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Molecular identification and histopathological of *Cysticercus tenuicollis* infestation among small ruminants

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Abstract

Small ruminant infections by the *Cysticercus tenuicollis* result in financial losses because of the condemnation of infected organs, which can happen in unsanitary conditions and is regarded as an ecotoxicological risk factor. Therefore, a cross-sectional study was conducted to investigate the infection rate of *Cysticercus tenuicollis*, risk factors, and morphological, histological, biochemical, and molecular characterization of the genetic sequence of cysts collected from the affected organs of 139 sheep and 261 goats in the Allam-ELrom abattoir, Matrouh Governorate, Egypt. The overall infection rate was 19.25%, with a higher prevalence in goats 23.40% compared to sheep 11.50%, indicating that species served as a significant risk factor for infection. However, no notable significant differences in infection rates were observed based on age or sex, with the main symptoms being pulmonary crackles by auscultation and visceral pain. The cysts were most frequently found in the omentum 72.76%, followed by the liver 18.18%. there were significant differences in biochemical parameters such as liver enzymes and lipid profile of the cyst fluid among different organs. Molecular identification through PCR and sequence investigation of the cox1 gene in goats and 12S rRNA in sheep confirmed the cysts as *C. tenuicollis*. The retrieved sequences (OL470129 for sheep and OL470130 for goats) were nearly identical to those reported from other countries, highlighting the genetic similarity of this parasite across regions.

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Introduction

Small ruminants are considered an important livestock especially in the developing countries as the primary supplies of meat, milk, and wool, and in the year 2022, the total capital head of sheep and goats in the Matrouh governorate, Egypt, was 288,000 head, so the parasitic infection among Small ruminants are considered serious economic issues due to the development of *C. tenuicollis* as fluid filled cysts in the internal organs of small ruminants,

which may end in inadequate milk production, death or lead to condemnation at meat inspection (1-4). The Predicators, like canines, are served as the final host; on the other hand, the sheep, goats, and other herbivorous animals serve as intermediate hosts (5). The biological cycle of *Taenia hydatigena* begins with the eggs laying from adult worms in final host's feces (dogs) that contaminated the pasture, which are then consumed by the ruminant's host through feeding or fecal-oral route for direct transmission (6). When eggs reach the small intestine, they hatch into oncospheres, which are then transported by blood to the liver, where they migrate before emerging on the organ's surface and attaching to the peritoneum, as well as other visceral organs like the kidney, heart, and lung. Then develop into the larval stage. The final host becomes infected by consuming the larval stages in infected organs of the intermediate host, which causes their scolex to liberate and adhere to the intestinal mucosal layer until the worm becomes adult (7). Therefore, the main ecotoxicological risk factors are poor community health education, unsanitary practices, such as contaminating food with predicators' feces, and illegally slaughtering intermediate hosts. Appropriate action to address these risk factors by assessing soil environmental risks may mitigate the likelihood of endo-parasite infection (8). *Cysticercus tenuicollis* has morphologically thin, translucent, or milky white walls that range in size from an apple to a walnut. Each of the four suckers on a single, tiny scolex contains rostellum hooks, and there are two rows of hooks with the large and small hooks (9). In small ruminant animals infected with *Cysticercus tenuicollis*, the pathological abnormalities in infected livers include swelling and congestion of the hepatic tissue in acute cases. In chronic cases, however, liver slices from the right and left hepatic lobes are affected. Additionally, histological examination reveals biliary hyperplasia, and prominent vein dilation without evidence of an inflammatory response with clinical symptoms that are included that sudden recumbency, visceral pain, and respiratory crackles have been reported along with sudden death (10,11). Also, migrating channels of *Cysticercus tenuicollis* induce parenchymatous hemorrhages in several organs, including the liver and lung, associated with focal areas of necrosis. Emphysemas with severe inflammatory reaction were diagnosed in cases infected with *Cysticercus tenuicollis* in the lung. Variable organs such as lungs, spleen, liver, omentum, heart, and genital organs such as the uterus, cervix, and vagina revealed the attachment of *C. tenuicollis* (12). The biochemical components of *C. tenuicollis* fluid vary according to the cyst habitat but generally contain a variety of chemical parameters, such as minerals, liver enzymes like alanine aminotransferase (AST), and Low-density lipoprotein (LDL) (13). Furthermore, conventional morphological identification of the cyst confirms infection during meat inspection, but the rapid progress in molecular biology diagnostic reconstruction followed by phylogenetic analysis is often believed to be more reliable and sensitive in preventing misdiagnosis and cross-reactivity with other metacestode Taeniidae (14-16).

Therefore, the current research done to investigate the prevalence, morphology, biochemical properties, histological characteristics, and gene sequencing of C. *tenuicollis* cysts in small ruminant. The research hypothesis said that there was a different gene sequence of *C. tenuicollis* cyst among sheep and goats and also with regard to cyst fluid in different organs, liver and omentum. This research provides novel insights into the parasitic infection in Matrouh Governorate, Egypt, contributing valuable data on its biological and genetic features in this region.

Martials and methods

Ethical approval

All particular animal procedures were revised and granted by the state ethics commission and the ethics committee of Alexandria University, Egypt (serial number 0306905 at 13/10/2024; FWA no. 00018699; and IRB no. 00012098) (17).

Study setting

In the abattoir of Allam-ELrom, Matrouh governorate, Egypt, which has coordinates (27.237316 E, 31.354343 N), during the period from January to October 2024.

Sample size

The abattoir of Allam-ELrom had 4216 cases of slaughtered small ruminant animals at year 2020 according to the registration record, of which 1400 were sheep and 2816 were goats (18). The infection frequency among sheep was 16% and in goats were 19%, in Upper Egypt (19). Therefore, the minimum total sample size required to conduct the current study were 139 sheep and 261 goats at CL 95% with 5% types I error by using of raosoft software programs sample size calculator.

Animal sampling

Using a multistage randomization sampling technique, 139 sheep and 261 goats were selected out of the 4216 small ruminant animals that were slaughtered. The first stage of the technique involved cluster randomization, which divided the animals into two clusters: the sheep cluster contained 1400, and the goats cluster contained 2816. The second stage involved random systematic technique, which selected 139 out of the 1400 sheep by selecting one sheep every 10 sheep that recorded in the Allam-ELrom abattoir, and the goats cluster selected 261 out of 2816 goats by selecting one goat every 11 goats that recorded in Allam-ELrom the abattoir, until 400 sheep and goats were selected as the total sample size.

Before slaughter inspection of small ruminant animals

In order to observe the animals both individually and collectively at rest and in action, 400 small ruminants should be subjected to before slaughter examination in an appropriate lighting condition. It is important to record general symptoms like abdominal pain and use auscultation to check for pulmonary crackles.

After slaughter inspection of small ruminant animals

Post-mortem macroscopic inspection of the visceral organs of 400 small ruminants, including both genders of sheep and goats, including the liver, omentum, lungs, and other organs, was conducted over the course of ten months by veterinary doctors at the Allam-Elrom abattoir. This inspection adhered to the regulations outlined in Law No. 517 of 1988, issued by the Egyptian Ministry of Agriculture and published in the Egyptian Gazette.

Measurement of cyst size

Cyst sizes were determined by using an electronic digital measuring pin micrometer. The measurement face was pressed onto the cyst by rotating the spindle and closing the ratchet, then securing the dimension with a locking nut.

Morphological identification and staining of *C tenuicollis cysts*

Cysticercus tenuicollis cysts were extracted from sheep and goats that were washed with saline (0.9%) and then in two separate sterile bottles containing 70% ethanol per each species, preserved collected cyst samples for gene sequences. Then the other two separated sterile bottles contained 10% formalin-preserved collected cyst samples for morphological investigation. A morphological examination of cysts and a count of the total length of hooks, either small and large, and the number of hooks of rostellum (NUH) was performed after mounting the scolex through compression between two slides, then submerged in 10% formalin followed by staining using carmine solution, later rinsed in water before being moved to ascending alcohol then descending alcohol for drying. Finally, the slides were mounted in Canada balsam (20-24).

Histological architectures of *C tenuicollis* collected from liver, omentum and lung

21 tissues specimens included 10 from liver, 10 from omentum and one from lung either infected with *Cysticercus tenuicollis* cysts or apparent healthy tissues were extracted from sheep and goats and then washed with normal saline (0.9%). The specimens were fixed in 10% phosphate-buffered formaldehyde, then and transferred to the pathology department of Alexandria University High Institute of Scientific Research for histopathological architectures. The fixed specimens were processed for paraffin sectioning. Serial sections (5µm) were prepared and stained using the following stains: Mayer's hematoxylin and eosin stain, Masson's trichrome stain, and Periodic acid Schiff's reagent (PAS) stain (25).

Biochemical analysis of *C. tenuicollis* cyst fluid

Simple random selection for twenty fresh cysts (10 cysts from liver and 10 cysts from omentum) from total cysts that were collected from carcasses, then their fluid was collected by using sterile syringes and analyzed for biochemical analysis (26).

Primers, solutions and genes

Cysticercus tenuicollis cysts were subsequently sent to the GEBRI in Borg Elarab City, Alexandria 21934, Egypt (29°41′47″E, 30°55′04″N) for DNA extraction. DNA extraction was done according to the weight of the scolex of cysts for sheep and goats. Tri-Fast™ solution method used for *C. tenuicollis* cysts scolex from sheep and traditional method using CTAB extraction buffer from goat. Dream Tag Green PCR Master Mix (2x) (K 1081, Thermo Fisher, USA) was used. The primers from Macrogen (Korea) were utilized to amplify size13490 with target gene 12SrRNA from sheep under accession number (MT784871, Genebank registration link, [available at] and utilized to amplify size 444 with target gene (cox1) from *C. tenicollis* of goats under accession number (MW316694, Genebank registration link, [available at] (27-29).

Visualization of the amplified PCR Products

To separate the amplified PCR fragments, the PCR product was put to a 1% agarose gel in 1X TBE with ethidium bromide stain. The Multi Image gel documentation system was then used to visualize the results.

DNA sequencing and phylogenetic analysis

The BLAST online tool was used to survey the acquired sequences and define the related percentage between them and the sequences of other species were registration in GenBank under accession numbers that were presented in the database. After that, the MEGA7 technique was used to align the sequences. A phylogenetic tree was created depend on Euclidean distance between them with a bootstrap test (1,000 replicates) and *Entamoeba. Nuttalli* (GenBank accession number, AB282671) was used as an out-group species (30-33).

Statistical analysis

By using SPSS version 26 at significant level 5% to analysis results as following the Chi square test, Monte Carlo test were performed to assess the difference in prevalence between the main risk variables (34). Also, after normality tested by Kolmogorov-Smirnov then applied the Mann-Whitney U test to compare the mean of quantitative measurement of hooks between both animal species and the biochemical analysis between different organs with discrimination analysis to confirm the percentage of overall

accuracy of biochemical analysis and which element gave the highest F value element of biochemical analysis which indicated the greatest biochemical element can different between organs. Logistic regression was used to estimate the odds ratio of risk factors.

Results

Before slaughter inspection

According to clinical examination of the small ruminants, visceral pain and respiratory crackles occurred in 17.5% of the examined cases. In addition, there were no other abnormal findings on the clinical examination. Table 1 and 2 displayed that the overall infestation rate of *Cysticercus tenuicollis* in small ruminants was 19.25%, with goats showing a higher prevalence 23.40% compared to sheep 11.50%. A significant difference was observed between animal species and infection rates (X^2 = 8.208, P = 0.004). Regarding age and sex, the infection rate was higher in females 23% compared to males 18%. In terms of age, animals younger than 3 years had an infection rate of 19.3%, which was nearly equal to those older than 3 years was 19.1%. However, insignificant associations were found between infection rates in relation to sex and age (P = 0.272 and P = 0.985, respectively). According to the multivariate risk factors analysis, the logistic regression of the omnibus tests of model coefficients was significant (P = 0.006), which means the total risk factors studied in the current model were significant, and the animal species was the only risk factor, which the odds ratio was 3.341 Cl 95% (1.500-7.444, P=0.003). That means the odds of chance of infection occurring in goats were significantly higher than in sheep by 3.341 times. On the other hand, the other risk factors, including animal sex and age, were insignificant (P = 0.809 and 0.469, respectively) (the odds ratio, 0.809 Cl 95% (0.405-1.615) and 0.469 Cl 95% (0.138-1.600), respectively).

Table 1: The infestation rate of metacestode obtained among the sheep and goats

Variables		No	Infestation	%	Chi square test	
					X^2	P
Small ruminants species	Sheep	139	16	11.5	8.208	0.004
	Goats	261	61	23.4		
Small ruminants gender	Males	300	54	18.00	1.206	0.272
	Females	100	23	23.00		
Small ruminants age	< 3 years	353	68	19.3	3.497^{E-4}	0.985
	≥3years	47	9	19.10		

Chi square test at 5% level of significant.

Table 2: Illustrations binary logistic regression for multivariate risk factors analysis and the probability of infestation rate of *Cysticercus tenuicollis* among examined animals

Risk factors	St. error	Significant	Omnibus tests of model coefficients	Hosmer and Lemeshow test
Animal species	0.409	0.003	0.006	0.962
Animal sex	0.353	0.547		
Animal age	0.626	0.227		
Constant	0.373	0.000		

Variable(s) entered: animal species, animal sex, and animal age.

Monte Carlo test at 5% level of significant

Table 3 showed that the distribution of *Cysticercus tenuicollis* in slaughtered animals was predominantly observed in the omentum 72.76%, followed by the liver 18.18%, then both the liver and omentum membrane 5.19%, and less frequently in the lungs, large intestine, and diaphragm 1.25%. A significant difference in distribution was noted (X^2_b = 11.56, P = 0.003). Table 4 said that the size of *C. tenuicollis* cysts varied by location. The largest cyst size observed in the liver was 1 cm, while in the omentum, it was 2 cm, in the lungs 4 cm, large intestine 1.8 cm, and diaphragm 2.3 cm.

Table 5 exhibited that, morphologically, the cyst appeared rounded with a thin, whitish wall. Inside, it contained a scolex with four suckers and a series of rostellum hooks, both small and large, arranged alternately in cysts from both sheep and goats. The average number of large hooks ranged from 13 to 15, while the small hooks numbered between 12 and 15. Other morphometric measurements, such as the length of the small and large hook blades and the length of the small and large hook handles, showed no significant differences (P<0.05) between those found in sheep and goats, as described in (Figure 1).

Table 3: Demonstrations the distribution of infestation of *Cysticercus tenuicollis* among infected small ruminant organs

Carcasses organs	Carcasses species		Total		Monte Carlo test	
	Goat	Sheep	No	%	X^2_b	P
Liver	8	6	14	18.18		
Omentum membrane	47	9	56	72.76		
Both liver and omentum membrane	3	1	4	5.19		
Lung	1	0	1	1.29	11.56	0.003_b
Large intestine	1	0	1	1.29		
Diaphragm	1	0	1	1.29		
Total	61	16	77	100		

Table 6 disclosed that the classification and validity of all biochemical analyses were 95%. This indicated that the biochemical elements were differentiated between live and omentum by 95%, and the highest elements different between the two organs were, in descending order according to the value of the F test, total protein, Ca, Na, and AST. The overall levels of the measured elements in the cyst fluid of the liver and omentum were significantly different, with 46.50±3.02; 59.50±3.02 U/l for ALT and 249.50±3.02; 210.50±9.08 U/l for AST. The concentrations of lipid profile were 163.50±3.02; 188.00±6.05 mmol/l for triglyceride and 80.50±3.02; 99.76±5.06 mmol/l for cholesterol. The mineral concentrations were 9.02±0.0605; 9.91±0.02 mmol/l for Ca, 2.35±0.302; 2.35±0.302 mmol/l for P, 43.95±0.302; 45.88±0.0302 mmol/l for Na, and 15.05±0.302; 15.808±0.045 mmol/l for K. The glucose concentration was 396.50±3.027; 408.50±3.02 mmol/l. The concentrations of total protein were 5.69±0.0302; 5.12±0.03 g/l, and urea was 5.55±0.302; 6.25±0.302 mmol/l. Creatinine was 0.605±0.0302; 0.60±0.0605 mmol/l, uric was 2.01±0.0302; 2.15±0.302 mmol/l, and LDH was 17.50±3.02; 21.00±6.05.

Table 4: Measurements of *C. tenuicollis* size among different of carcasses organs

Specimens examined	size of cyst category	No	%
Liver	1cm -	7	50
	2cm-	4	28.58
	3cm-	2	14.28
	≥4cm-	1	7.14
Omentum membrane	1cm -	16	28.57
	2cm-	24	42.85
	3cm-	6	10.73
	≥4cm-	10	17.85
liver and omentum membrane	1cm -	0	0.00
	2cm-	0	0.00
	3cm-	1	33.34
	≥4cm-	3	66.66
Lung	4.0 cm	1	100
large intestine	1.8 cm	1	100
Diaphragm	2.3cm	1	100

Table 5: shows the measurements of *C. Tenuicollis* hooks of sheep and goat

Hooks	Animal species	Mean± SD	Mann-Whitney U	
			U	P
Large hooks (um)	Sheep	218.33±30.13	4.000	1.000
	Goats	218.40±30.12		
Total		218.36±26.95		
Small hooks (um)	Sheep	147.70±12.904	3.000	0.700
	Goats	142.70±18.89		
Total		145.20±14.72		
Large hooks (blade/um)	Sheep	84.06±14.33	3.000	0.700
	Goats	93.26±18.63		
Total		88.66±15.702		
Large hooks (handle/um)	Sheep	111.73±11.75	3.500	0.700
	Goats	104.61±25.68		
Total		108.17±18.28		
Small hooks (blade/um)	Sheep	69.86±5.201	3.500	0.700
	Goats	67.66±6.806		
Total		68.76±5.55		
Small hooks (handle/um)	Sheep	58.56±4.81	3.000	0.700
	Goats	61.66±2.88		
Total		60.11±3.93		

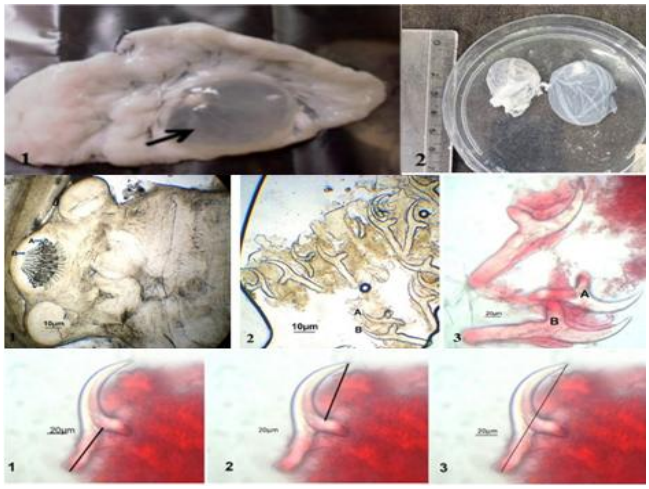

Figure 1: Shows the measurements of *C. Tenuicollis* scolex and hooks.

Table 6: Expressions the biochemical analysis of cyst fluids among different cysts locations

Elements	Cyst's location		Mann-Whitney U		Discrimination analysis	
	liver	omentum	U	P	F	Cross-validated percentage
Na (mmol/l)	43.95±0.302	45.88±0.0302	20.11	0.000	404.417	95.0%
K (mmol/l)	15.05±0.302	15.808±0.045	7.828	0.000	61.272	
Ca (mmol/l)	9.02±0.0605	9.91±0.02	42.654	0.000	1819.354	
Ph (mmol/l)	2.35±0.302	2.35±0.302	0.00	1.000	0.000	
Glucose (mmol/l)	396.50±3.027	408.50±3.02	8.863	0.000	78.545	
Urea (mmol/l)	5.55±0.302	6.25±0.302	5.17	0.000	26.727	
Creatinine (mmol/l)	0.605±0.0302	0.60±0.0605	-0.234	0.818	0.055	
Uric (mmol/l)	2.01±0.0302	2.15±0.302	1.403	0.178	1.968	
Cholesterol (mmol/l)	80.50±3.02	99.76±5.06	10.321	0.000	106.523	
Triglycerides (mmol/l)	163.50±3.02	188.00±6.05	11.444	0.000	130.964	
ALT (U/l)	46.50±3.02	59.50±3.02	9.601	0.000	92.182	
AST (U/l)	249.50±3.02	210.50±9.08	-12.881	0.000	165.927	
Lipoprotein (mmol/l)	17.50±3.02	21.00±6.05	1.635	0.119	2.673	
Total protein (g/l)	5.695±0.0302	5.12±0030277	0.000	0.000	1772.182	

PCR sequencing and phylogenetic analysis

Table 7 show that the both sequences were submitted to GenBank and identified as *T. hydatigena* larval stage, with accession numbers OL470129 (registration in Genebank link [available at] for sheep and OL470130 (registration in Genebank link [available at] for goat. Notably, the *T. hydatigena* isolates in this study were found to cluster with closely related isolates in distinct lineages. For example, the *T. hydatigena* from sheep (OL470129) formed a well-supported subclade with strains from sheep, goats, and cattle in Iran (KX084714), while the goat isolate (OL470130) grouped in a separate clade with the sheep isolate from Egypt (KU671388). 453 bp was generated by PCR amplification of the small subunit of the cox1 gene from DNA extracted from goat cysts, while a 446 bp for the small subunit of the 12S rRNA gene from DNA extracted from goat cysts.

Gene sequences of sheep

Furthermore, the sequence obtained from the sheep (OL470129) exhibited the highest similarity (99.25%) with *T. hydatigena* larval stage strains from sheep, goats, and cattle in Iran (KU745526, KU750812, and KU745527, respectively) and from sheep in Iraq (LC749826, LC746809). A comparison of our isolate with *T. hydatigena* larval stage from sheep in Iran (JQ717210, JQ717221) revealed 99.24% nucleotide similarity. It also showed 99.23% similarity with strains from sheep in Iran (KX084714), 98.99% with sheep from Iran (JQ717246) and China (MT784876), 98.74% with sheep from Iraq (OR063932), (Figure 2).

Gene sequences of goat

The sequence of *T. hydatigena* larval stage from goat (OL470130) showed 94.74% identity with *T. hydatigena* from sheep in Egypt (KU671388; KU671396), and 94.49% identity with sequences from sheep, goat, and cattle in Iran (KU745526, KU750812, KU745527) as well as from sheep in Iraq (LC749826, LC746809). It shared 93.96% identity with strains from sheep, goat, and cattle in Iran (KX084714), and 93.85% with sheep from Iran (JQ717210). The similarity was 93.62% with sheep strains from Iran (JQ717221, JQ717246), 93.66% with sheep from China (MT784876, MT784880), 93.43% with sheep from Iraq (OR063932), and 94.09% with sheep from Iran (JQ717223).

Table 7: *Taenia hydatigena* > 93.66 % identity and query cover >83% obtained from Genebank and used for phylogenetic analysis of recovered sequence of cox1 of goats and 12SrRNA of sheep in the current study

Accession No.	Host	locality
*OL470129	sheep	Egypt
KU745526	Sheep/Goat/Cattle	Iran
KU750812	Sheep/Goat/Cattle	Iran
LC749826	Sheep	Iraq
LC746809	Sheep	Iraq
MT784877	Dog	China
JQ717210	Sheep	Iran
KU745527	Sheep/Goat/Cattle	Iran
JQ717221	Sheep	Iran
JQ717246	Sheep	Iran
MT784876	Sheep	China
OR063932	Sheep	Iraq
FJ518620	Human	China
MT784893	Dog	China
*OL470130	Goat	Egypt
JQ717223	Sheep	Iran
MT784896	Dog	China
MT784880	Sheep	China
MK858249	Sheep	Iraq
KX084714	Sheep/Goat/Cattle	Iran
KU671388	Sheep	Egypt
KU671396	Sheep	Egypt

*Accession obtained in the current study.

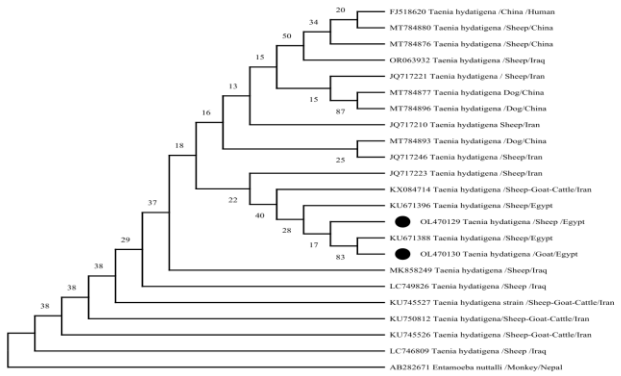

Figure 2: Shows the Dendrogram of neighbor-joining analysis of the12S rRNA gene sequences of the *T.hydatigena* larval stage of sheep and (cox1) genes of goat represented in black circle (OL470130 and OL470129), with out-group and bootstrap confident values were calculated at 500 repetitions.

The histopathological features

The histopathological features in lung parenchyma were large areas of caseous necrosis with severe inflammatory reaction of mononuclear cell infiltration. Thickening of alveolar septa was clearer resulting from inflammation with peripheral emphysematous reaction resulting in interstitial pneumonitis. Large areas of lung tissue replaced with fibrous thick-walled cavities with macrophage and lymphocyte aggregation as well as collagen bundles. A clear filled double layered cyst was attached to the wall of the omentum that the outer layer infiltrated with lymphocyte while the inner layer attached to the omentum wall in addition to progressive inflammation and in some areas vacuolar degenerative changes were appeared. A clear filled double layered cyst was attached to the wall of the omentum that the outer layer infiltrated with lymphocyte while the inner layer attached to the omentum wall in addition to progressive inflammation and in some areas vacuolar degenerative changes were appeared (Figures 3-7).

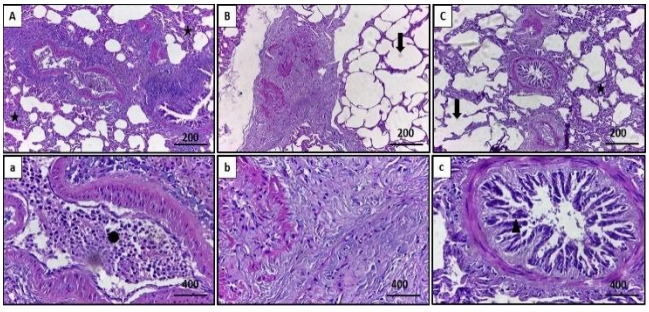

Figure 3: Histopathological lesions of *Cysticercus tenuicollis* in lung showing infiltration of inflammatory cells (oval) leading to thickening of the alveolar wall (star) as well as papillary hyperplasia of the bronchiolar alveolar wall (arrow head) and peripheral emphysema (arrow), H&E stain.

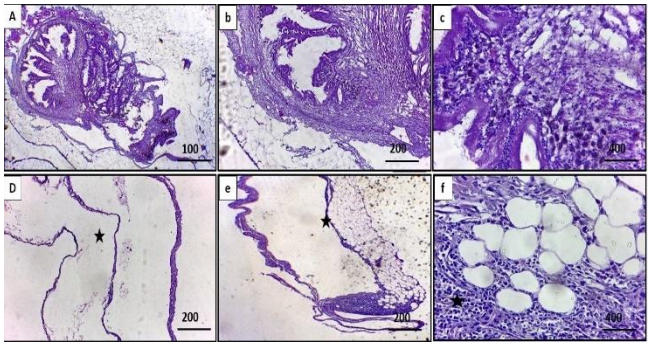

Figure 4: Histopathological lesions of *Cysticercus tenuicollis* in omentum showing double layered cyst adhered to omentum (Star) with mononuclear cell inflammatory reaction (oval), H&E stain.

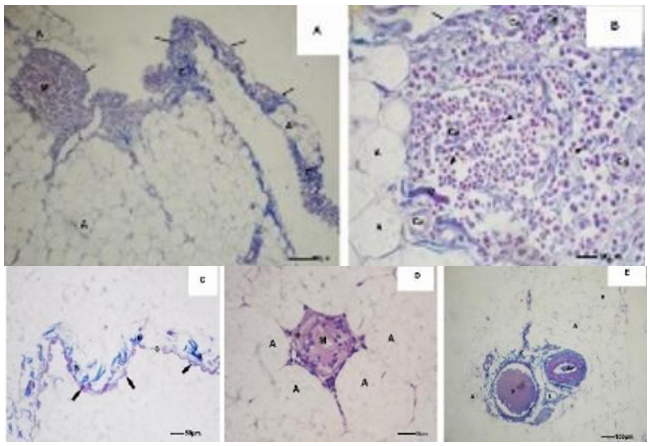

Figure 5: Histological characteristics of the omentum of the goat: A) A photomicrograph of the goat's omentum showing the mesothelial sheet (arrows) which encloses predominantly adipocytes (A) embedded in a loose connective tissue mainly collagen fibers (C). In the omentum, the leukocytes aggregate in the perivascular area to form what are termed milky spots (M) (L) lymphatic vessel. Masson's trichrome stain. Bar 100 µm. B) Higher magnification of the previous image shows that the milky spot comprises numerous convoluted capillaries (Ca) surrounded by leukocyte aggregates (arrowheads) (C) collagen fibers, (A) adipocytes, and (arrows) mesothelial sheet. Masson's trichrome stain. Bar 50 µm. C) The mesothelial sheet (arrows) resting on an interrupted basement membrane (B), and the presence of gaps (G) between mesothelial cells. Masson's trichrome stain. Bar=50 µm. D) The mesothelial sheet (arrows) resting on an interrupted basement membrane (B), and the presence of gaps (G) between mesothelial cells. Masson's trichrome stain. Bar=50 µm. E) A photomicrograph of the goat's omentum showing branches of the gastroepiploic system, artery (Ar), and vein (V). L=lymphatic vessel, A= adipocytes. Masson's trichrome stain. Bar 100 µm.

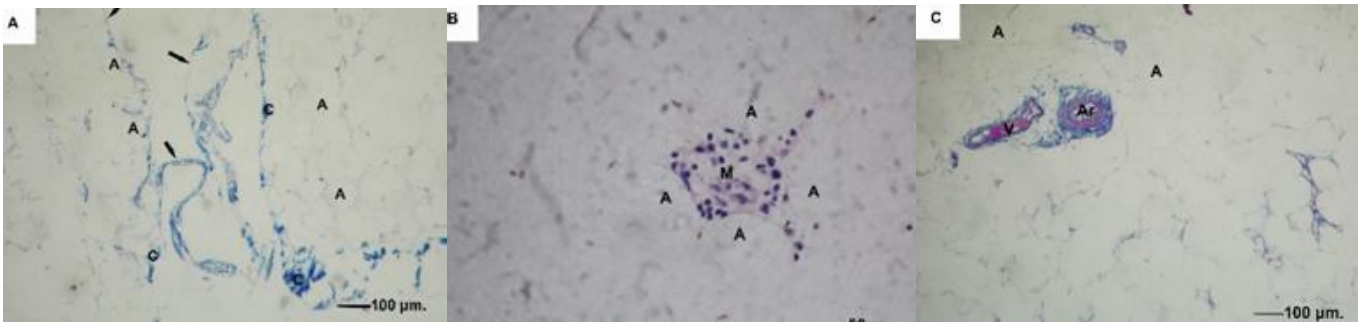

Figure 6: Histological characteristics of the omentum of the sheep: A) A photomicrograph of the sheep's omentum showing the mesothelial sheet (arrows) which encloses predominantly adipocytes (A) embedded in a loose connective tissue mainly collagen fibers (C). Masson's trichrome stain. Bar 100 µm. B) A photomicrograph of the sheep's omentum showing a milky spot (M) star in shape located between white adipocytes (A) without mesothelial cover. H&E stain. Bar=50µm. C) A photomicrograph of the sheep's omentum showing branches of the gastroepiploic system, artery (Ar), and vein (V). L=lymphatic vessel, A= adipocytes. Masson's trichrome stain. Bar 100 µm.

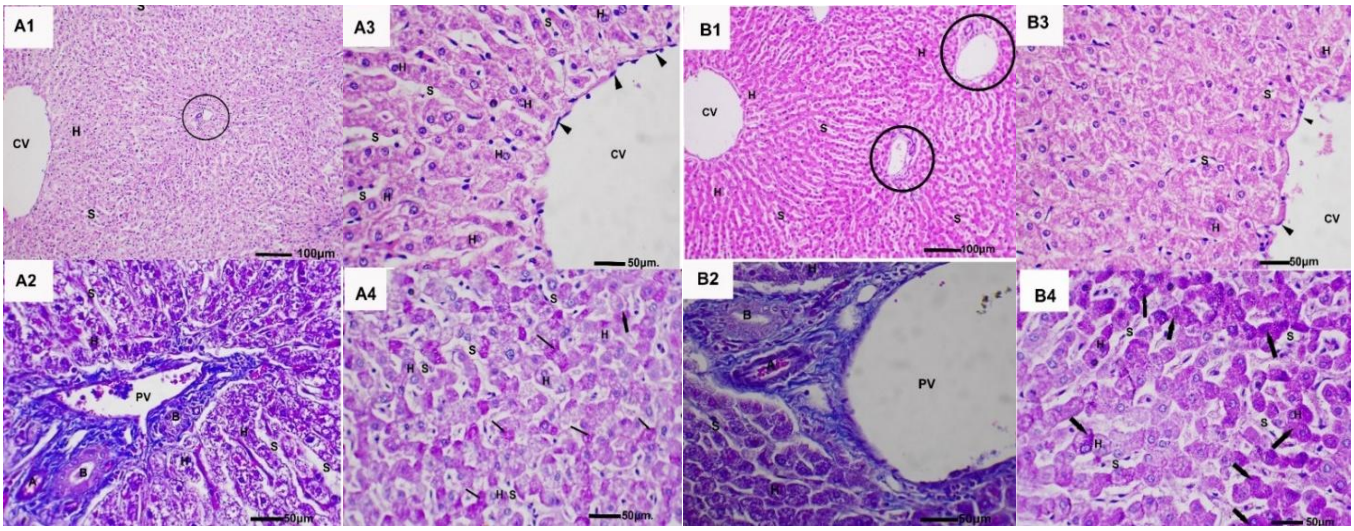

Figure 7: Microscopic picture of the apparently normal liver of goat (A) and sheep (B): A1) The liver of goats showing normal hepatocytes (H) separated by blood sinusoids (S). CV= central vein, and circle= the portal triad. Hematoxylin and eosin stain. Bar 100 µm. A2). The portal triad is composed of a branch of the portal vein (PV), a branch of the hepatic artery (A), in addition to a branch of the bile duct (B). Masson's trichrome stain. Bar 50 µm. A3). The central vein (CV) is lined by endothelial cells (arrowheads) in the center. Hematoxylin and eosin stain. Bar 50 µm. A4) The hepatocytes (H) contain glycogen granules (arrows) and blood sinusoids (S) present between hepatocytes. PAS stain. Bar 50 µm. B1) The sheep's liver has normal hepatocytes (H) separated by blood sinusoids (S). The central vein (CV) presents in the center of the hepatic lobule and the portal triad (circle) presents at the periphery. Hematoxylin and eosin stain. Bar 100 µm. B2) The portal triad is composed of a branch of the portal vein (PV), a branch of the hepatic artery (A), in addition to a branch of the bile duct (B) (H) hepatocytes, and (S) blood sinusoids. Masson's trichrome stain. Bar 50 µm. B3) The central vein (CV) is in the center and is lined by endothelial cells (arrowheads). H= hepatocytes, and S= blood sinusoids. Hematoxylin and eosin stain. Bar 50 µm. B4) The hepatocytes (H) contain glycogen granules (arrows) and blood sinusoids (S) present between hepatocytes. PAS stain. Bar 50 µm.

Discussion

Although there was no sudden death as reported by Abdollahi and his colleagues, which same line to current study which demonstrated that the ante-mortem examination were instances had respiratory crackles and visceral pain (35). The distribution of *C. tenuicollis* cyst infections and the microscopic morphological or molecular features of the *C. tenuicollis* metacestode and associated non-modified risk factors were among the several issues covered in the current study. In the present work, the total infestation rate is 19.25%, but goats had twice the opportunity to get infected

than sheep because goats were better garbage scavengers; the primary organs affected were the omentum membrane 72.76%, followed by the liver 18.18% because this was the first organ to which the embryo attached; the cysts also spread throughout the omentum membrane and the lung, large intestine, and diaphragm, all of which were recorded once. This discrepancy could result from variations in how much time animals spend in contaminated surroundings. One of the main causes of the high infection rate in Matrouh, Egypt, is the widespread illicit slaughtering of small ruminants outside of government abattoirs without veterinary examination. The existence of stray dogs closes to small ruminant farms, which frequently do not have a deworming prevention program that involves the routine administration of anthelmintic medications, is another issue. Furthermore, compared to sheep, goats' immune systems grow more slowly, which may be the reason for their increased infection rate. Stray canines and farms with small ruminants that have not instituted a dewormed preventive program by the periodic administration of anthelmintic medications, especially for Platyhelminthes like Praziquantel, are additional contributing causes, so according to comparable research conducted in 2020 by Aboulailaa M, Minoufiya, Egypt has an 18% infection rate with the same cause (36). Additionally, the infection rate is higher in the Nile Delta, Dakahlia governorate, Egypt (21%). However, contrary to recent findings regarding the organ distribution of carcasses, as reported by Essa *et al.* (37), the hepatic is the main organ of infection, then other organs like the omentum, because the liver is highly rich in protein; this leads to the cyst surviving for at least 4 days in the environment). According to current research by Dyab AK *et al.* (38), infection rates in Aswan, Egypt's slaughterhouses are reported to be 13.3% for sheep and 24.2% for goats. This finding may have contributed to the slower immune development of goats. 28 Because the parasites are widespread in Iraq due to sheep and goats are exposed to surroundings with infected dogs (the definitive hosts) for longer periods of time, the infection rate is highest in the Karbala abattoir in Iraq, affecting goats at 35.41% and sheep at 32.5%.29 In the same direction as the ongoing research Goats have a higher frequency of *C. tenuicollis* 39% compared to sheep 17.4% in Paraíba, Brazil. The primary organs where *C. tenuicollis* cysts are seen are the omentum and mesentery.

Furthermore, although the difference was not statistically significant, the infection rate was higher in females than in males. This is in line with the results of Omar *et al.* (24), who found that females had noticeably greater infection rates. Given that females are frequently held for reproduction for a longer period of time than males, who are often killed early, the increased infection incidence in females may be the result of their prolonged use in breeding.

The current study showed that cyst numbers varied depending on the organ. In the liver, 42.85% of the cysts were single, followed by 28.59% of double cysts and 21.42% of cysts greater than four. In the omentum membrane, this disparity may be explained by the fact that the omentum is the first organ to which the embryo attaches, which facilitates the formation of cysts there. On the other hand, the percentage of single cyst infections was 69.64%, the percentage of double cyst infections was 12.51%, and the percentage of cysts greater than four was 10.71%. The most frequently measured size in the liver, according to the current study, was one centimeter 50%; on the other hand, the mesenteric membrane had a maximum size of 8.4 cm and a minimum size of 0.9 cm, with one centimeter being the most frequently recorded size 28.57%. The number of cysts detected varied according to several criteria, such as the number of hexacanth embryo infections, the length of the infection, the location of the infection, the species differences, and the immunity of intermediary hosts (39).

Similar to the current study, all cyst sizes fall between one to seven centimeters. In Northeastern Tunisia, Khaled *et al.* (40) and Corda *et al.* (41) discovered that about 70% of cyst sizes fall between one and three centimeters. This leads to the liquid inside the variable-volume, double-membraned cysts that is under pressure. The calcified cysts, which range in diameter from 0.5 to 0.7 cm, are situated in several organs.

According to Al-Hamzawi's (42) study on the quantity and size of cysts in Al-Diwaniyah Province, Iraq, 98% of infected organs, such as the liver or omentum, have one to two cysts, while only 2% of carcasses have three to six cysts. However, when it comes to the size of the cysts, it is discovered that they vary depending on the organs and animal species. For example, the liver has cysts that are smaller than the omentum in sheep (2 cm and 11 cm, respectively). However, in goats, the size of the cysts in the omentum is smaller, about 8 cm. This difference in size can be attributed to the cyst infection duration, the distribution of the cysts in carcasses, and the possibility that the cysts have spread to the lungs.

The present study discovered that the average number of large hooks is 13-15 and the average number of small hooks is 12-15. The average length of sheep and goats' large hooks was 218.33±30.13; 218.40±30.12; the average length of their large hooks was 84.06±14.33; 93.26±18.63; the average length of their large hooks handle was 111.73±11.75; 104.61±25.68; the average length of small hooks was 147.70±12.9; 142.70±18.89; the small hooks blade was 69.86±5.201; 67.66±6.806, and the small hooks handle was 58.56±4.81; 61.66±2.88). These results contrasted with those of Mokhtaria *et al.* (43), who reported that the average length of the large hooks on sheep and goats was 15±1.12; 14.9±0.85, whereas the average length of the small hooks was 15.1±0.72; 15.1±0.79.

According to Radfar *et al.* (44), the averages for sheep and goats' large hooks were 15.33±1.33; 14.66±0.5, whereas the averages for their tiny hooks were 15.44±1.42; 14.77±0.66. Additionally, Singh *et al.* (45) observed that the

average number of hooks was 29-31 for sheep and 28-31 for goats, while the total length of large hooks for sheep and goats was 193.0-207.0; 194-205.0, the large hook blade was 91.0-99.0; 92.0-99.0, the total length of small hooks was 131.0-147.0; 137-147, and the small hook blade was 70.5-77.0; 72.0-78.0, respectively. It's interesting to note that there was no discernible morphometric difference between sheep and goat cysts, since this result concurs with all earlier investigations.

Compared to previous studies in Algeria (46), Iraq (47), and Iranian sheep (48), the biochemical analysis values in the current study were greater. On the other hand, as respects minerals and total protein, they were lower than those stated by previous researchers. The liver enzyme levels in both organs were greater than what Nazifi *et al.* (47) reported for Iranian sheep. The variations in the parasite strain, the organ from which the cyst was extracted, or the animal breed may be the cause of the variations in the biochemical data. The difference in cyst size is caused by differences in the volume of the liquid and its biochemical makeup across different organs and species; for instance, sheep have larger means of triglycerides (g/l) and cholesterol (g/l) than goats, but goats have a higher mean value of glucose (g/l). The length of large versus small hooks is another factor contributing to this disparity (43).

The closest values of base pair and 12S rRNA showed greater *C. tenuicollis* intra-taxon variation than cox1. This was due in part to the phylogenetic tree, which showed the current accession number OL470129 of *C. tenuicollis* cyst isolated from sheep in Matrouh governorate, Egypt, found at base pair 446, and is close to a cyst isolated from delta zone, Egypt, among sheep, with accession number KU671396 at base pair 394. Additionally, *C. tenuicollis* and other members of the Taenia genus can be distinguished from one another using highly discriminative variable portions of the nuclear 18S rRNA gene (49,50).

Regarding the gene sequence accession number OL470130 of *C. tenuicollis* cysts isolated from goats in the governorate of Matrouh, Egypt, at base pair 453, it was discovered that, even though the cysts were isolated from goats in the governorate of Basrah, Iraq, accession number OK356792, at base pair 225, the related gene sequences contributed to the same total amount of protein, which is estimated at cyst antigen and finds 3.4 nm in both Egypt and Iraq. Furthermore, even though the sequencing can confirm our findings and the goat species, the Cox1 gene is amplified and sequenced to determine the genetic pattern of *C. tenuicollis* isolated from goats (51). In addition, compared to earlier research done in other countries, our study revealed greater genetic variation in the Cox1 gene (52). The lack of infection control and prevention measures and the high frequency and rate of *C. tenuicollis* transmission in Matrouh, Egypt, are thought to be the likely causes of this. Furthermore, the parasite's proliferation between sheep and goats may result in greater genetic variation across the several groups of this species found throughout the world (53,54). Certain bands in the PCR reaction were successfully identified by the cox-1 and 12S rRNA primers. The sheep strain's 12S rRNA sequence was remarkably similar to sequences from Iraq and Iran. The cysts collected from sheep in Matrouh, Egypt, were confirmed to be *Cysticercus tenuicollis* by the goat's cox-1 sequence, which was nearly identical to sequences from Egypt and Iran. Additionally, as dogs are the definitive hosts of T. hydatigena, which was also isolated in the current study, it is imperative to explore other parasites linked to dogs (55-57).

In agreement to current study by Dehghan Rahimabadi P, 2024 is found that; the *Cysticercus tenuicollis* infection in the liver, lung, and omentum revealed that; verminous pneumonia is diagnosed through emphysema, thickened inflammatory alveolar wall, and interstitial inflammation. In the omentum, the cyst of the parasite was attached to the omentum, resulting in a proliferating inflammatory reaction. Adhesion of the parasite to the omentum wall, which is highly vascularized, revealed adhesion to the surrounding organs resulting from the massive inflammatory reaction of eosinophils, macrophages, lymphocytes, and plasma cells. The infection of *Cysticercus tenuicollis* in the lung and omentum lead to fibrosis and inflammatory reaction were the most significant pathological findings and may result in peritonitis (58).

Conclusion

The goats were twice as likely as sheep to contract larval stages of *T. hydatigena* because of their scavenging activities, and animal species was the higher risk factor for infection. Therefore, early molecular diagnosis and identification of *Cysticercus tenuicollis* gene sequences among intermediate hosts are essential for developing preventive strategy measures. Also, applied HACCP systems or ISO 22000 and ISO 14001 were essential programs for infection control.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

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التحديد الجزيئي والتشريح المرضي لإصابة عدوى الكيسانية المذنبة رقيقة العنق في المجترات الصغيرة

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الخلاصة

تؤدي عدوى المجترات الصغيرة بواسطة الكيسَةُ المُذَنَّبَةُ الرَّقيقَةُ العُنُق إلى خسائر مالية بسبب إدانة الأعضاء المصابة، وهو ما يمكن أن يحدث في ظروف غير صحية ويعتبر عامل خطر على السمية البيئية. لذلك، تم إجراء دراسة مقطعية لبحث معدل الاصابة الطفيلية للمراحل اليرقية الكيسَةُ المُذَنَّبَةُ الرَّقيقَةُ العُنُق، وعوامل الخطر، والتوصيف الشكلي والنسيجي والكيميائي الحيوي والجزيئي للتسلسل الجيني للأكياس التي تم جمعها من العضو المصاب لـ ١٣٩ خروفاً و ٢٦١ ماعز في مسلخ علام الروم، محافظة مطروح، مصر. بلغ معدل الإصابة الإجمالي ١٩,٢٥%،

مع انتشار أعلى في الماعز (٢٣,٤٠%) مقارنة بالأغنام (١١,٥٠%)، مما يشير إلى أن الأنواع كانت بمثابة عامل خطر كبير للإصابة. ومع ذلك، لم تتم ملاحظة فروق ذات دلالة إحصائية في معدلات الإصابة على أساس العمر أو الجنس، وكانت الأعراض الرئيسية هي الطقطقة الرئوية عن طريق التسمع والألم الحشوي. تم العثور على الأكياس في أغلب الأحيان في الثرب (٧٢,٧٦٪)، تليها الكبد (١٨,١٨٪). كانت هناك فروق ذات دلالة إحصائية في المعايير البيوكيميائية لسائل الكيس بين الأعضاء المختلفة. أكد التحديد الجزيئي من خلال تفاعل البوليميرات المتسلسل والتحقيق التسلسلي لجين cox1 في الماعز و 12S rRNA في الأغنام أن الأكياس هي الكيسَةُ المُذَنَّبَةُ الرَّقيقَةُ العُنُق، وكانت التسلسلات المسترجعة (OL470129 للأغنام وOL470130 للماعز) مطابقة تقريبًا لتلك المبلغ عنها من بلدان أخرى، مما يسلط الضوء على التشابه الوراثي لهذا الطفيل عبر المناطق.