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## **SOME STUDIES ON PARASITIC LIVER AFFECTIONS OF SHEEP IN ASSIUT GOVERNORATE**

(With 5 Tables and 2 Plates)

By

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**بعض الدراسات على إصابة الكبد الطفيلية في الأغنام في محافظة أسيوط**

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تم إجراء مسح على طفيليات الكبد لعدد ٢٥٠ من الأغنام مختلفة الأعمار والجنس ذبحت بمجازر أسيوط المختلفة وذلك لاستبيان نوع ونسبة الإصابات الطفيلية للكبد. بلغت نسبة الإصابة الكلية بطفيليات الكبد ٢٩,٦% وتم تشخيص الأطوار الطفيلية التالية: الديدان الكبدية (فاشيولا جايجانتিকা ١٤% وفاشيولا هيبنتاكا ٩,٢%) وسيستمرسكس تينيكوللس ٤,٨% والحويصلات المائيه ٢,٤% تم تشخيص الإصابة بالديدان الكبدية عن طريق فحص عينات البراز والحويصلات المرارية لوجود البويضات بالإضافة إلى فحص الكبد للتعرف على نسبة الإصابة بالديدان الكبدية. أوضحت الدراسة أن أمثل طريقة لتشخيص الديدان الكبدية هي عن طريق الكشف عن البويضات في الحويصلات المرارية حيث بلغت نسبة الإصابة بها ٢١,٢% يليها عن طريق التعرف على الديدان الكبدية في الكبد ١٩,٦% ثم عن طريق الكشف عن البويضات في البراز ١٢,٤%. بلغت نسبة الخصوبة والعقم والستكلس للحويصلات المائية (أكياس العطش) ٣٣,٣% ، ١٣,٣% و ٥٣,٣% على التوالي وأوضحت الدراسة أن نسبة الإصابة بطفيليات الكبد تزداد مع تقدم العمر كما أنها تكون في الإناث أعلى من الذكور.

### **SUMMARY**

A parasitological survey was carried out on the livers of 250 sheep of different ages and sex slaughtered in Assiut slaughter houses for studying the prevalence of different hepatic parasites. The overall prevalence rate of infection with hepatic parasitic infection was 29.6%. The detected parasites were: *Fasciola spp.* 19.6% (*F.gigantica* 14%, *F.hepatica* 9.2%), *Cysticercus tenuicollis* 4.8% and hydatid cysts 2.4%. Diagnosis of fascioliasis was carried out through faecal, gall bladder

examination in addition to comparison of these methods with P.M. examination for detection of adult worms in the liver. Diagnosis of fascioliasis by detection of eggs in the gall bladder was the best method of diagnosis (21.2%) followed by detection of worms in the liver (19.6%) and by detection of eggs in the faeces (11.6%). The prevalence rate of fertile, sterile and calcified hydatid cysts was 33.3%, 13.3% and 53.3% respectively. The present study showed that the prevalence rate of different parasites encountered in the liver was comparatively higher in females than in males. On the other hand it was higher in aged animals than in young ones.

*Key words: Sheep, Parasitic liver affections, Diagnosis of fascioliasis.*

## INTRODUCTION

Sheep are one of the major important sources of animal protein for human consumption. Hepatic affections of sheep are of great effects on the general body health. Moreover the parasitic affections of the liver lead to decrease of milk, wool and meat production beside the condemnation of the affected livers (Cawdery, 1984). The common parasites affecting liver of sheep were: *Fasciola spp.*, *Cysticercus tenuicollis* of *Taenia hydatigena*, hydatid cysts beside *Stilesia hepatica* (Soulsby, 1982).

Nowadays human fascioliasis is becoming an increasingly important global problem in many countries. In Egypt, fascioliasis is an increasing problem in man (Haseeb *et al.*, 2002) and animals (Haridy *et al.*, 1999). Fascioliasis is an important zoonotic disease in the world wide and infected animals are considered the main source for human fascioliasis (El-Shazly *et al.*, 2002). Fascioliasis causes great economic losses to the livestock industries due to both direct effects, actual liver condemnation at slaughter houses and indirect effects such as decrease in feed efficiency, weight gains, milk production and reproductive performance (Malone, 1986).

*Cysticercus tenuicollis*, the larval stage of *Taenia hydatigena* was reported in the liver, mesentery and omentum of sheep (Jones *et al.*, 1997). Liversey *et al.* (1981) noticed sudden death among 1-3 month old housed lambs naturally infected with *Cysticercus tenuicollis*.

Hydatidosis due to *Echinococcus granulosus* is a chronic disease

of zoonotic importance occurring in several herbivorous animals and man, and it is known to be endemic in the Middle East (FAO 1993). It has a worldwide distribution (Craig *et al.*, 1996). Hydatidosis has been reported in both urban and rural communities where dogs, the main definitive host, have access to raw offal through home slaughter, poorly regulated abattoirs, scavenging carcasses and discarded offals (Watson-Jones *et al.*, 1997). Echinococcosis is a cyclozoonosis that require two vertebrate hosts to uphold the life cycle, while most cysts develop in the liver and lungs, other organs and tissues may become affected. It has a high economic losses from the condemnation of affected organs (Soulsby, 1982).

The aim of the present work is to investigate the various species of liver parasites and their prevalence in slaughtered sheep at Assiut Governorate. In addition to evaluate different methods for diagnosis of fascioliasis through faecal, gall bladder examination and comparison of these methods with P.M. examination for detection of adult worms in the liver.

## **MATERIALS and METHODS**

### **Animals:**

A total number of 250 sheep (native breeds) of different age and sex from different abattoirs at Assiut Governorate were subjected to examination for parasitic liver infection.

### **Samples:**

Faecal and gall bladder samples were collected from each animal with regarding to age and sex. The collected samples were immediately transported to parasitology laboratory and examined for the presence of various parasitic liver infections.

Faecal samples and gall bladder scrapings were microscopically examined for the presence of *Fasciola spp.*eggs.

Faecal samples were microscopically examined by both direct and concentration sedimentation techniques according to Soulsby (1982).

P.M. examination was carried out on livers of slaughtered sheep. The liver's capsule was examined grossly and many incisions were made through the whole hepatic tissue. Gross picture of the infected livers was described.

Animal gall bladders were firstly inspected for presence of adults of *Fasciola spp.* The collected *Fasciola* worms were processed, mounted and identified according to Soulsby (1982) and Georgi (1985).

Collected cysticerci and hydatid cysts were described grossly for the size, shape, dimensions and nature of their contents.

The fertility of the hydatid cysts was judged by the presence of protoscolices, or brood capsules and the cysts which did not show them were described as sterile cysts (Soulsby, 1982).

Viability of protoscolices of hydatid cysts was assessed by muscular movements, motility of the flame cells and by 0.1% eosin exclusion test (evaluated under light microscope). Staining was done by putting 0.01 ml of pooled protoscolices over a slide, mixed by 0.01 ml eosin (0.1%) and was evaluated by low power microscopy after one minute. Dead protoscolices absorbed eosin and colored red while alive ones remained colorless (Himonas *et al.*, 1994).

#### **Statistical analysis:**

Obtained data were subjected to a software program (SPSS) according to Borenstein *et al.* (1997).

## **RESULTS**

Out of the 250 examined cases 67 cases (26.8%) proved to be affected with parasitic infections in their livers (Table 1).

The examined livers of sheep in this study showed that they were infected with the following parasitic stages: *Fasciola spp.* (19.6%) (*Fasciola gigantica* 14%, *Fasciola hepatica* 9.2%), *Cysticercus tenuicollis* (4.8%) and hydatid cyst (2.4%) (Table 1)

#### **Fascioliasis:**

Out of the 250 examined sheep 49 (19.6%) were infected with *Fasciola spp.* Both *Fasciola gigantica* (14%) and *Fasciola hepatica* (9.2%) were detected in the infected livers. (Plate I Fig. 1).

#### **Gross picture:**

Most of the infected livers were enlarged, dark brown in color, hard and tough in consistency. The gall bladder was greatly distended with bile (Plate, I Fig. 3). Thickened fibrous and dilated bile ducts were filled with adult flukes, while immature flukes were found in the liver parenchyma.

On cut section of the liver it revealed presence of mucoid greenish inspissated bile accompanied with immature flukes.

Table (2) summarized the prevalence of fascioliasis infection rate among slaughtered sheep by various methods of diagnosis. The overall infection rates were 29 (11.6%), 53 (21.2%) and 49 (19.6%) out of the 250 examined sheep as analysed by detection of eggs in faeces, gall bladder and worms in liver respectively. *Fasciola spp.* that were detected in this study were *F.hepatica* and *F.gigantica* depending upon the morphological criteria of the adult flukes extracted from the bile duct and gall bladder of the examined slaughtered sheep (Plate I Fig. 1).

Total liver condemnation was recorded in 15 cases. The average number of fluke burden ranged from 40-250 fluke per liver.

In the present study in the liver of one infected sheep a hazelnut sized cysts containing a yellowish purulent gelatinous material were found in which *Fasciola spp.* eggs were detected by impression smears.

#### **Cysticercosis:**

*Cysticercus tenuicollis* (the larval stage of *Taenia hydatigena*) was recorded in 12 cases (4.8%) out of the 250 examined sheep.

#### **Gross picture:**

*C.tenuicollis* appeared as relatively large thin walled cysts attaining a diameter of 30-50 mm. The cyst was transparent grayish white in color and attached to the surface of the liver by long neck and has an invaginated scolex. (Plate II Fig. 1).

The affected liver was enlarged and dark in color with multiple cysts attaching to liver tissues along the contact surface.

Hepatitis cysticercosa caused by massive infection with *Cysticercus tenuicollis* was recorded during the present study (Plate II Fig. 2)

**Hydatid cyst:** The prevalence rate of hydatid cysts in livers of slaughtered sheep was 2.4%. Most of the cysts were of 5 mm to 3cm in diameter, but it reached the size of 5 cm in some cases. The cysts were oval, rounded or of variable size and shape. Viable cysts were grayish, soft in texture, contained turbid fluid either white or pale yellowish in color and splashed on cutting.

Degenerated or dead cysts were surrounded by thick fibrous capsule containing gelatinous material. Older cysts were hard in consistency and calcified with pus in the center. Concerning the condition of the hydatid cysts which were recovered from the examined animals, the presence of protoscolices or brood capsules as shown in (Plate II Fig. 5) indicated fertility of the cysts. On the contrary the

aspirated fluid did not contain any protoscolices or brood capsules in case of sterile hydatid cysts.

It was found that the total percentages of fertile, sterile and calcified sheep hydatid cysts were 33.3%, 13.33% and 53.3% respectively (Table 3). The percentage of the viability of protoscolices of fertile hydatid cysts was 75.82%, the viability of protoscolices was assessed by muscular movements, flame cells motility and by 0.1% eosin stains (evaluated under light microscope). Dead protoscolices absorbed eosin and colored red while alive ones remained colorless.

The effect of both age and sex on the prevalence rate of various parasites recovered from the liver of slaughtered sheep was summarized in (Table 4, 5), which points out that the rate of infection was higher in the aged animals than in young ones. On the other hand it was comparatively higher in females than in males.

**Table 1:** Prevalence of various parasites recovered from the livers of 250 slaughtered sheep.

No. of examined sheep (250)	<i>C.tenuicollis</i>	Hydatid cyst	Fascioliasis				Total <i>Fasciola spp.</i> infected livers	Total
			<i>F.gigantica</i>	<i>F.hepatica</i>	Mixed infection			
No.of infected animals	12	6	26	14	9	49	67	
% of infected cases	4.8%	2.4%	10.4%	5.6%	3.6%	19.6%	26.8%	

**Table 2:** Fascioliasis infection rate among slaughtered sheep by detection of eggs in faeces, gall bladder and worms in liver

No. of sheep	Fascioliasis among sheep					
	Eggs in faeces		Eggs in gall bladder		Worms in liver	
	No + ve	%	No. + ve	%	No. + ve	%
250	29	11.6%	53	21.2%	49	19.6%

**Table 3:** Condition of the hydatid cysts recovered from the liver of slaughtered sheep.

No. of cysts examined	Fertile		Sterile		Calcified		Viability
	No.	%	No.	%	No.	%	
15	5	33.3%	2	13.33%	8	53.3%	75.82%

**Table 4:** Relationship between infection rate with different parasites recovered from the liver and age of slaughtered sheep.

Slaughtered sheep		Type of parasites							
		<i>Fasciola spp.</i>		<i>C.tenuicollis</i>		Hydatid cyst		Total	
Age	Total No.	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%
Adults (over one year old)	200	45	22.5%	11	5.5%	6	3%	62	31%
Lambs (aged up to one year)	50	4	8%	1	2%	0	0	5	10%
Total	250	49	19.6%	12	4.8%	6	2.4	67	26.8%
Statistical analysis		$X^2 = 5.33$ $P < 0.05$		$X^2 = 0.85$ $P > 0.05$		$X^2 = 1.53$ $P > 0.05$			

**Table 5:** Relationship between infection rate with different parasites recovered from the liver and sex of slaughtered sheep.

Slaughtered sheep		Type of parasites							
		<i>Fasciola spp.</i>		<i>C.tenuicollis</i>		Hydatid cyst		Total	
sex	Total No.	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%
Male	75	12	16%	2	2.66%	1	1.33%	15	20%
Female	175	37	21.14%	10	5.71%	5	2.85%	52	29.7%
Total	250	49	19.6%	12	4.8%	6	2.4%	67	26.8%
Statistical analysis		$X^2 = 0.88$ $P > 0.05$		$X^2 = 1.07$ $P > 0.05$		$X^2 = 0.52$ $P > 0.05$			

\* No mixed infection was detected.

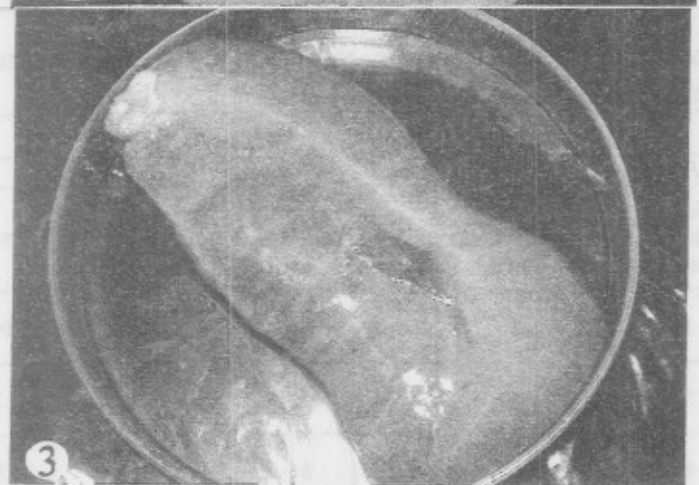
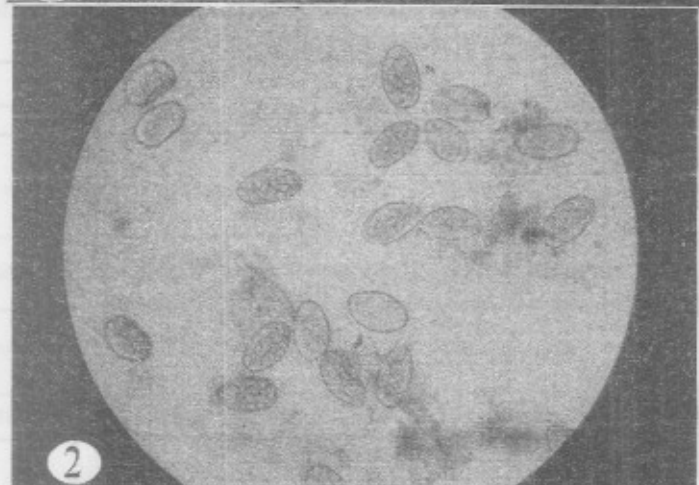
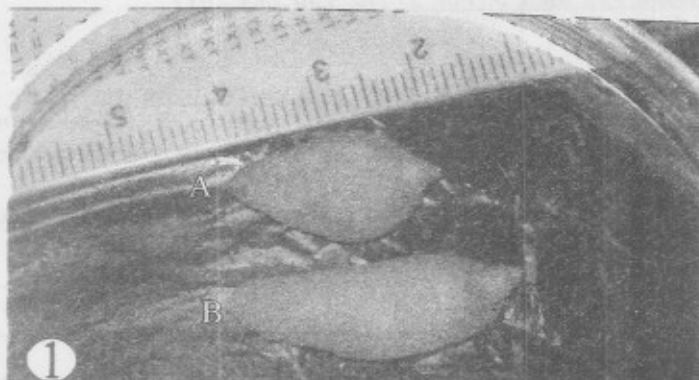


Plate I: Liver of sheep infected with fascioliasis

1-Adult *Fasciola* worm    A) *F. hepatica*    B) *F. gigantica*

2-*Fasciola* spp. eggs recovered from the gall bladder. X40

3-Gall bladder greatly distended with bile in sheep infected with fascioliasis.



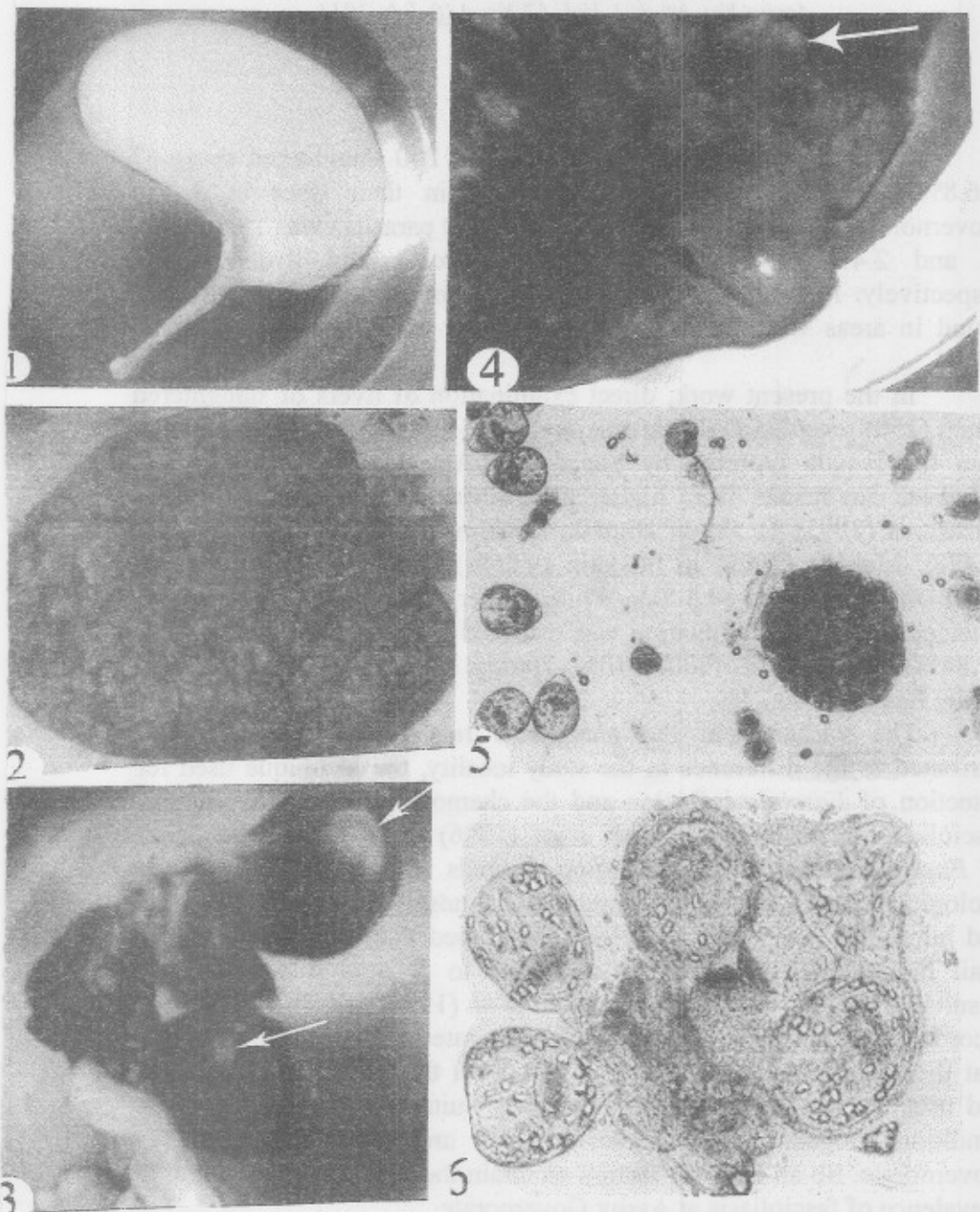


Plate II: Liver of sheep infected with *Cysticercus tenuicollis* and hydatid cysts.

1- *Cysticercus tenuicollis* removed from the liver of infected sheep.

2- Hepatitis cysticercosa caused by massive infection with *C.tenuicollis*.

3-Normal hydatid cyst in sheep liver (arrow)

4- Calcified Hydatid cyst in sheep liver (arrow)

5-Hydatid sand of fertile H.cyst showing protoscolices, brood capsules, and calcareous corpuscles (unstained X 40)

6-Protoscolices attached to the wall of the brood capsule by slender stalk (unstained X100)

## DISCUSSION

The present study revealed that out of 250 slaughtered sheep 67 (26.8%) cases showing various parasites in their liver in Assiut Governorate. The rate of affections of different parasites was 19.6 %, 4.8 % and 2.4% of *Fasciola spp.*, *C.tenuicollis* and hydatid cysts respectively. Fascioliasis as parasitic disease of zoonotic importance is found in areas where sheep, cattle and other herbivorous animals are raised.

In the present work, direct examination of livers of slaughtered sheep (250) revealed *Fasciola spp.* worms in 49 (19.6%) which is lower than the results reported by Sanad and Al. Megrin (2005) 21.9%. However our results were higher than those recorded by Nwosu and Strastava (1993) 15.5% in Nigeria, Ibrahim *et al.* (2002) in Matrouh (8.4%), Mahran (2005) in Shalatin (4.66%) and El-Bakry and Arafa (2008) in Alexandria (9.5 %). While higher prevalence of fascioliasis detected by faecal examination was recorded by El-Shazly *et al.* (2002) 17.84%, Morsy *et al.* (2005) 40%, Haridy *et al.* (2006) 30-40% and Abdel Rady (2007) 25%.

The variation in the prevalence rate of infection may be attributed to the difference in the study locality, the technique used for detection of *Fasciola* infection and the chemotherapeutic used against fascioliasis for animals. Urquhart *et al.* (1996) reported that prevalence of *Fasciola* infection in any region depends on presence of suitable ecological conditions for the intermediate hosts, suitable temperature and humidity. Esteban *et al.* (1997) mentioned that the high ability of snail intermediate host and larval stages to resist and adapt severe unsuitable ecological conditions. Salem *et al.* (1990) and El-Bahy (1997) recorded that the higher *Fasciola* infection rate in sheep may be due to that these animals usually graze in low level than cattle and buffaloes and usually changing from place to place. Suitable ecological, climatic conditions together with *Lymnea* snail are available in Assiut Governorate. So all of these factors ascertain the present work regarding prevalence of fascioliasis at Assiut Governorate.

Concerning the methods of diagnosis of fascioliasis in sheep, it was found that detection of eggs in the gall bladder was the best method of diagnosis (21.2%) which was higher than those depending on detection of eggs in the faeces (11.6%) and worms in the liver (19.6%). These findings agreed with that reported by Abdel-Aal *et al.* (1999) who

recorded that the overall infection rates of fascioliasis in sheep were 12.4% and 22.9% as diagnosed by faecal and gall bladder examination respectively. The present results confirms the results of Sanad and Al-Megrin (2005) who reported that the infection rate depending on detection of eggs in the faeces (13.5%) was less than that depending on detection of worms in the liver (21.9%). They interpreted their results by the associating pathological changes in the liver and biliary ducts that may hinder passage of eggs to the intestine or it may be due to damage of eggs during their passage due to their thin shell. Haseeb *et al.* (2002) reported that *Fasciola* eggs are usually excreted at small number and at irregular pattern and may not appear in the faeces of lightly infected hosts. The interpretation reported by Haseeb *et al.* (2002) was more satisfactory and this may be interpreted by presence of immature worms in liver after recent infection hence no detection of eggs in the faeces or may be due to presence of small numbers of adult worms in bile ducts and gall bladders.

On the other hand, the infection rate depending on detection of eggs in the gall bladder (21.2%) was higher than that depending on detection of adult worms in the liver (19.5%). This may be interpreted by the fact that animals exposed to infection with fascioliasis and treated by chemotherapeutical drugs leading to improvement, disappearance of clinical signs and elimination of the adult flukes from the liver parenchyma and bile ducts whereas *Fasciola spp.* eggs are still attached to the mucous membrane of the gall bladder. The present result confirms the results of Abd el Wahed *et al.* (1997) who reported that some eggs of *Fasciola spp.* appeared in faecal samples of three sheep after 14 days from treatment and disappeared after that and they attributed that to the discharging of remaining eggs in the gall bladder.

In the present work detection of the characteristic *Fasciola spp.* eggs in the contents of the cysts found in the liver of an infected sheep may confirm the records of Urquhart *et al.* (1996) who mentioned that sometimes fluke eggs provoke a granuloma like reaction which can result in obliteration of the affected bile ducts.

### **Cysticercosis:**

The present study showed that 4.6% of sheep were infected with *Cysticercus tenuicollis*. Nearly similar results were recorded by Ibrahim *et al.* (2002) 4.6%, Cabrera *et al.* (2003) 5 % and Mahran (2005) 4%. Our results were lower than those reported by Fathi and Abdel-Hasseb (2005) 9.2% and El-Bakry and Arafa (2008) 11.6%. Trees *et al.* (1985)

reported that locally *Taenia hydatigena* causes very high rates of liver condemnation in a North Yorkshire farm and it is probable that nationally the major cause of lesions and losses in lamb's livers.

Hepatitis cysticercosa caused by massive infection with *C. tenuicollis* which was recorded during the present work agreed with the records of Urquhart *et al.* (1996). The gross picture of *Cysticercus tenuicollis* described in the present work was similar to those reported by Soulsby (1982); Urquhart *et al.* (1996); Ibrahim *et al.* (2002).

### **Hydatidosis:**

The prevalence rates of cystic echinococcosis in livestock animals are most reliable indicators of the extent of environmental contamination with *Echinococcus granulosus* eggs in the investigated area and may indicate a potential risk for human infection (Ibrahim and Craig, 1998). The prevalence of hydatid cysts detected in sheep livers in the present study was 2.4%. Nearly similar findings were reported by Rahman *et al.* (1992) 1.33%, Anwar *et al.* (1993) 2.83 %, and Mahran (2005) 3%. Our results were higher than those reported by other workers in Egypt (Sedik *et al.*, 1976) 0.2%, El-Mossalami *et al.* (1986) 0.13% and FAO (1993) 0.27%). Meanwhile our results were lower than those reported by Bortoletti *et al.* (1990) 91.3%, Magzoub *et al.* (1992) 32%, Ibrahim and Craig (1998) 15.8% and Abu-Eisha (1999) 4.8%. Such variation may be due to the fact that such animals were reared at different localities. Moreover the high incidence of hydatidosis among sheep may be attributed to their close association with dogs. In addition to the fact that sheep graze close to the ground over a very wide area where the abundance of stray dogs and the chance of ingesting contaminated herbage are high (Ibrahim and Craig, 1998). During the present study dogs were frequently observed inside and around the slaughter houses including areas where offals were kept. In the present work the fertility rate of hepatic hydatid cysts of sheep was 26.6%. This result was lower than that recorded by Hafeez *et al.* (1994) 84.9% , Hosseini and Eslam (1998) 88%, Ibrahim and Craig (1998) 86.3%, Abou-Eisha (1999) 34.1%, Fathi and Abdel-Hasseb (2005) 30% and Mahran (2005) 33.3% on the other hand Bortoletti *et al.* (1990) recorded that 75.7% of examined sheep harbored sterile hydatid cysts. Saad and El-Abin (1982) attributed the higher percentage of fertile cysts in sheep to selective permeability of the cyst membrane. The degree of the rates of cyst fertility determine the real role of a particular species of food animals in the cycle of infection with echinococcosis (Gusbi *et al.*,

1990). Concerning the viability of protoscolices of fertile cysts of sheep, the present results (75.82%) were lower than that reported by Himonas *et al.* (1994) 81.3% Hosseini (1995) 83.7% and Mahran (2005) 85.3%.

In this study effect of sex and age on the rate of infection of various parasites recorded in the liver of slaughtered sheep points out that the incidence was comparatively higher in females than in males. This may be related to males are kept in relative low number for fattening purposes so the chance of infection was very low while females were kept for breeding in small flocks and most of them graze near canals, as well as the prohibition of slaughtering of young female ones. On the other hand the incidence in the aged animals was higher than in young ones. The high incidence of various parasites among the aged animal may be attributed to the exposure period of aged animals to the infection.

Salem *et al.* (1990) concluded that the adult animals have more infection rate with *Fasciola spp.* than young animals. This may be attributed to the long prepatent period of *Fasciola spp.* to produce the eggs (diagnostic stage). El-Shazly *et al.* (2002) incriminated female sheep as the most important farm animal in the dissemination and spreading zoonotic fascioliasis. In the present study it was found that aged sheep were more susceptible to infection with hydatid cysts than young ones as no infection was recorded in lambs. These results agreed with the findings of El-Bassiouny *et al.* (1988); Anwer *et al.* (1993); Mahran (2005). Gemmell and Roberts (1995) described a model in which the intensity of cystic echinococcosis infection in sheep increases linearly with age due to the lack of immunity.

## CONCLUSION

This study revealed higher prevalence rate of infection with different parasites recovered in the livers of slaughtered sheep (26.8%). The obtained results revealed the detection of some parasites which have public health significance (*Fasciola spp.* and cystic echinococcosis). Sheep play an important role in the dissemination, spreading and maintenance of both fascioliasis and cystic hydatidosis to man and animals, on the other hand these diseases cause great economic losses due to condemnation of the infected organs. The higher percentage of fertile hydatid cysts in sheep suggests that these animals could be an important intermediate host for the maintenance of the domestic life cycle of *Echinococcus granulosus* in Assiut Governorate.

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