

Hydatidosis in Menoufia's three Abattoirs, Egypt: Epidemiological, Histopathological, Economic, and Molecular Insights in Cattle and Buffaloes

Sally Abdelrahem Mohamed, Nasr Mawoud Elbahy, Ahmed Osman Elkhatam*

Department of parasitology, Faculty of veterinary medicine, University of Sadat City.

*Corresponding author: ahmed.osman@vet.usc.edu.eg Received: 2/12/2023 Accepted: 14/1/2024

ABSTRACT

This study aimed to assess the prevalence, economic losses, molecular characterization, and histopathology of hydatid cysts in slaughtered cattle and buffaloes across three abattoirs (Al-Shuhada, Ashmoun, and Khalij Al-Arab) in Menoufia governorate, Egypt over two years from March 2021 to May 2023. The overall prevalence of hydatid cysts was 0.96%, with cattle exhibiting a substantially higher prevalence rate (1.05%) compared to buffaloes (0.55%). The higher prevalence rate was observed in older female cattle (1.90%). The liver exhibited a greater predilection site for infection (0.63%) versus the lungs (0.33%). Our study estimated economic losses due to organ condemnation totaled 50600 EGP. Morphological and histopathological examinations were recorded. PCR and Sequence analysis of NADH dehydrogenase gene showed that the sequence of hydatid cyst from slaughtered cattle in the present study was *E. orteppi*. It had high identity percent (93.33%-99.57%) with sequences of *E. orteppi* from Egypt, Sudan, Namibia, India, China, Japan, Germany, Ukraine and Argentina and occurred in the same clade. According to these results, hydatidosis poses a significant veterinary and economic risk to the cow and buffalo populations under study in Egypt, necessitating better preventive and control measures.

Keywords: Hydatid cyst, Prevalence, Economic impact, histopathology, PCR, Menoufia.

INTRODUCTION

Hydatidosis is a parasitic disease that has significant consequences for both Veterinary Medicine and public health worldwide (Mathivathani et al., 2023). The disease is caused by the metacestode larval stage or hydatid cyst of the tapeworm *Echinococcus granulosus*, which belongs to the family Taeniidae (El Hassan and El-Bahr, 2022). The adult *E. granulosus* resides in the small intestines of definitive hosts, which include dogs and other canids. Gravid proglottids release eggs in the feces, which

contaminate the environment. Intermediate hosts such as livestock, wild herbivores, and humans ingest the eggs accidentally, leading to the development of hydatid cysts in internal organs like the liver, lungs, kidney and rarely other sites (Njoroge et al., 2000; Moro and Schantz, 2009; Boufana et al., 2015).

The global distribution of cystic hydatidosis mirrors that of the definitive canid hosts and correlates to regions with extensive livestock production utilizing open grazing practices (Craig and Larrieu, 2006;

Moro and Schantz, 2006; Ibrahim et al., 2016). Hyperendemic regions include the Mediterranean littoral, northern and eastern Africa, the Middle East, China, Central Asia, Russia, South America, Australia and New Zealand (Deplazes et al., 2017). The annual global livestock-associated economic losses are estimated at over US\$ 141,605,195 (Jafer et al., 2020). Cysts reduce meat, milk and wool production. Condemnation of offal and carcasses further escalates the economic detriments and compromises food safety (Torgerson and Dowling, 2001). In hyperendemic developing regions, human infection rates can be as high as 5-10% in some communities (Moro et al., 2011). In Egypt, previous studies noted hydatidosis prevalence up to 31.4% in slaughtered herbivores (Haridy et al., 2000). However, there have been limited recent comprehensive analyses of hydatidosis covering aspects like genetic diversity, detailed organ tropism, risk factors and economic impact in Egyptian livestock.

The pathogenesis of hydatid cysts encompasses two phases - early acute phase due to rapid cyst enlargement and a late chronic phase associated with slow growth and cyst degeneration (Rinaldi et al., 2014). The early phase manifests as an inflammatory reaction modulated by host immunity and the parasite's development. The chronic phase develops as the parasite evades host defenses through various mechanisms like antigen B-mediated inhibition of neutrophil chemotaxis and suppression of dendritic cell maturation (Nono et al., 2012). Other evasive strategies include molecular mimicry to evade immune detection and manipulation of host E-cadherin for reduced immunogenicity (Brehm, 2010; Hemer et al., 2014). A key feature is cysts' laminated acellular and germinal epithelium lining that protects the protoscolices (Soleymani et al., 2021). The immune response can lead to caseation,

fibrosis, and eventual cyst degeneration with time. Calcification indicates dead cysts (Rinaldi et al., 2014). In some cases, hydatid cysts are found only in the lungs or liver, while in others, they are present in both organs (Armiñanzas et al., 2015). The clinical course of cattle hydatidosis varies depending on the location and characteristics of the cysts. Clinical signs and symptoms may be related to the mass effect of the cyst, its superinfection, or anaphylactic reactions secondary to its rupture (Boukhatem and Bouzar kouadri, 2017).

E. granulosus demonstrates considerable genetic diversity, characterized by distinct genotypes (G1-G10) with differing host specificities and geographical distributions (Nakao et al., 2013). The predominant strains include *E. granulosus* sensu stricto (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6-G10) (Romig et al., 2015). The diversity of strains contributes to the complex epidemiology and transmission patterns of hydatidosis worldwide.

This study aimed to investigate the current epidemiological, financial loss, histopathology, molecular and morphological characteristics of hydatid cysts in slaughtered cattle and buffaloes from three abattoirs in the Menoufia governorate of Egypt over two years.

MATERIALS AND METHODS

1. Sample Collection

This study was conducted from March 2021 to March 2023 to determine the prevalence of hydatid cysts in slaughtered animals at 3 abattoirs (Al-Shuhada, Ashmoun, and Bay Al-Arab) in Menoufia Province, Egypt. Complete postmortem inspection was conducted on 4791 carcasses, including cattle (N=3890) and buffaloes (N=901). The cattle comprised 2100 males

and 1790 females aged 1.5-3 years and 5-15 years, respectively. The buffaloes included 486 males and 415 females aged 1.5-3 years and 5-10, respectively.

2. Detection of Hydatid Cysts

The liver and lungs of each carcass were meticulously inspected by visual examination and palpation to detect the presence of hydatid cysts. The liver and lungs were visually examined. Palpation was performed to evaluate the texture and consistency of organs. The lungs were palpated by applying light pressure, covering the entire organ from apex to base. The liver was first visually examined; then, two incisions were made at right angles and diagonally to the lobes to expose the bile channels. All identified cysts were collected and stored at -80°C for subsequent molecular analysis.

3. Histopathological Examination

The excised cysts were transported to the Pathology Lab. Tanta University, Egypt. The cyst wall sections were preserved in 10% neutral buffered formalin. The liver, lung, and nearby tissues that were affected by infection were also preserved for a duration of 3 days. After fixation, the tissues were processed and embedded in paraffin blocks. Subsequently, 5µm slices were stained with hematoxylin-eosin (H&E) (Bancroft and Layton 2013).

4. Molecular Characterization

The total DNA was isolated from frozen cyst samples utilizing a commercially available extraction kit following the manufacturer's protocol. Polymerase chain reaction (PCR) was performed to amplify a 500 base pair fragment of the NADH dehydrogenase subunit 1 (NADH-1) gene using previously published PCR primers and thermal cycling conditions according (Bowles and McManus, 1993). The PCR amplicons were

analyzed by agarose gel electrophoresis and visualized under ultraviolet light after ethidium bromide staining. Bands corresponding to the expected size were excised from the gel, and the DNA was extracted using a commercial gel extraction kit. The purified NADH-1 gene fragments were sequenced by a commercial sequencing facility in both directions using the forward and reverse primers for the NADH-1 gene. The resulting NADH-1 sequences were evaluated by BLAST analysis (blast.ncbi.nlm.nih.gov) and aligned to NADH-1 sequences available in GenBank. Phylogenetic analysis and tree construction were performed utilizing the neighbor-joining method.

5. Economic Analysis

The economic loss (EL) caused by organ disapproval was determined by application of the subsequent 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)

6. Statistical Analysis

The Chi-square test using the SPSS program version 20 was employed to evaluate the correlations among animal species, locality, season, age, gender, and the occurrence of hydatid cysts. Significance was attributed to P values <0.05.

RESULTS

1. Prevalence of Hydatid cysts:

A total of 4791 slaughtered animals were thoroughly examined over two years, including 3890 cattle and 901 buffaloes from three abattoirs in Menofia governorate, Egypt. Detailed postmortem inspection was undertaken to determine the prevalence, organ distribution, and associated

histopathological and molecular characteristics of hydatid cyst infections within this population.

Out of the 4791 animals that were examined, 46 were recorded as having hydatid cyst infections, indicating an overall prevalence of 0.96% (as detailed in Table 1). Cattle exhibited a much greater infection rate of 1.05% compared to buffaloes, with an infection rate of 0.55%. This difference in infection rates was determined to have a significant statistical difference ($p < 0.05$, $\chi^2 = 8.26$). After doing a relative risk assessment, it found that cattle had a 1.9-fold increased chance of getting hydatid cyst infections compared to buffaloes (RR = 1.9, 95% CI: 1.2-3.1). These data highlight the heightened susceptibility of cattle to hydatidosis.

The infection rate of hydatid cysts for the three examined abattoirs were summarized in Table 2. The prevalence rates at the Al-Shuhada, Ashmoun, and Bay Al-Arab abattoirs for cattle were 1.14%, 1.08%, and 0.87%, respectively, while for buffaloes were 0.71%, 0.43%, and 0.41% respectively. The chi-square test analysis indicated that there were no statistically significant

variations in prevalence across the various sites for both cattle ($\chi^2 = 1.33$, $p = 0.512$) and buffaloes ($\chi^2 = 0.26$, $p = 0.874$). The data indicated a uniform geographic spread of hydatidosis in the investigated areas.

The occurrence of hydatid cysts was noticeably higher in female cattle aged 5-15 years (1.90%) than males aged 1.5-3 years (0.33%). The observed disparity demonstrated a significantly greater risk, with a 5.8-fold increased probability ($p < 0.001$, RR = 5.8). Regarding buffaloes, there was a slightly higher occurrence of 0.72% in older female animals (aged 5-10 years) than in younger males (0.41%). However, this difference was not statistically significant ($p = 0.056$, RR = 1.8) as shown in Table 3.

The prevalence of the hydatid cysts in cattle was the highest during Autumn (2.13%) and the lowest during Spring (0.82%). Also in buffaloes, the highest prevalence was seen during the Autumn season, with a prevalence rate of 0.70% and the lowest infections was recorded in spring (0.36%). There was no significance in seasonal occurrence of hydatid cysts in both cattle ($p = 0.123$) and buffaloes ($p = 0.874$) as shown in Table 4.

Table 1. Prevalence of the hydatid cyst in slaughtered cattle and buffaloes in abattoirs of Menoufia governorate, Egypt.

Animal species	No. examined	No. infected	% of infection
Cattle	3890	41	1.05
Buffaloes	901	5	0.55
Total	4791	46	0.96

Table 2. Prevalence of bovine hydatidosis during the period of 2021-2023 in 3 abattoirs of Menoufia governorate, Egypt.

Locality	Cattle				Buffaloes		
	No. examined	No. infected	% of infection	No. examined	No. infected	% of infection	
Al-Shuhada	1,835	21	1.14	425	3	0.71	
Ashmoun	1,015	11	1.08	235	1	0.43	
Khalij Arab	1,040	9	0.87	241	1	0.41	
Total	3,890	41	1.05	901	5	0.55	

Table 3. Effect of age and sex on the infection rate of hydatid cysts in slaughtered bovines from in 3 abattoirs of Menoufia governorate, Egypt.

Species	Sex	Age (years)	No. examined	No. infected	% of infection
Cattle	Female	5-15	1,790	34	1.90
Cattle	Male	1.5-3	2,100	7	0.33
Buffalo	Female	5-10	415	3	0.72
Buffalo	Male	1.5-3	486	2	0.41
Total	-	-	4,791	46	0.96

Table 4. Seasonal Prevalence of hydatid cysts in slaughtered bovines from in 3 abattoirs of Menoufia governorate, Egypt.

	Cattle			Buffaloes		
	No. examined	No. infected	% of infection	No. examined	No. infected	% of infection
Winter	1265	11	0.87	293	2	0.68
Spring	1215	10	0.82	281	1	0.36
Summer	800	7	0.88	185	1	0.54
Autumn	610	13	2.13	142	1	0.70
Total	3890	41	1.05	901	5	0.55

2. Organ distribution

Concerning the distribution of hydatid cysts in different organs of infected slaughtered animals, the most infected organ was the liver, followed by the lung, with an infection rate of 65.22% (30/46) and 34.78

% (16/46) respectively (Table 5). In cattle, the frequency of hydatid cysts was 65.85 % for the liver and 34.15 % for the lungs (Table 5). While the infection with hydatid cysts was 60 % in the liver and 40 % in the lungs of slaughtered buffaloes (Table 5).

Table 5. Organ distribution of the hydatid cysts in slaughtered cattle and buffaloes.

Organ	Cattle		Buffaloes	
	Liver	Lung	Liver	Lung
Infected	27	14	3	2
% of infection	65.85 %	34.15%	60 %	40 %
Total	41		5	

3. Histopathological examination

Macroscopic examination revealed the presence of fluid-filled hydatid cysts in the affected organs, the lungs of cattle (Fig. 1 A, B), Buffalos (Figure 1 C, D) and liver (Figure 1F). The cysts demonstrated a white laminated outer membrane enclosing the clear cyst fluid content. Small cysts were often clustered as grape-like aggregates that distorted the organ architecture. The host tissue reaction manifested as a surrounding fibrous capsule.

Microscopic analysis highlighted the laminated, a cellular outer cyst wall

composed of condensed host extracellular matrix. The inner germinal layer was cellular and nucleated. In fertile cysts, the inner layer had brood capsules and protoscolices with hooklets. Areas of inflammation, necrosis and fibrosis were discernible in the adjacent organ parenchyma. Hepatocytes adjacent to hepatic cysts exhibited atrophy and necrosis. In pulmonary cysts, the surrounding lung parenchyma collapses. Overall, these morphological findings were confirmatory of hydatid cyst infections (Figure 2).

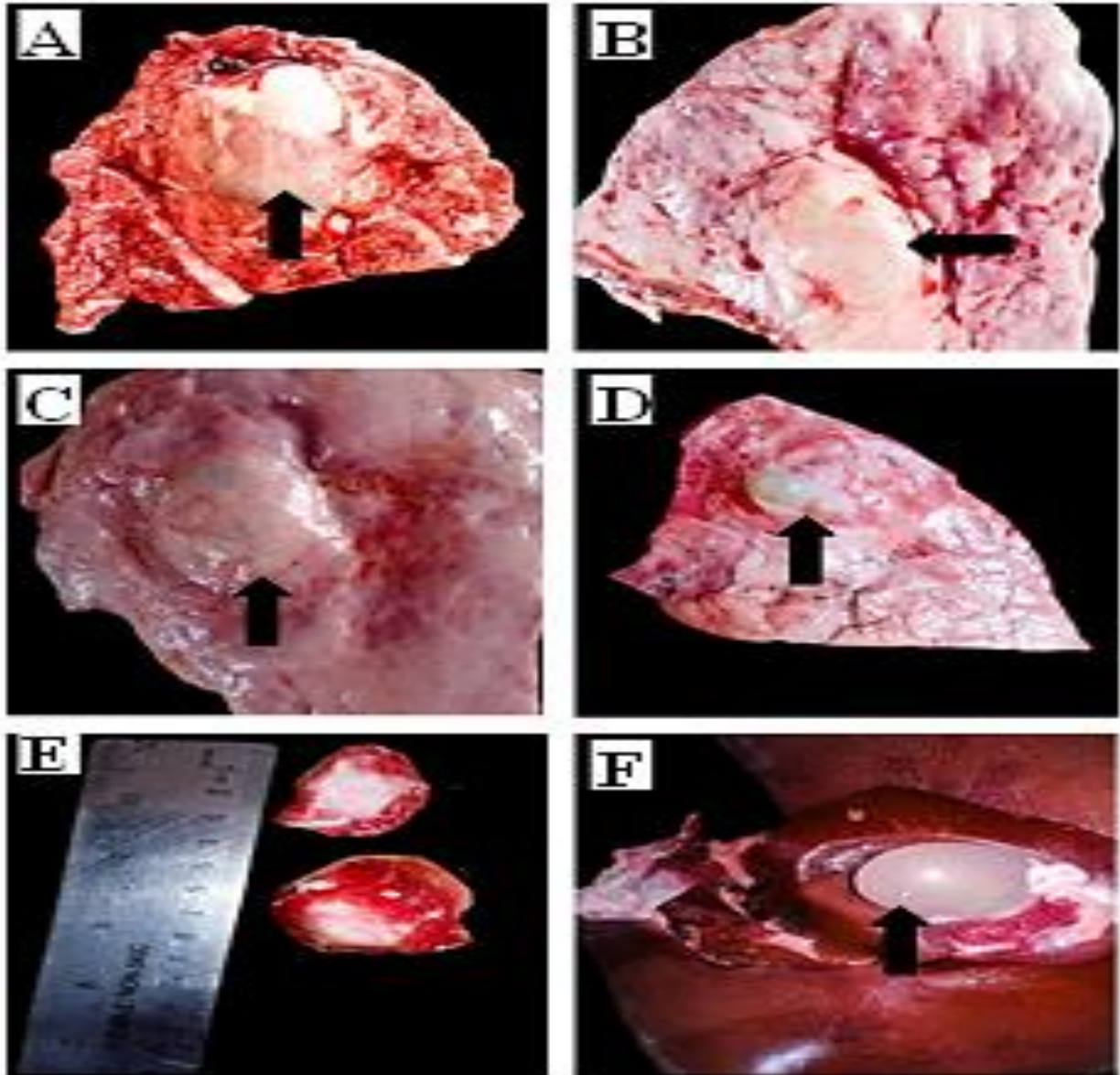


Figure 1. Gross examination of hydatid cysts. Photographs (A-D) showed different sizes of hydatid cysts in the lungs of slaughtered bovines. Photograph (E) showed excised hydatid cysts from the lungs which have spongy appearance. (F) cow's liver which has large hydatid cyst.

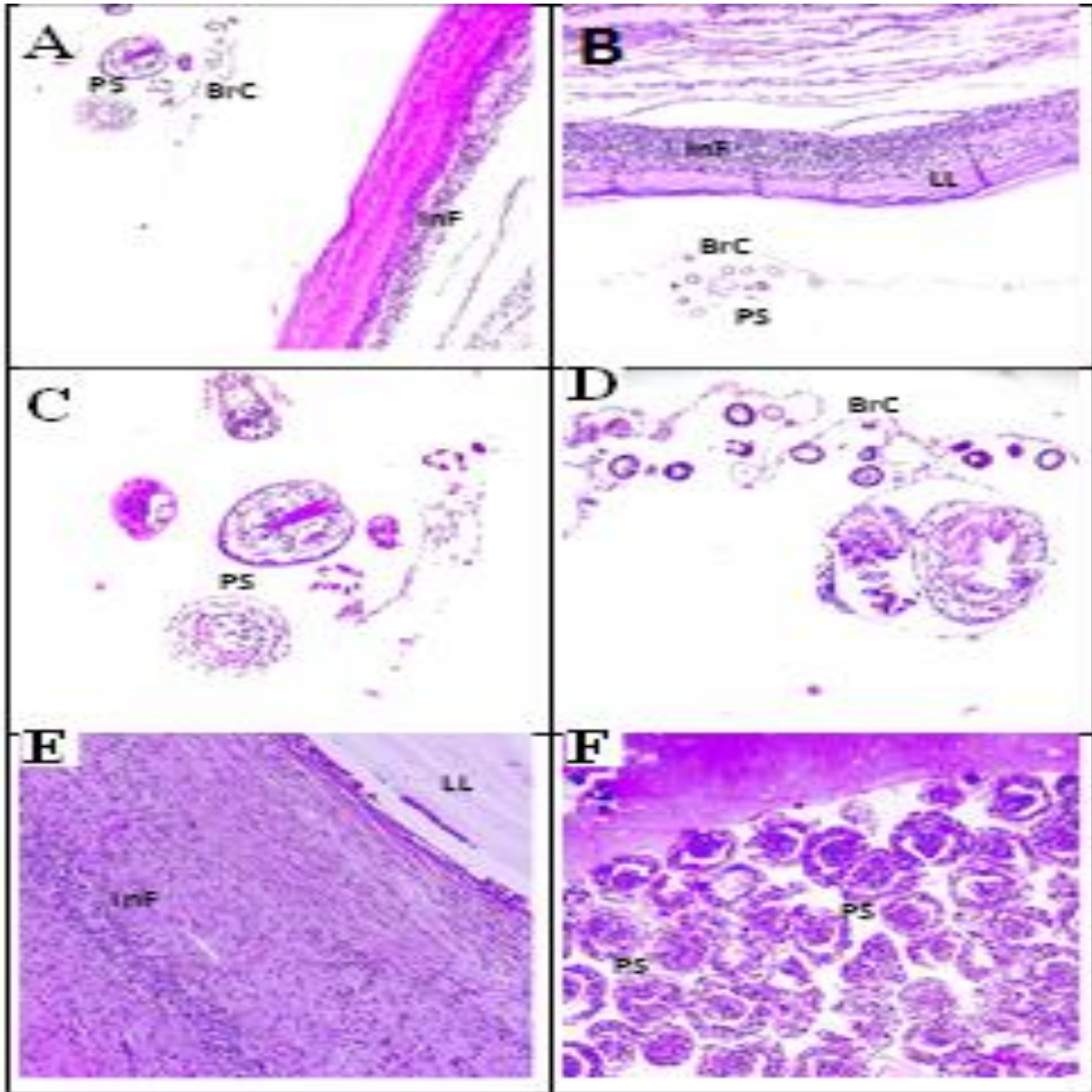


Figure 2. Histopathological examination of hydatid cysts found in cattle lungs (A, C), buffalo (B, D) and livers (F). Images A to F include (A) a picture of daughter cysts (Brood capsule; BrC) filled with multiple protoscolices (PS). (B) an image of Hydatid cyst wall, highlighting its outer laminated layer (LL) followed by inner germinal layer (GL) and protoscolices (PS). (C, and D) Higher magnification of daughter cysts (Brood capsule; BrC) filled with multiple protoscolices (PS). (E) shows a thick laminated layer followed by an outer layer made of collagen fibers that peel away from the laminated layer (AD), with a notable expansion of the outer layer due to an intense inflammatory response (InF).

4. PCR and sequence analysis

Polymerase chain reaction using forward and reverse primers for NADH-1 gene detected the expected band size of 500 bp from the DNA of hydatid cysts which collected from slaughtered cattle in this study (Figure Lane 3 and Lane 4). No bands were detected from double-distilled water as control negative (Figure Lane 2).

In the present study, The sequence of NADH-1 gene of *E. ortleppi* from Menoufia has high identity percent of 99.57% with *E. ortleppi* from buffaloes from India (KY766906, KY766907, KY766908), 99.57% with *E. ortleppi* from

cattle from Germany (KU842045, KU842044, KX010904), 99.57% with *E. ortleppi* from pig from China (OP471634 , OP471631) , 99.57% with *E. ortleppi* from human from China(OP471633, OP471632), 99.57% with *E. ortleppi* from yak from China (OP471630), 99.57% with *E. ortleppi* from cattle from Argentina (KC579444), 99.57% with *E. ortleppi* from cattle from (NC011122), 96.64%% with *E. ortleppi* from buffalo from Egypt (LC758545). The *E. ortleppi* sequence in the present study clustered with *E. ortleppi* sequences from Egypt and other countries (Figure 4).



Figure 3. Polymerase chain reaction (PCR) amplifies the NADH-1 gene of *E. ortleppi* in cattle from Menoufia governorate, Egypt. Lane 1, Control positive, lane 2 and 3, the hydatid cysts from cattle from Menoufia governorate, and lane 2, negative control double-distilled water. M is a DNA molecular size marker.

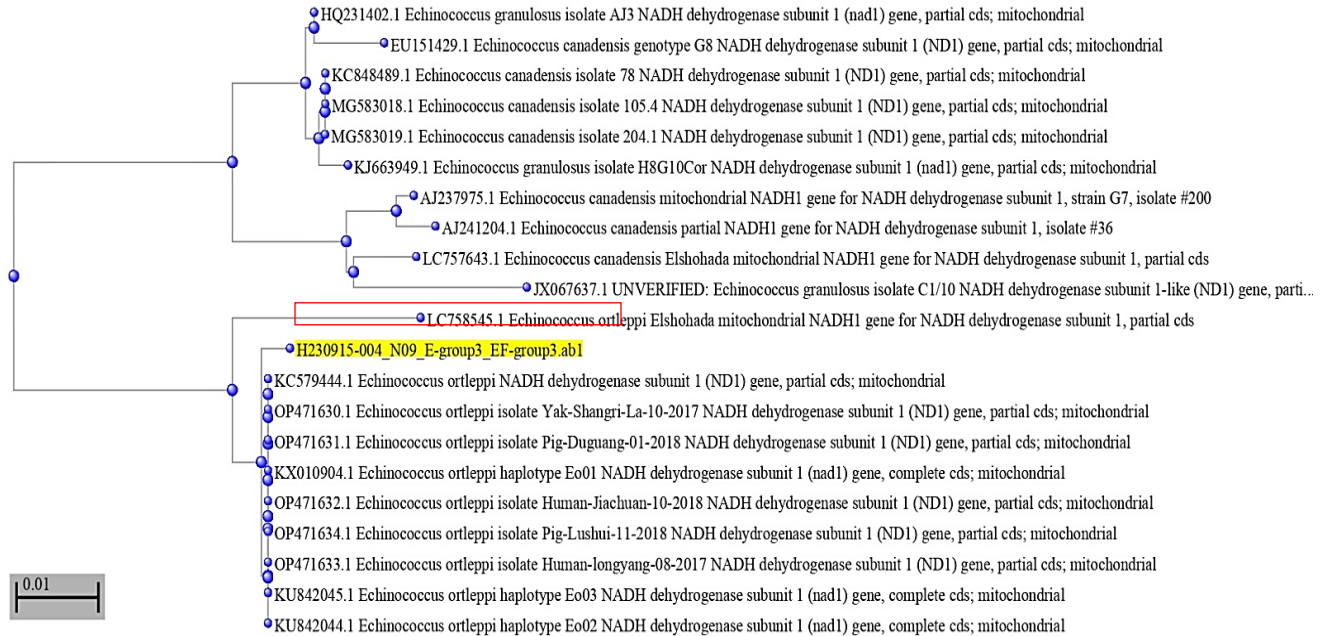


Figure 4. Phylogenetic tree of NADH-1 gene of *E. ortleppi* in cattle from Menoufia governorate, Egypt. The sequence obtained in the present study is boxed in red. Phylogenetic tree was constructed using Neighbor-joining method.

5. Economic impact of hydatid cyst

In the present study, the economic losses due to the condemnation of infected organs with hydatid cysts were 86600 EGP

(Table 6). The condemnation of the infected liver and lungs with hydatid cysts caused a financial loss of 81000 and 5600 EGP (Table 6).

Table 6. Economic losses of hydatid cysts in slaughtered cattle and buffaloes.

Condemned organ	Infected	Weight (kg)	Total condemned (kg)	Price/kg (EGP)	Total loss (EGP)
Liver	30	6	180	450	81000
Lung	16	5	80	70	5600
Total	46	-	-	-	86600

DISCUSSION

This investigation was undertaken to elucidate the current epidemiological scenario of hydatidosis among slaughtered cattle and buffaloes from three abattoirs in the Menoufia Governorate of Egypt. The findings provide vital insights into the prevalence, organ predilection patterns,

histopathological features, genetic diversity, economic losses and risk factors associated with hydatid cyst infections within the studied livestock populations.

The overall prevalence of hydatid cysts among the examined animals was 0.96%, with cattle exhibiting a significantly higher infection rate of 1.05% compared to buffaloes at 0.55%. This disparity highlights

the increased vulnerability of cattle to hydatid infections in the study's context, and our results align with previous studies comparing the prevalence of hydatid cysts in Egyptian cattle and buffaloes have reported rates of 1.83% and 2.34% by Hassanin et al. (2013), 0.61% and 0.2% by Borai et al. (2013), and 0.4% for both species by Barghash et al. (2017). The observed prevalence aligns closely with these previous Egyptian reports. However, substantially higher positivity rates have been recorded in other regional studies, including 56% in Moroccan cattle, 40% in sheep and 7% in goats (Azlaf and Dakkak, 2006), and 7.39% overall across Ethiopian ruminants (Hailu et al., 2019). This variability may be attributable to differences in local environmental contamination with eggs, canine definitive host populations, livestock rearing practices and climate.

Hydatid cysts exhibited a clear preference for female cattle, with the highest prevalence of 1.90% observed in females aged 5-15 years, in contrast to the lower rate of 0.33% in younger males. This pattern of higher prevalence among older female livestock has been noted in previous studies (Azlaf and Dakkak, 2006; Hassanin et al., 2013). This may reflect cumulative exposure over prolonged lifespans. The chances of ingesting infective eggs while grazing increase over time. Furthermore, enhanced immune response in younger animals may contribute to the decrease in infection rates (Lahmar et al., 1999).

The liver was determined to be the main location for cyst growth, with a notably higher occurrence compared to the lungs. Previous research has shown that the liver is the first place where parasite eggs are filtered after being ingested. This is where most oncospheres get trapped, leading to the development of hepatic cysts (Eckert et al., 2000; Moro and Schantz, 2009;

Siracusano et al., 2009). To locate cysts in the lungs, it is necessary to avoid them being captured by the liver and then spreading throughout the body. The reduced occurrence in the lungs indicates that a lesser percentage of parasites effectively evade the liver. The observed pattern aligns with observations from different countries where liver cysts are more prevalent in livestock (Lahmar et al., 1999; Roostaei et al., 2017; Hailu et al., 2019).

Detailed morphologic analysis revealed characteristic features of hydatid cysts, including the laminated outer membrane, internal germinal layer, brood capsules and protoscolices. The surrounding inflammatory reaction and adjacent tissue damage concurred with the described host response against enlarging cysts (Rinaldi et al., 2014; Jiménez et al., 2020). Overall, the microscopic findings were confirmatory of active hydatid infections.

Molecular genotyping using NADH-1 PCR and sequencing successfully identified the cyst isolates from cattle as *Echinococcus ortleppi* G5 genotype. Phylogenetic analysis reflected homology between the sequences obtained in this study and global isolates of *E. ortleppi*. Earlier reports have documented a predominance of *E. canadensis* G6 in Egyptian camels and *E. ortleppi* G5 in buffaloes, affirming the present genetic identities (Amer et al., 2015; Miambo et al., 2022). However, an intriguing finding was the occurrence of *E. canadensis* in cattle, contrary to expectations of the more common *E. granulosus sensu stricto*. Instances of untypical *Echinococcus* species and genotypes infecting atypical host animals are being increasingly recognized, signaling evolving transmission dynamics (Alvarez Rojas et al., 2014). Further molecular epidemiological investigations across diverse Egyptian

regions can elucidate the range of genotypes circulating among livestock.

The economic analysis conducted in this study revealed substantial losses totaling 86600 EGP throughout the two-year research period due to the condemnation of organs caused by hydatidosis. Previous studies in endemic areas have reported annual losses of US \$2 million in Morocco and US \$214 million in Iran (Azlaf et al., 2007; Rokni, 2008). Such detriments can substantially impact macro-economic, particularly in developing nations with resource-constrained Veterinary and public health systems. Cost-benefit analyses clearly indicate that investments into cystic echinococcosis prevention and control programs can lead to major cost savings and socio-economic benefits in the long term (Torgerson and Dowling, 2001; Majorowski et al., 2005). This underscores the need for evidence-based, context-specific control policies tailored to regional epidemiological patterns.

CONCLUSIONS

The findings of this investigation highlight the significant veterinary and economic impact of hydatidosis among Egyptian livestock. The elevated prevalence among older female cattle, hepatic localization patterns and identification of *E. canadensis* and *E. ortleppi* genotypes provide vital epidemiological insights relevant for control policy. The data suggest that focusing interventions towards older livestock, active canine surveillance, regulated slaughterhouse practices, deworming programs and public awareness campaigns can assist in curtailing transmission. Additionally, the economic analysis provides a rationale for prioritizing hydatidosis control. Further studies can explore regional variations, urban-rural disparities, risk modeling and cost-effectiveness assessments to devise

integrated national control frameworks against this economically relevant parasitic zoonosis.

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CONFLICT OF INTEREST

No conflict of interest

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