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Prevalence and pathological findings of some parasitic infections of sheep in Egypt

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ABSTRACT

Sheep is reared for their productivity of meat, milk and wool. Parasitic infections are big hazards which threat their productivity. In this study, we examined macroscopically 16605 sheep with infection rates of *Cysticercus tenuicollis* (*C. tenuicollis*), *Cysticercus ovis* (*C. ovis*) and *Fasciola hepatica* (*F. hepatica*) as 4.4%, 1.95% and 0.04%, respectively. The results showed that females and adult sheep are highly infected than males and young sheep. *F. hepatica* was founded in liver, while *C. tenuicollis* was founded in liver and omentum and *C. ovis* infected both cardiac and skeletal muscles (lead to total condemnation in heavily infected sheep) which caused high losses. The pathological examination of liver infected with *F. hepatica* revealed pseudo-lobulation and bile duct hyperplasia while *C. ovis* in muscles showed calcification and granulomatous inflammation and cardiac muscles showed some different shapes of microcysts of *Sarcocystis* spp. The aim of this study was to determine effect of different factors (age, sex and seasons) on prevalence *C. tenuicollis*, *C. ovis* and *F. hepatica* and pathological characters of them on different organs of sheep.

1. INTRODUCTION

Sheep are a major source of meat, milk, and wool around the world, but they are susceptible to various parasitic infections that can negatively impact their health and productivity. Among the key parasites affecting them are *Fasciola hepatica, Cysticercus ovis and Cysticercus tenuicollis.*

Fasciola hepatica (commonly referred to as liver flukes) is trematode that inhabits the liver and bile ducts of sheep. Their life cycle involves an intermediate snail host, and their infections result in fasciolosis, characterized by liver damage, anemia, and reduced productivity. This disease has significant economic consequences, including liver condemnation and reduced growth rates [1].

Cysticercus ovis and *Cysticercus tenuicollis* are larval stages of tapeworms (*Taenia ovis* and *Taenia hydatigena*, respectively) that infect sheep. *C. ovis* forms cysts in skeletal and cardiac muscles called (sheep measles), leading to carcass condemnation in case of heavy infection, while *C. tenuicollis* primarily affects the peritoneum, causing economic losses through organ damage [2]

Although *C. ovis* is neither a flock health nor zoonotic issue, it does impact food quality. *Cysticerci*, both viable and degenerate, are visible in the meat of infected animals rendering it unacceptable for human consumption. As a result, carcasses are condemned at slaughter using guidelines provided by the Food and Agricultural Organization of the United Nations[3, 4]

The aim of this study was to determine effect of different factors (age, sex and seasons) on prevalence of parasitic infection on sheep and pathological effects of parasitism on affected organs.

2. MATERIALS AND METHODS

2.1 Study area and sample collection:

Samples were collected from automated slaughterhouse in Al-Bassateen, Cairo, Egypt, during period from November 2023 to November 2024

2.2. Postmortem (PM) inspection of the slaughtered sheep

Slaughtered sheep were subjected to routine inspection. The liver, lung, heart, omentum, diaphragm, tongue and major skeletal muscles were investigated for the presence of any Fasciola sp (liver flukes), C. ovis and C. tenuicollis by visual inspection, incisions and palpation. The infected organs and muscles with these cysts or flukes were transferred to the laboratory of the Parasitology Department, Faculty of Veterinary Medicine, Menoufia University.

2.3. Mounting of Trematodes and Cestodes samples:

Collected samples were fixed in formalin 10% after compressed between 2 slides and stained with acetic carmine stain then mounted as previously mentioned by. [2].

2.4. Histopathological Examination

Liver, muscle and heart tissue samples were collected and fixed in 10% neutral buffered formalin. Tissue samples were processed and embedded in paraffin wax. Sections were cut with a thickness of 5 μm and stained with hematoxylin and eosin for microscopical evaluation. [5]

2.5. Statistical analysis

A statistical application (GraphPad Prism 9) was used to examine statistical significance differences using Chi-square test.

Chi-square (x2) statistical test was used to determine the associations between the various potential risk factors like age, sex and season, and the prevalence of Fasciola hepatica, Cysticercus tenuicollis, Cysticercus ovis in the examined sheep. A statistically significant association among variables was considered to exist if P value was less than (0.05).

3. RESULTS

1-Prevalence of parasitic infection in the inspected sheep:

Out of the 16605 examined sheep, there were 730 (4.4%) *C. tenuicollis* in liver and omentum, 323 (1.95%) *C. ovis* in cardiac and skeletal muscles and 7 (0.04%) *Fasciola* spp. in liver

Infection rate of *F. hepatica* in males was (0.02%) while in females was (0.9%) and in young (0.01%) while in adult (0.69%). In this study infection of slaughtered sheep with *C. tenuicollis* was higher in female and adult (26.2% and 24.5%) than male and young (3.9%, 3.4%) respectively. Cardiac and skeletal muscles of slaughtered sheep were affected by *C. ovis* in males (1.75%) while in female the infection rate was (11.4%). There were totally condemned 5 rams due to heavy infestation in cardiac and all skeletal muscles of body by *C. ovis* in which a higher infection rate in adult compared to young sheep as 8.8% *vs.*1.6%. The age and gender had a significant effect on infection rate of *F. hepatica*, *C. tenuicollis* and *C. ovis* as shown in **Table 1**.

The infection rate differs in different seasons as in case of *F. hepatica* there is no infection in winter while infection is high in spring and summer. Infection with *C. tenuicollis* is higher in winter (5.6%) than spring (4.6%), summer (4.4%) and autumn (3.5%). The infection rate in *C. ovis* was similar to *C. tenuicollis*, which winter is the highest (2.2%) and autumn is the lowest (1.8%). The season had a significant effect on infection rate of *C. tenuicollis*, while there is no significant relationship between season and infection rate of *F. hepatica* and *C. ovis* as shown in **Table 2.**

2-Gross and microscopic morphological characters of detected parasites:

a- *Fasciola hepatica* in liver:

Grossly liver showed numerous whitish tortuous tracts ranging from (0.5 – 2) cm length that caused by migration of immature and adult worm. Bile duct also contains brown fluids and some adult worms. Their length is from (2 to 3) cm (Fig.1 A,B &C).

Under dissecting microscope the worm has an oral and ventral suckers with uterus filled with golden yellow eggs (Fig.1D).

b- Cysticercus tenuicollis:

c- Grossly, liver and omentum of infected sheep showed *Cysticercus tenuicollis* which is a bladder with different sizes that filled with fluids with the invaginated scolex inside it. The cyst is hanged superficially to these organs not embedded inside it (Fig. 2 A&B).

Under dissecting microscope the structure of scolex appear which contain 4 suckers and hooks (Fig. 2 C).

Table (1): Infection rate of *Fasciola* hepatica, *Cysticercus. tenuicollis* and *Cysticercus. ovis* in slaughtered sheep according to sex and age:

	Inspected /infected (%)	Male	Female	X^2	P- value	Young (9m-2y)	Adult (>2:4y)	X^2	P- value
F .hepatica	Ins. 16605	16273	332			15881	724		
	Inf. 7 (0.04%)	4 (0.02%)	3 (0.9%)	86.82	0.0003*	2 (0.01%)	5 (0.69%)	75.54	<0.0001*
C. tenuicollis	Ins. 16605	16273	332			15881	724		
	Inf. 730 (4.4%)	643 (3.9%)	87 (26.2%)	383.4	<0.0001*	552 (3.4%)	178 (24.5%)	734.2	<0.0001*
C. ovis	Ins. 16605	16273	332			15881	724		
	Inf. 323 (1.9%)	285 (1.75%)	38 (11.4%)	160.3	<0.0001*	259 (1.6%)	64 (8.8%)	188.2	<0.0001*

(Ins.: inspected, Inf.: infected, m: month, y: year, *: significant P- value <0.005)

Table (2): Infection rate of *Fasciola* hepatica, *Cysticercus. tenuicollis* and *Cysticercus. ovis* in slaughtered sheep according to season:

	Inspected /infected %	Winter	Spring	Summer	Autumn	X^2	P-value
F. hepatica	Ins. 16605	1856	4492	6610	3647	1.638	0.6518
	Inf. 7 (0.04%)	0 (0%)	3 (0.06%)	3 (0.04%)	1 (0.02%)		
C. tenuicollis	Ins. 16605	1856	4492	6610	3647	13.97	0.003*
	Inf. 730 (4.4%)	104 (5.6%)	209 (4.6%)	289 (4.4%)	128 (3.5%)		
C. ovis	Ins. 16605	1856	4492	6610	3647	1.883	0.5970
	Inf. 323 (1.9%)	41 (2.2%)	94 (2.09%)	122 (1.8%)	66 (1.8%)		

d- Cysticercus ovis:

Grossly, old *C. ovis* appeared as whitish solid mass like rice grains that embedded in muscles due to calcification and losing of fluids (Fig 3 A&B) while the fertile cyst is a bladder like and contains fluids with scolex inside it (Fig.3C).

Under dissecting microscope the cyst appears containing invaginated scolex with suckers and hooks (Fig 3 D).

3- Histopathological findings:

a- Fasciola hepatica in liver

Histopathological examination of liver tissue samples from sheep infected with *F. hepatica* showed the migrating parasite within hepatic tissue forming migratory tunnels (Fig.4 A) with fibrous connective tissue thick wall. The portal area showed

severe bile duct hyperplasia, dilatation of lymphatics and proliferation of fibrous connective tissue (Fig. 4 B &C).

The hepatic parenchyma showed pseudo-lobulation due to sever fibrosis (Fig. 4 B) and focal areas of necrosis infiltrated with mononuclear cells (Fig.4 D).

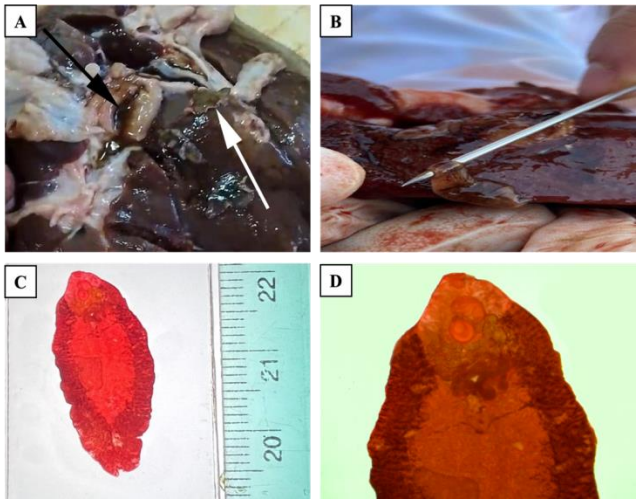

Fig. 1. *Fasciola hepatica* in liver of sheep: (A): infected liver of sheep showing adult worm and tunnels that it makes. (B): Adult *F. hepatica* from infected liver (on needle). (C): stained whole adult worm (*F. hepatica)* measuring 2.5 cm. (D): stained worm under microscope showing oral, ventral suckers and uterus filled with eggs (x4).

b- *Cysticercus ovis* in muscles:

Histopathological examination of muscular tissue samples from sheep infected with old *Cystcercus ovis* showed granulomatous inflammation with old necrotic calcified areas surrounded by thick connective tissue capsules (Fig. 5A&B).

c- *Sarcocystis sp.* in cardiac muscles:

Histopathological examination of cardiac muscles from sheep showed elongated microcyst of *Sarcocystis* sp. packed with banana-shaped bradyzoites (Fig. 6 A, B &C).

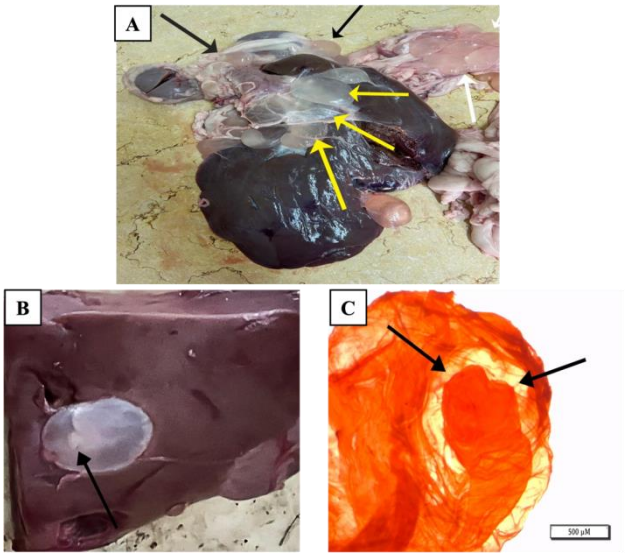

Fig.2. *Cysticercus tenuicollis* in infected sheep: (A): viscera (liver, omentum & kidney) of old ewe heavily infested with *C. tenuicollis*(black and yellow arrows) (B): liver of ram infested with *C. tenuicollis* appear as a bladder filled with fluids with scolex in the whitish fluid(black arrow). (C)Stained and mounted *C. tenuicollis* showing invaginated *scolex* with suckers and hooks (black arrows).

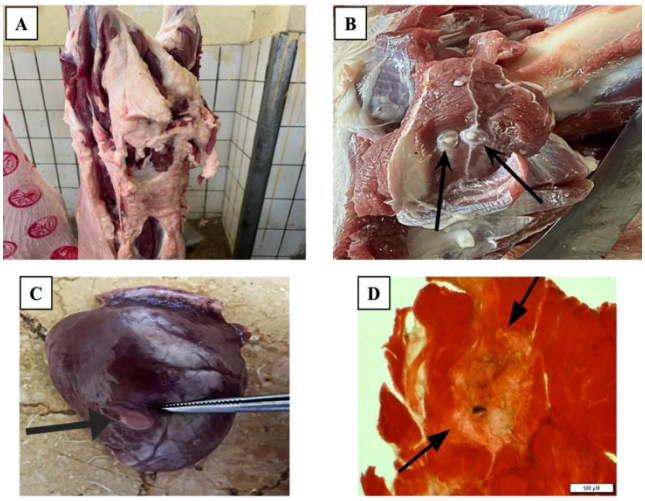

Fig.3. *Cysticercus tenuicollis* in infected sheep: (A): viscera (liver, omentum & kidney) of old ewe heavily infested with *C. tenuicollis*(black and yellow arrows) (B): liver of ram infested with *C. tenuicollis* appear as a bladder filled with fluids with scolex in the whitish fluid(black arrow). (C)Stained and mounted *C. tenuicollis* showing invaginated *scolex* with suckers and hooks (black arrows).

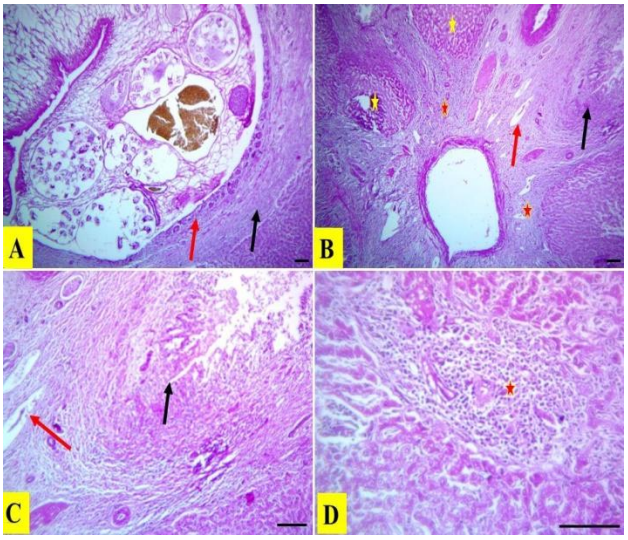

Fig. 4. A: *Fasciola* spp. (red arrow), connective tissue (black arow) (H&E X 10). **B**: bile duct hyperplasia (black arow), dilatation of lymphatics (red arrow) and proliferation of fibrous connective tissue (red star) and pseudo lobulation (yellow star) (H&E X 10). **C**: Higher magnification from B (H&E X 20). **D**: focal areas of necrosis infiltrated with mononuclear cells (red star) (H&E X 40). Scale bar =100 µm.

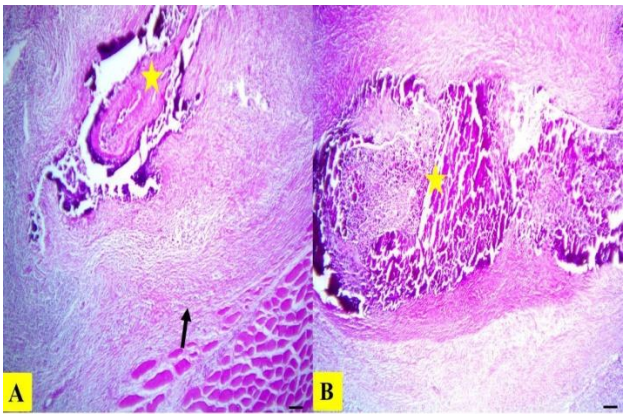

Fig. 5. Old *Cystcercus ovis* granuloma; necrotic calcified areas (yellow star), thick connective tissue capsules (black arrow) (A & B) (H&E X 10). Scale bar= 100 µm.

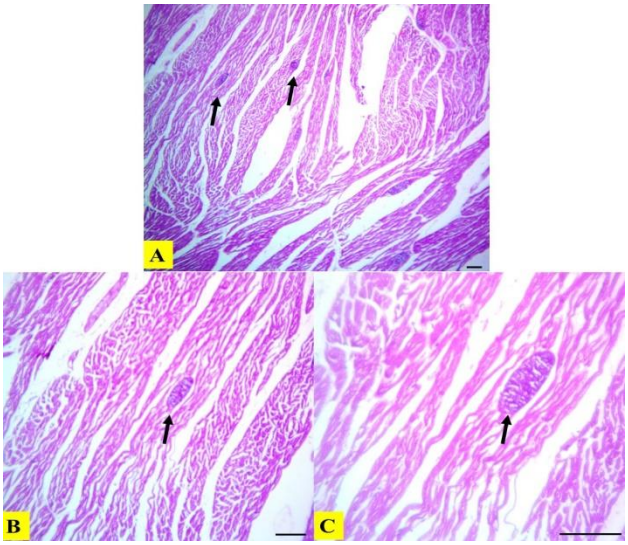

Fig. 6. cardiac muscles of sheep; *Sarcocystis* sp. packed with banana-shaped bradyzoites (black arrow) (A, B &C) (H&E X 10, 20 &40 respectively). Scale bar 100= µm.

4. DISCUSSION:

The result of infection rate of *F. hepatica*(0.04%) was lower than infection rates of (14.7%&0.41%) previously reported in slaughtered sheep in Egypt by [6] &[7] and 0.67% in slaughtered sheep in Jeddah which reported by [8]. The high difference in the infection rates between now and the pervious time may be due to the high awareness of people of importance to use the good control &deworming program in rearing of sheep which lead to decrease infection rates of *Fasciola spp*.

In this study, The highest infection rate of *F. hepatica* was observed in spring (0.06%) followed by summer (0.04 %), fall (0.02%), this agreed with [8] in which The highest infection rate was observed in spring (2.43%) followed by summer (2.15%), fall (1.59%) and then winter (0.96%).There was no statistically significance of *F. hepatica* in regards to seasons (P>0.05).

In this study, liver shows numerous whitish tortuous tracts ranging from (0.5 – 2) cm length that caused by migration of immature and adult worm. Bile duct also containing brown fluids and some adult worms and calcified in cut section. This previously reported by [8] and [9].

Histopathological findings of liver infected with *F.hepatica* in this study showed that the migrating parasite within hepatic tissue forming migratory tunnels with fibrous connective tissue thick wall. The portal area showed severe bile duct hyperplasia, dilatation of lymphatics and proliferation of fibrous connective tissue. The hepatic parenchyma showed pseudo lobulation

due to sever fibrosis and focal areas of necrosis infiltrated with mononuclear cells and eosinophiles. This is previously reported in [8] and [9].

Cysticercus tenuicollis infection rate in this study is (4.4%) which is similar to reported by [10] (4%) and [11] in India, while it is lower than the infection rate reported by [12] 13.4% [13] 16% and [14] 51.9%.

Infection rate *of C. tenuicollis* is higher in female (26.2%) than male (3.9%) this result agreed with [13], [15] but differ from the result reported by [10] and [16] which found infection rate is higher in males (26%) and (2.7%) than females (23.9%) and (2.15%) respectively. The infection rate is always recorded due to high association between dogs and sheep in different parts of world so the chances of infection are possible.

The infection rate of *C. tenuicollis* was higher in adult sheep (24.5%) than in the young ones (3.4%) This result agreed with, [15], [13] and [12].but differ from [16] which found that infection rate in young (4.1%) than adult (1.9%). High infection in adult is maybe due to adult live more than young so the risk of infection and opportunity of association with dogs is high.

According to seasonal variation in this study, the highest rate of infection is in winter (5.4%) and the lowest rate is in autumn (3.5%). This result is differ to [12] in which the highest rate was in the autumn. There was very highly significant differences in slaughtered sheep ($P<0.0001$) while the result of [12]was non-significant ($P>0.05$).

The result of infection rate of *Cysticercus ovis* in this study was (1.9%) which agreed with the result of [16] in Iran and [17] in Egypt which was 1.27% and 2.02% respectively.

In this study the infection rate of *C. ovis* was higher in females (11.4%) than males (1.75%), this agreed with [12] but differ with [17] as they reported high infection in males than females.

Older sheep (8.8%) is more susceptible to infection with *C. ovis* than younger one (1.6%) which agreed with [12] and [18]. This may due to adults live more than young and chances of infection is higher in them.

According to seasonal variation the highest infection rate is in winter (2.2%) followed by spring (2.02%) , then summer and autumn with the same rate (1.8%), this differ with[12] and [16] which found the highest rate in spring(1.8%) (4.1%) and the lowest was in autumn and winter respectively. There was no statistically significance of *C. ovis* in regards to seasons ($P>0.05$).

C. ovis appear as small whitish cysts filled with fluid that contain an immature worm this was agree with [13] and [12] ,While old *C. ovis* appear as whitish solid mass that embedded in muscles due to calcification and losing of fluids.

Histopathological findings showed that granulomatous inflammation with old necrotic calcified areas surrounded by thick connective tissue capsules which agreed with [18].

During histopathological examination for cysticercosis in sheep heart, the filed showed some microcyst (*Sarcocystis sp.*) with different shapes &length which maybe oval and elongated. These microcysts contain banana shape bradyzoites that is previously reported by [19] in Egypt and [20] in Peru.

5. CONCLUSIONS

. During this survey on slaughtered sheep in the slaughterhouse of El-basateen, cysticercosis is the most prevalent parasitic disease followed by Fasciolasis. Infection rate with *F. hepatica* in this study was lower when compared with previous studies. This indicates that the control of this parasite increased due to its economic importance to avoid its losses. Cysticercosis (*Cysticercus tenuicollis* and *Cysticercus ovis)* had high infection rate. The preventative measures against them should be increased to avoid its losses because they lead to condemnation of many parts of sheep meat which become unacceptable by people for consumption.

6. ACKNOWLEDGEMENTS

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Authors' declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

Conflict of interests.

All authors have stated the absence of any conflicts of interest.

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Authors' contributions.

M.A.G.: Data collection, Conceptualization, Formal Analysis, , Resources, Writing
S.A.: Data collection
R.M.F.: Data collection
M.A.M.: Writing, Editing and Supervision
A.R.E.: Review, Editing and Supervision
E.K.B: Conceptualization, Investigation Supervision, Formal Analysis, Review Editing
All authors have read and agreed to the published version of the manuscript.

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