definitive hosts or on a dog's coat can infect humans and intermediate hosts [3]. The highly resistant echinococcus eggs can live in the environment for up to eight months before being washed away, transported by flies and other vectors, or adhering to shoes, animal feet, or other surfaces. This can lead to a more extensive distribution and contamination of the environment, including dwellings [4]. The organs and tissues of intermediate hosts are habitats to hydatid cysts which mostly found lungs of camels and livers of cattle and sheep, while in human detected in lungs, liver, spleen and brain. The hydatid cysts filled with fluid, which contains nutrients and other elements necessary for the development of the larval cyst and the surrounding fibrous capsule, composed of aggregates of plasma cells and

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lymphocytes and a thick layer of connective tissue, formed because of the inflammatory response between the parasite and the host [5].

Hydatidosis is a serious illness that affects both humans and livestock, since postmortem inspection is mostly used to diagnose it in intermediate host animals and the disease is typically a symptomatic in its early stages (6). Imaging methods, such as X-rays, computed tomography (CT) and magnetic resonance imaging (MRI) and ultrasounds for the lungs and liver, are crucial for diagnosing human hydatid cysts since they are portable and reasonably priced. Both methods are employed for follow-up, population screening, and diagnosis [7]. The socio-economic impact is considerably high since both man and livestock are involved as intermediate hosts [8]. The economic losses in animal production as lowered meat, milk and wool production and health hazards along with treatment costs of cystic echinococcosis in man are enormous [9]. Hydatid cysts can be spread in different organs of the host such as the liver, lung, heart, spleen and brain that may result in death [10]. In Egypt, infested cases involve humans and livestock animals, particularly camels. Because hydatidosis has no symptoms, it typically identified in necropsy, lung and liver hydatidosis injuries in livestock animals cause economic loss due to the condemnation of tissues [11].

Hydatid cyst infections are one of the many illnesses that affect domestic animals, including camels. These infections are significant since they lower the animals' production [12]. The potential for camels to act as disease vectors are extremely concerning due to many factors, including the expanding camel herds in wildlife with non-domestic species, the lack of biosecurity laws and biosafety in many areas, the growing human demand for meat, particularly in Egypt and other African and Asian countries, and others [13]. Camels (*Camelus dromedaries*) mainly found in hot, dry regions such as the Middle East, Africa, and India. There are more than 37 million camels in the world, and they provide a significant amount of milk and meat in many marginal and desert areas [14]. Hydatid cysts influence 1–220 people per 100,000 people, depending on the region. The disease is prevalent in Argentina, Australia, China, New Zealand, Eastern Europe, Eastern Africa, and the Mediterranean and Middle East regions [15]. Roughly, 110 million people are living in the North African nation of Egypt. Approximately 57% of Egyptians live in rural areas, and 43% live in urbanizing cities [16]. Egypt boasts a huge agricultural industry, comfortable temperatures, and an amazing biodiversity. Many diseases, including parasites like the endemic Echinococcus granulosus parasite in Egypt, can spread easily in these conditions [17].

A morphological examination of the hydatid cysts was conducted to ascertain their size, shape, vitality, and state. The usual morphology of hydatid cysts appears to have an exterior layer and an inner germinal layer, as well as cellular infiltration and a fibrous tissue capsule around the liver and lung tissue afflicted by the infection [18]. Several investigations have been carried out to enhance the effectiveness of diagnostic examinations and enable the identification of low concentrations of antibodies against cystic echinococcosis. To improve the specificity and sensitivity of these serological approaches for the early diagnosis and confirmation of the disease, antigens must be purified [19]. The serologica diagnostic tests' use of crude hydatid cyst antigens is insufficient. Thus, to rule out the other cross-reactive proteins, the isolation of hydatid cyst antigens is required [20]. Immunodiagnosis is helpful in the early detection of infectious diseases, and in the case of cystic echinococcosis helpful in the post-treatment follow-up of patients [21]. It was thought that *E. granulosus* belonged to a single species until recently. It is now recognized, however, to have a significant genetic variety since different genotypes and strains show different patterns of immunization, treatment reactions, and disease [22]. Molecular characterization resulted in ten numerous genotypes (G1-G10) that have been described in the world based on nucleotide sequence analysis of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*), ADH dehydrogenase subunit 1 (*ND1*) genes and intra-transcribed spacer 1 rDNA (ITS1), 12S rDNA, and nuclear actin II (*ACT II*) genes. These different genotypes have been associated with distinct, intermediate hosts sheep, pigs, cattle, horses, camels, goats and cervides [23]. *E. granulosus* is a complex of distinct strains that vary in a wide range of parameters, hence influencing the epidemiology and management of CE. The camel (G6) strain was shown to be involved in human infection through molecular characterisation of human and animal isolates, despite some studies suggesting that the sheep (G1) strain was the most significant strain linked to human CE [24].

Cystic hydatid disease is a major worldwide illness among the most neglected zoonotic parasitic diseases that affects both humans and animals caused by numerous strains s of *Echinococcus granulosus* isolates [25]. Thus, the objective of this study was to investigate the occurrence of hydatiditosis in humans and camels in Egypt, with particular reference to the morphological and macroscopic examination of hydatid cysts in infected tissues and the molecular identification of isolated human and camel hydatid

cysts using PCR and DNA sequencing to assess their zoonotic significance.

Material and Methods

1.1. Study area

Between December 2022 and November 2023, an epidemiological survey was carried out to determine the yearly prevalence of hydatidosis in camels. Slaughter of surveyed camels in the Kom-Hamada slaughterhouse in Beheira, Governorate which is situated on Egypt's west coast (X/Y coordinates: 30.343551/ 30.848099), served as the study's location. Human samples were taken from individuals complaining of stomach problems, fever, and abdominal pain at private clinics in the Beheira Governorates, as well as from the outpatient clinic at Kasr Alainy Hospital, hepatology division of Endemic Medicine Department, Faculty of Medicine, Cairo University

1.2. Tissue and blood sample

During postmortem examination, the hydatid cysts collected from the livers and lungs of camels that have slaughtered and carefully examined using standard meat inspection techniques. Fluid from human hydatid cysts aspirated from infected patients during a therapeutic operation before the aseptic injection of any scolicidal medication using Puncture, Aspiration, Injection and Re-aspiration technique (PAIR).

Camel blood samples had been taken at the time of slaughter; positive samples were obtained from infected camels that had lung cysts, while negative samples were collected from healthy camels that had no cysts, as determined by a veterinarian inspection following the slaughter. Human blood samples were chosen based on medical history, and laboratory tests. Negative blood samples from healthy individuals who did not have hydatidosis were IHAT negative and positive samples from patients who had been diagnosed with the disease by a sonographer.

1.3. Sample preparation

Positive and negative camel and human blood samples were collected and aliquoted in 1.5 ml Eppendorf tubes then labeled and stored at -20 ^{o}C until used in the molecular analysis [26].

To verify the fertility of each collected camel hydatid cyst, a drop of cyst fluid was inspected for the presence of protoscolices after the cyst's contents were cut open. The cyst was regarded as sterile since it lacked protoscolices and had pus that had either calcified or degenerated [27]. The biological characteristic of protoscolices' muscle activity (invagination and evagination), as seen by direct microscope examination at x 40, was used to assess the vitality and fertility of the recently obtained protoscolices. Furthermore, the vital stain (Eosin dye) was employed to determine the vitality of protoscolices indirectly [28]. The aspirated human or camel hydatid fluid was centrifuged for 10 minutes at 400xg, and the sediment was then examined under a light microscope for the presence of protoscolices [29].

1.4. Molecular identification

Molecular analysis has been used to characterize the genetic structure of isolated hydatid cyst species and assessment of their zoonotic significance using conventional polymerase chain reaction (PCR) and DNA sequencing [30]. Twenty (13 camels and 7 humans) samples of protoscolices, germinal layers and external layers from camel and human hydatid cysts. Tissue samples were rinsed in physiological saline, transferred into sterile tubes (each from a single cyst) and fixed in 70% ethanol. Blood samples from *E.granulosus* serologically positive and negative human and camels were also collected (Table 1). The positive control specimen prepared from purified hydatid cyst protoscolices from infected camel.

Table 1: Hydatid cyst and blood samples collected from human and camel

Sample No.	Species	Type
H1, H2 and H3	Camel	HC protoscoleces
H4, H5 and H6	Camel	HC Germinal layer
H7, H8 and H9	Camel	HC Outer layer
H10, H11 and H12	Human	HC protoscoleces
H13, H14, H15 and H16	Camel	Positive Serum
H17, H18, H19 and H20	Human	Positive Serum

1.4.1. DNA extraction

All hydatid cyst samples were kept until DNA extraction in 70% ethanol. Using the MagMAXTM CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific), 5823 Newton Drive, Carlsbad, California, USA). samples were pre-digested and DNA was extracted, eluted in 50 µL of the elution buffer provided in the kit, using 200 µl of blood samples or 25 mg of hydatid cyst samples (external layer, internal germinal layer, and protoscoleces) as per manual instructions with various changes. Every sample (isolate) will be a pool taken from a single cyst [31].

1.4.2. Primers

The forward primer 5'-TTTTTGGGCATCCTGAGGTTTAT-3' and the reverse primer 5'-TAAAGAAAGAACATAATGAAAATG-3' (Creative Biogene, Shirley, NY 11967, USA) were used to amplify the mitochondrial *cox1* gene in accordance with the protocol that was described. This amplifies specific markers for the various genotypes within Echinococcus through PCR, using a 25µl reaction mixture that contains 12.5 µl of 2x COSMO PCR RED Master Mix (Azura Genomics Inc, Massachusetts, USA), 0.5 µL of each primer and 2 µL of target DNA [32].

1.4.3. Agarose gel electrophoresis

On a 1.5% agarose gel, the PCR products were resolved and stained with 10 mg/ml ethidium bromide (*Infitek Co., Ltd.* Ligaoguojihuayuan, Lixia District, Jinan, Shandong, China). The PCR results, which were predicted to be 450 bp, were inspected using the InGenius3 gel documentation system and assessed at 100V for one using a 100 bp ladder plus (*Sigma–Aldrich*, St. Louis, MO, USA). [33]. For maximum band resolution and repeatability, the parameters for Single-strand conformation polymorphism (SSCP) electrophoresis have been established. The gels will then be dried on blotting paper and auto-radio-graphically examined [34].

1.4.4. Phylogenetic tree construction

The QIAquick PCR Purification Kit (Cat. no. 28104. QIAGEN, Germany) will be utilized for the purification of amplification products. Using an Applied Biosystems Big Dye Terminator Kit, automated sequencing will be used to ascertain the sequences (Perkin Elmer, Norwalk, CT, USA) The Genetics Computer Group Sequence Analysis Software Package was utilized to sequence the *cox1* gene based on the positive PCR results obtained [35].

2. Results

2.1. Prevalence of hydatidosis in slaughtered camels

Examination of 1220 camels, slaughtered during the routine postmortem inspection, showed that a total of 106 were infected with hydatid cysts with an overall prevalence of 8.7%. Concerning the site of infestation, the lungs revealed the highest prevalence of 6.9% (84/1220) while the liver showed the lowest prevalence of 1.8% (22/1220). The examined camels showed the highest infestation rate during spring (3.03%) followed by summer season (2.55%) then autumn (1.98%), and the lowest infestation rate in Winter season (1.23%) (Table 2).

Table 2: Prevalence rate of hydatidosis among slaughtered camels

Exam. camels	Infested Liver		Infested Lung		Total infested	
	No.	%	No.	%	No.	%
Winter	3	0.25	12	0.99	15	1.23
Spring	8	0.65	29	2.4	37	3.03
Summer	6	0.50	25	2.04	31	2.55
Autumn	5	0.40	18	1.46	23	1.89
Total prev.	22	**1.8 %**	84	**6.9 %**	106	**8.7 %**

Macroscopic investigation of hydatid cysts in both camel liver and lung by visual examination and palpation for detection of mature fertile hydatid cysts (Figure 1 a & b).

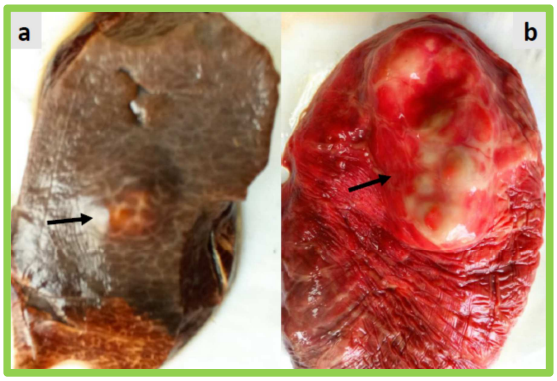

Figure 1: mature fertile hydatid cysts (black arrows) in camel liver (**a**) and lung (**b**)

Microscopic examination of aspirated hydatid internal fluid for the presence of protoscolices in fertile cysts (Figure 2 a & b).

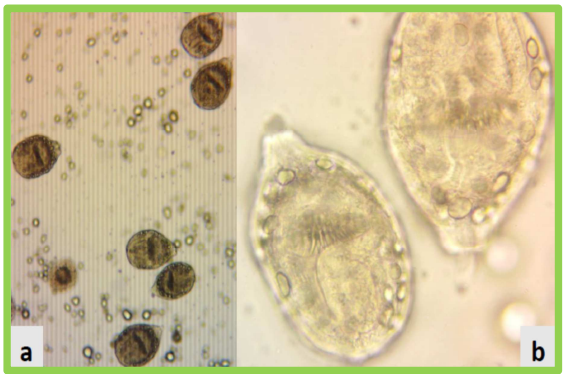

Figure 2: protoscolices of fertile hydatid cysts, x10 (**a**) and x40 (**b**).

The fertility rate of hydatid cysts was 45% and 69% in camel liver and lung, respectively with an overall hydatid cysts fertility rate of 65.5% (Figure 3).

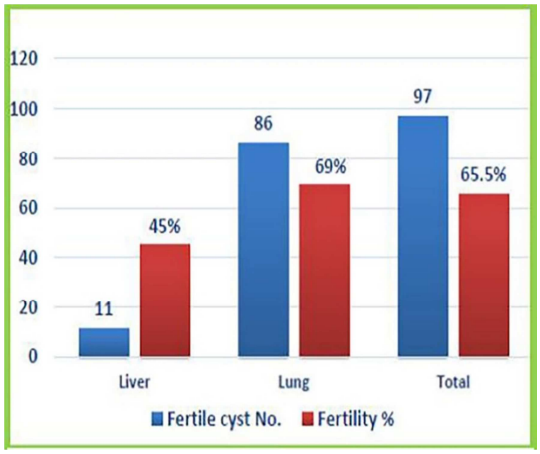

Figure 3: fertility rate of camel hydatid cysts

2.2. *Molecular characterization*

2.2.1. Amplification of the mitochondrial cox1 gene

All human and camel cyst samples (H1-H12) and only one camel blood sample (H13) were PCR positive at 450 bp using amplified *cox1* gen, while the rest of the blood samples were negative (H14-H20) (Figure 4).

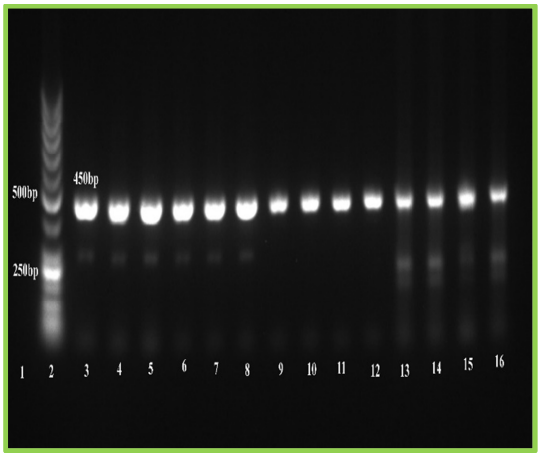

Figure 4: PCR analysis of the *cox1* gene. Lane 1: negative control, lane 2: 100 bp ladder, lane 3: positive control and lanes 4-16: positive human and camel hydatid cyst samples (450 bp)

2.2.2. Phylogenetic analysis

Three Human isolates nucleotide sequences generated in this study were deposited in GenBank under accession numbers MZ348904, MZ348905 and MZ348906 and three camel isolates nucleotide under accession numbers MZ348907 MZ348908 and MZ348909 for cytochrome c oxidase subunit I cox_1 sequences.

Regarding the *E .granulosus* species, the BLAST analysis showed that our human and camel isolate of *cox1* sequence (from MZ348904 to MZ348909) exhibited high homology (95%) with *E. granulosus* isolates (KU202240, KU359037, HF947565), while reference isolates from other species in the GenBank database (*E. canadensis , E. ortleppi , E. equinus, E. multilocularis*) formed other clades (Figure 5).

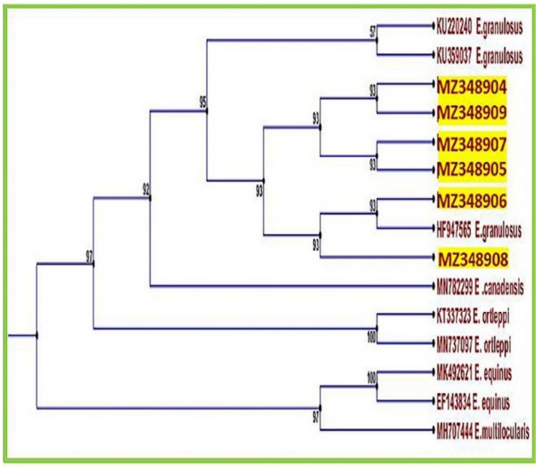

Figure 5: phylogenetic tree of representative sequences of *E. granulosus* (MZ348904 to MZ348909) isolated from human and camel in Egypt, exhibited high homology (95%) with other *E. granulosus* isolates

According to the phylogenetic tree, our isolates, were belongs to *E. granulosous* G6 (camel strain) and overall isolates sequences of mtDNA indicated 95 % homology with references G6 sequences in the GenBank database (KU359037 , KU220240) (Figure 6).

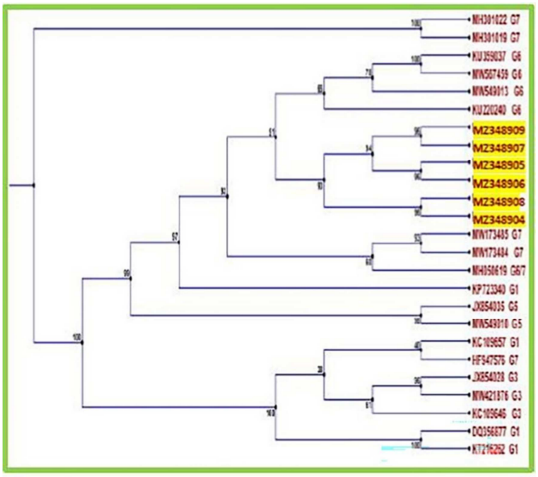

Figure 6: Phylogenetic tree of representative sequences of *E. granulosus* (MZ348904 to MZ348909) isolated from human and camel in Egypt, exhibited high homology (95%) with references G6 sequences

The BLAST analysis showed that human and camel isolate of *cox1* sequence exhibited high homology of 100% with reference sequences of *E. granulosus* in GenBank that were collected from camel (MW173484 , MW173485) (Figure 7).

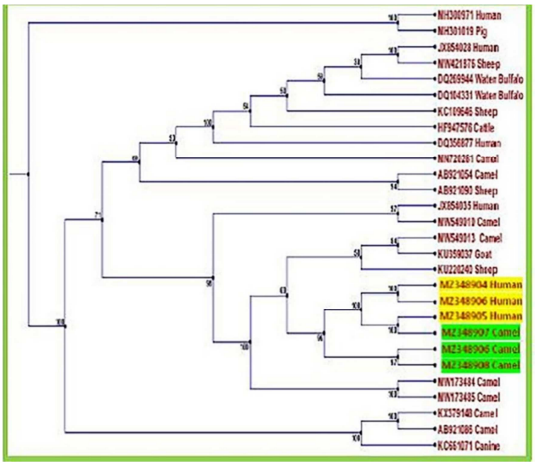

Figure 7: Phylogenetic tree of representative sequences of *E. granulosus* (MZ348904 to MZ348909) isolated from human and camel in Egypt, exhibited high homology (100%) with reference sequences of camel isolated *E. granulosus* deposited in GenBank

3. Discussion

Hydatidosis prevalence rates were epizootiological characteristics associated with infection rates in the intermediate host reservoir livestock [36]. In this study, the overall infestation rate of hydatidosis was 8.7% in camels slaughtered during the routine postmortem inspection at Kom Hamada abattoir, Beheira Governorate Egypt. These obtained results were similar to those of 8.32% in camels slaughtered at in Aswan Governorate, Southern Egypt [37] and nearly closed to 9.64% reported in slaughtered camels in Iraq [38]. In contrast, a significantly lower camel hydatidosis in Egypt, 1.7 % [39]. The variation in the prevalence of hydatidosis infection in camels throughout different countries may attributed to various factors, such as the implementation of control measures, the degree of community awareness regarding the disease, the educational and economic standing of the populace, the farming community, temperature variations [40]. Furthermore, the kind of pasture, exposure levels, having a dog, the final host of *E. granulosus*, camel and the camel's owner come into contact with the dog's eggs, and viability of eggs could aid in the disease's transmission [2].

The liver and lungs were the only organs affected concerning cysts' organ distribution. Otherwise, the current study's distribution of hydatidosis throughout the affected organs showed that the lung was the most often infected organ, with a n incidence of infection of 6.9%, followed by the liver at 1.8%. These findings agreed with a many of previous research [37, 39] they established that camels' lungs contained a higher mean number of hydatid cysts than their liver, along with the majority of favoring seats. Lung inclined for camel hydatidosis may cause by the lung tissue's soft, smooth texture, which encourages the cyst to grow more quickly. When parasite oncospheres are present, it is difficult for them to continuously multiply due to the stiff and inflexible nature of camel liver tissue [38].

A highest infestation rate in this investigation were detected during spring (3.03%) followed by summer season (2.55%) then autumn (1.98%), and the lowest infestation rate in winter season (1.23%). Similar results had notably that the high prevalence of hydatid cysts observed in spring and summer followed by autumn and winter [39, 41]. On the other hand, other studies revealed that winter had the highest rate of hydatidosis infection followed by autumn while, a lower rate of hydatidosis infection was reported in summer and spring (9, 37). In general, the climate has an impact on the incidence of hydatidosis in camels; data on seasonal prevalence indicated a year-round presence of the disease with non-significant statistical changes, and seasonal fluctuations could not be particularly recognized as a risk factor [42]. An accurate assessment of a species' potential to infect dogs can be done using data on the number of calcified, sterile, and fertile cysts in camels [18].

In this study, the macroscopic and microscopic investigation of hydatid cysts, showed fertility rate of 45% and 69% in camel liver and lung, respectively with overall fertility rate of 65.5%. These findings were consistent with other previous results [37, 43]. Conversely, the percentage of fertile cysts in the liver was higher than in the lung, and interestingly, the fertility rate of a single cyst was higher than that of several cysts [41, 44]. These differences ascribed to the variety of protoscolices origins, environmental variables, incubation temperature, the amount of time that elapsed between collecting and handling of the sample, and the criteria employed to assess reproductive viability [38].

Ten genotypes (G1 to G10) have recognized using mitochondrial DNA sequencing, and these genotypes have accurately identified in molecular epidemiological surveys of *E. granulosus* in various geographic contexts and host habitats [9]. Although, *E. granulosus,* it was formerly believed to be a single species, it is now known to have significant genetic variety since different genotypes and strains show different disease patterns, treatment responses, and vaccination histories [22]. The molecular analysis in

this work, revealed that all human and camel hydrated cyst samples and only one camel blood were given positive result at 450 bp by amplified using *cox1* gen and phylogenetic analysis exhibited high homology (95-100%) with reference sequences of *E. granulosous* G6 (camel strain) in GenBank. Similar study was identical to those of the G6 *E. granulosus sensu lato* genotype strain detected in camels from Sudan and Iran [45]. In addition, our results agreed with the previous reports, assuming that G6 is widespread in camel-raising countries of Africa, Asia, and the Middle East [46]. In Egypt, a considerable number of molecular studies have been performed to characterize hydatid cysts from several intermediate hosts by PCR amplification and sequencing of mitochondrial markers (cox1, 12S rRNA and nad1) [23]. The majority of camels slaughtered at abattoirs in Egyptian governorates are imported from Sudan where G6 infections have been reported in camels [9], it is likely to identify the same genotype in Egypt. Only three genotypes (G1, G6 and G7) have been identified from the Egyptian population and G6 was the dominant genotype among human isolates [47].

4. **Conclusion**

The current study indicates elevated neglected zoonotic hydatidosis among salaughtered camels from Beheira, governorates, Egypt. This obtained finding reflects the spread of the infection from dogs (final host), to camels and humans (intermediate hosts) in Egypt. The strong genotyping homology between the studied camel and human hydatid cyst samples with the *Echinococcus granulosous* camel strain (G6), indicates the necessity for a bigger investigation that analyzes several hydatid cyst isolates from various geographic locations.

5. Ethical approval

All experimental methods were carried out in compliance with ethical protocol no.18/234, the institutional rules established by the Animal Research Committee of the National Research Centre, Egypt.

6. **Conflict of interest**

The authors have declared no conflict of interest.

7. **Acknowledgments**

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