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PREVALENCE AND HISTOPATHOLOGICAL CHANGES OF BOVINE FASCIOLIASIS, WITH UNUSUAL MIGRATION TO LUNG IN NEW-VALLEY GOVERNORATE

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Received: 17 March 2019; **Accepted:** 27 March 2019

ABSTRACT

The main objectives of this study were to determine the prevalence of fascioliasis in cattle, and to describe the histopathological changes in liver and lung. A total of 600 slaughtered bull's l ≤ 2 years were examined grossly to investigate *Fasciola* infection, from abattoirs in New-Valley Governorate. The livers of 120 bulls (20%) were positive for fascioliasis, with unusual migration to lung. The results indicated that *Fasciola hepatica* was more prevalent than other flukes infections including *Fasciola gigantica* and *Fasciola hepatogigantica* (58.3 vs. 12.5 and 29.2%, respictivelly). The histopathological examinations illustrate the presence of acute fascioliasis (66.7%), chronic fascioliasis (33.3%), and ectopic lesion of Fascioliasis (3.3 %). From the above results it could be concluded that bovine fascioliasis is endemic in New –Valley, constitutes a major cause of economic losses and threat public health.

Key words: *Fasciola* infection, bulls, histopathological changes, ectopic lesion, New-Valley Governorate.

INTRODUCTION

Fascioliasis is a serious infectious parasitic disease infecting domestic ruminants and humans, worldwide. The mortality due to animal fascioliasis in Egypt, were estimated by about 190 million animals per year according to the Egyptian Academy of Scientific Research and Technology Report (Kuraa and Malek, 2014). Fascioliasis is responsible for immense economic losses in cattle in terms of condemnation of livers, reduces animal reproductively, weight gain, and the production of meat and milk (Elshraway and Mahmoud, 2017). Ruminant fascioliasis infection occurs after ingestion of forage containing metacercarial cyst, which excyst in the gut, and penetrate the intestinal wall migrate through the peritoneal cavity and enter the bile duct and liver parenchyma. Occasionally, some flukes wander in the peritoneal cavity and other ectopic areas, producing lesions in the eye, brain, lungs, skin and other ectopic foci of host tissues (Nappi, 2002). Meat inspection provides vital data and valuable information on the incidences and prevalence of animal diseases and conditions within any country to prevent the transmission of infectious and zoonotic diseases to humans. Documentation and collection of this information will make changes in animal husbandry practices and disease control (Phiri, 2006). This study is conducted to determine the prevalence of fascioliasis infections in cattle by studying the gross and microscopic morphological features of the parasitic stages, and describe the histopathological changes in liver and lung.

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MATERIALS AND METHODS

The study was conducted in the El-Kharga abattoirs in New Valley Governorate.

Samples collection
A total of 600 slaughtered bull at age ≤ 2 years, were daily inspected for the presence of liver fascioliasis from May 2017 to November 2018, which efficiently inspected by naked eye and palpation for the presence of gross lesion and the worms then further examinations.

Post mortem examination:
The livers and lungs inspected by making multiple cuts to check the presence of fascioliasis, palpated, and photographed for any *Fasciola*, all retained worms of *Fasciola* sp. were stained with acetic acid – alum carmine (Garcia and Bruckner, 2001), Then

identified by the morphological features of the agent (Arjmand *et al.*, 2015).

Histopathological examination:

Specimens from affected livers were immediately taken and immersed in 10 % formalin. After proper fixation, the specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, then cleared in xylene and embedded in paraffin .Thin sections about 4-6 microns in thickness were prepared and stained with haematoxylin and eosin stain for general microscopic examination according to (Bancroft and Cook, 1994). The prepared permanent slides were mounted and examined on light microscope and viewed at different magnifications.

RESULTS

From 600 slaughtered bull at age ≤ 2 years, the prevalence rate of the total *Fasciola* infection was 20%. Results in Fig.1**,** showed that the bovine fascioliasis was higher during autumn and spring than summer and winter (31 and 21.1% vs. 18.5 and 13.9%), respectively.

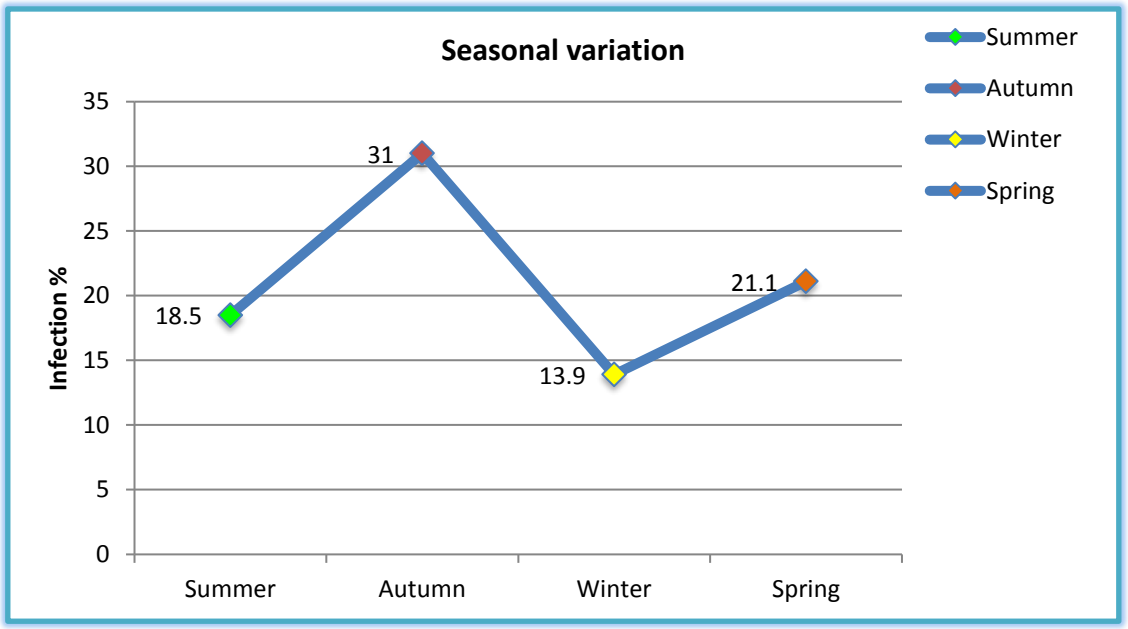

Fig. 1: Seasonal variation of fascioliasis in slaughtered cattle at El-kharga abattoir.

Post mortem examination

The results of examined 120 *Fasciola* infected livers on the current study (Table 1), refer that, *F. hepatica* was the most commonly parasite with higher prevalence rate (58.3%). However, the prevalence rate of *F. hepatogigantica* and *F. gigantica* was (29.2 and 12.5%), respectively. Macroscopic examination of retained *Fasciola* from tissue appeared grayish brown leaf- like. The measurements of *F. hepatica* was 2-3 cm in length and about 0.5-1.5 cm in breadth. The microscopic features of *F. hepatica* were the oral sucker at the conical anterior end equal in size with ventral sucker which located in at the level of the shoulder. The inner intestinal branches are rudimentary. Testes were highly branched, tandem in position and located at the middle third of the body. Macroscopic examination of *F. gigantica* was measured about 3-8 cm in length and about 0.5-1.5 cm in breadth, microscopically; the oral sucker was at the anterior end and ventral sucker was larger. The intestinal caeca were branched and characterized by T and Y shaped branches. Testes were highly branched, tandem in position and located at the middle third of the body. In case of *F. hepatogigantica*, it is an intermediate form having few morphological characters from *Fasciola hepatica* and *Fasciola gigantica* (Fig.2) which is genetically confirmed by (Khalifa *et al.*, 2013).

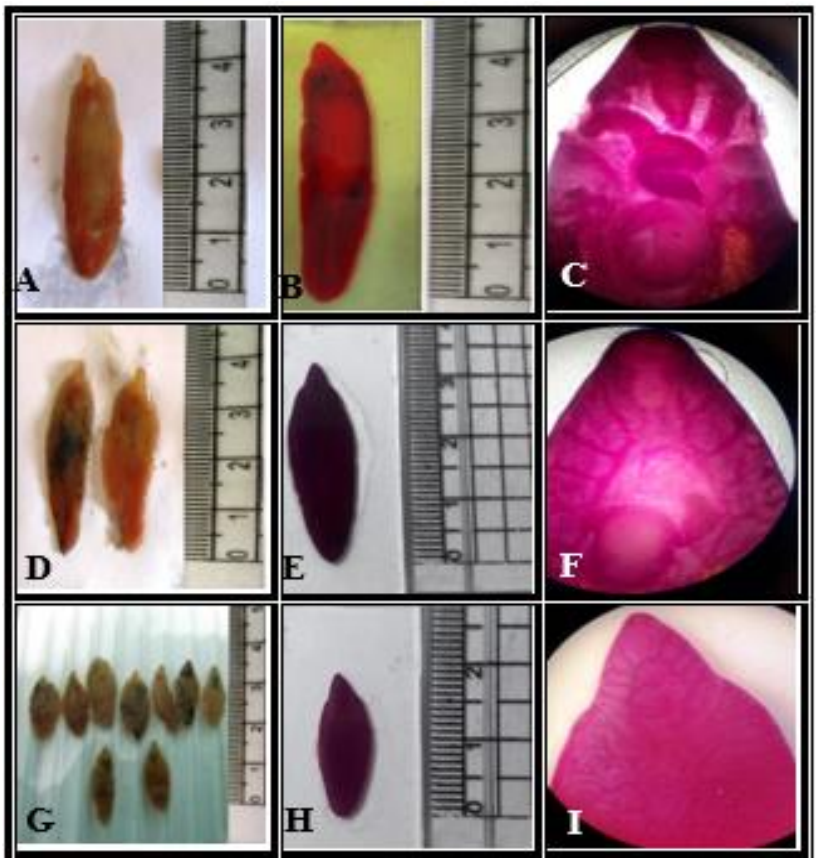

Fig. 2: Unstained
***Fasciola hepatica* (G), *Fasciola hepatogigantica* (D) and *Fasciola gigantic*a (A)**
- Stained with alum carmine
***Fasciola hepatica* (H), *Fasciola hepatogigantica* (E) and *Fasciola gigantic*a (B)**
- Anterior end of
***Fasciola hepatica* (I), *Fasciola hepatogigantica* (F) and *Fasciola gigantic*a (C)**

Table 1: Species of *Fasciola* encountered in infected livers.

Species of Fasciola	NO.	%
F. hepatica	70	58.3
F. hepatogigantica	35	29.2
F. gigantica	15	12.5
Total	120	100.00

normal sized liver showed areas of cirrhosis and thickened calcified bile duct wall (Fig.4). The ectopic lesion was found in 3.3% of animal's lung that suffered from chronic fascioliasis. The ectopic lesions in lung were detected when the affected liver contains huge number of *Fasciola*. The gross appearance of lung showed that the pulmonary parenchyma was firm in consistency and a few number of immature flukes were encapsulated in cysts (Fig.7A and 7B).

As illustrated in (Table 2) the percentage of acute fascioliasis was 66.7%. The gross pathological changes of the liver were enlarged, firm, congested and oozing blood freely from the cut surface (Fig.3). The percentage of chronic fascioliasis was 33.3%. Grossly the livers were mostly small in size and firm in consistency with corrugated capsule. Mature liver flukes were occasionally observed within the lumen of the thickened bile ducts. Black minute granules (hematoporphyrin pigment) of gritty sensation were found in the bile duct. On the other hand, some

Table 2: The results for the histopathological examination.

Pathological lesion	NO.	%
Acute fascioliasis	80	66.7
Chronic fascioliasis	40	33.3

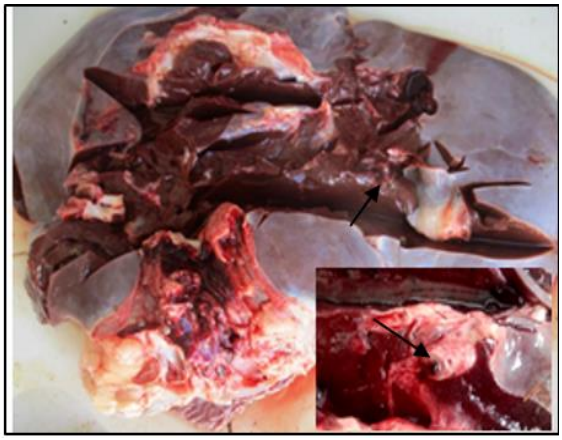

Fig. 3: Macroscopic liver fascioliasis showing enlarged and firm liver with *Fasciola* sp. in bile duct.

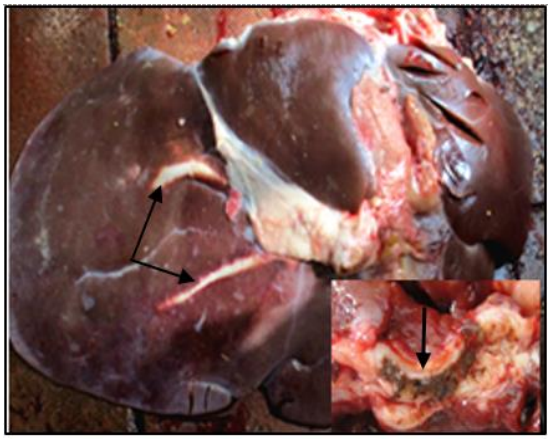

Fig. 4: Macroscopic liver fascioliasis showing engorgement of bile duct hematoporphyrin pigment.

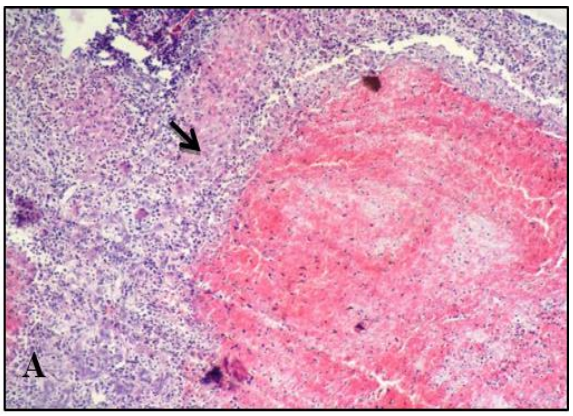

Fig. 5 A: Liver showing migratory tract of fasciola containing necrosis & haemorrhage (H & E x4).

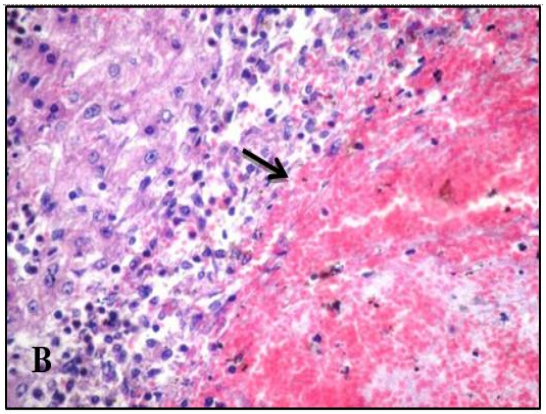

Fig. 5 B: Higher magnification of **(Fig. A)** H & E(x10).

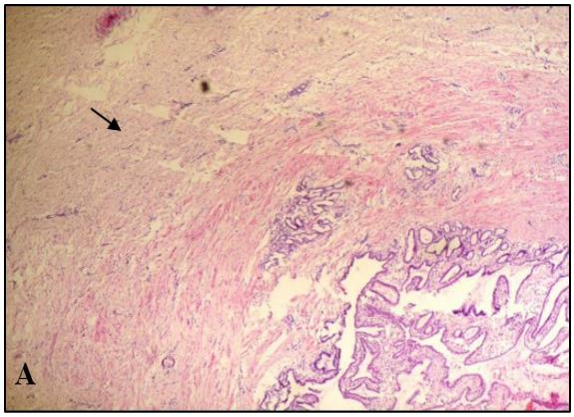

Fig. 6 A: Chronic cholangitis and bile duct hyperplasia H & E (x10).

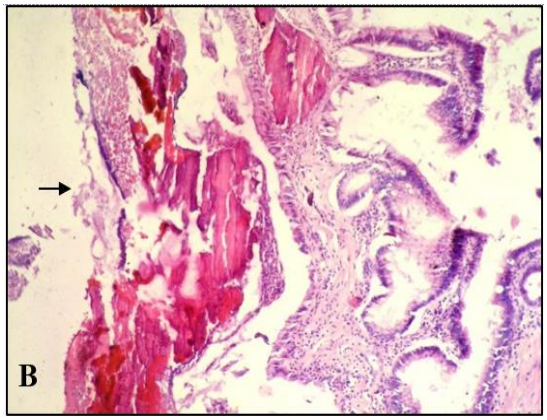

Fig. 6 B: Dead and calcified parasite H & E (x10).

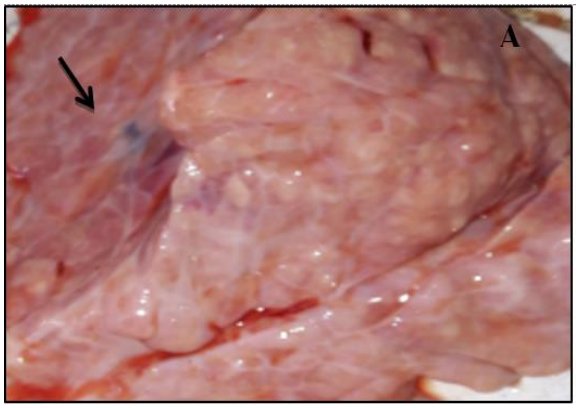

Fig. 7A: Lung showing encapsulated *Fasciola* sp.

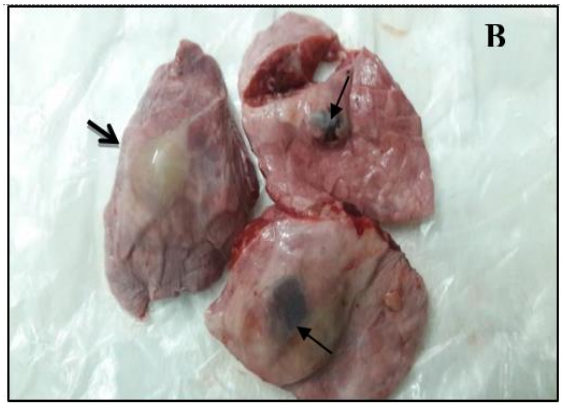

Fig. 7B: Lung showing cyst containing *Fasciola* sp.

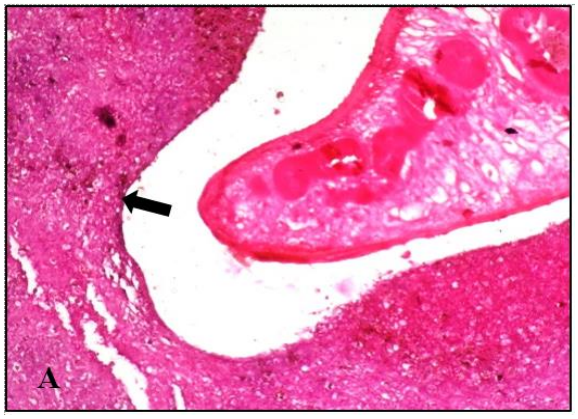

Fig. 8A: Lung showing immature fluke encapsulated by connective tissue H & E (x10).

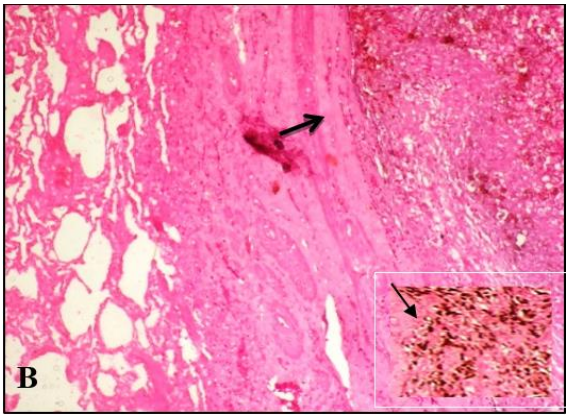

Fig. 8B: Lung showing hemosiderosis H & E (x10).

The histopathological examination of liver.

a) Acute fascioliasis revealed the presence of hemorrhagic migrating tracts formed from degenerated hepatocytes and erythrocytes (Fig.5A) & (Fig.5B).

b) Chronic fascioliasis was characterized by chronic cholangitis and hyperplasia in bile duct (Fig.6A), and the presence of dead and calcified parasite (Fig.6B).

The histopathological examination of the lung.

The immature flukes encapsulated by connective tissue (Fig.8A). and multiple area of necrosis with hemorrhage caused by young flukes which led to hemosiderosis were consistently observed (Fig.8B).

DISCUSSION

Fascioliasis is an important parasitic disease of domestic ruminants and occurs worldwide as a result of infection with liver fluke species (Hashemnia *et al.,* 2015). The overall prevalence rate of fascioliasis in the examined bulls slaughtered in the El-Kharga abattoir was about 20% which nearly agreed with Njoku-Tony (2011) who found 23.3% of examined cattle, in Nigeria. On other hand, higher frequency of fascioliasis have been recorded by Kuchai *et al.* (2011) who reported 51.42%, in Iran. Also, Iboyi *et al.* (2017) found that the prevalence was about 42% in Nigeria. Similar results was reported by Akoji *et al.* (2015) in cattle (41.7%). However, there were some remarkable lower results detected in Egypt by Elmonir *et al.* (2015) 0.2%, Borai *et al.* (2013) 2.67% and Youssef *et al.* (2013) 1.46%. In the current study the highest infection rate was observed in autumn season (31%) followed by spring and summer (21.1 and 18.5%), respectively. but the lowest rate observed in winter 13.9%. The results in our study was agreement with Rahman *et al.* (2017) who found that the proportion of fascioliasis cases was the highest in from September to November (28.7%) and the lowest rate in winter season (20.8%). However, Elshraway and Mahmoud (2017) reported that the highest fascioliasis infection was found in winter (35.04%). The distribution of *F. hepatica* depends upon many factors, such as eco-climatic factors and regional differences in management practices (Mehmood *et al.,* 2017). The prevalence rate of liver flukes in herbivores varies considerably throughout the world. The previous study by Dar *et al.* (2012) in Egypt observed that the prevalence rate of *F. hepatica* and *F. gigantica* in cattle (69.4 %) and (30.6 %), respectively. Post mortem examination on the 120 *Fasciola* infected livers of the current results indicated that *F. hepatica* and *F. hepatogigantica* were the main *Fasciola*

species in the survey regions, however, *F.hepatica* were found to be the predominant *Facsiola* species causing bovine fascioliasis. Similar results was confirmed by Arjmand *et al.* (2015) at Ahvaz Abattoir, Iran, he found that the percentage of *F. hepatica* in the infected livers was 67.54%.

The gross pathological lesions of acute fascioliasis among the affected livers reveal that the majority of livers were enlarged, firm, congested and oozing blood freely from the cut surface. These lesions were in complete agreement with those obtained by Borai *et al.* (2013), and in disagreement with Badr and Nasr (2009) who reported the presence of multiple soft abscesses on the liver surface and a viscous yellow material oozed from the cut ends, during the cut section. The abscesses were surrounded by hyperemic zone. The microscopically findings in the current study revealed that hemorrhagic migrating tracts formed from degenerated hepatocytes and erythrocytes. These lesions agree with those mentioned by Borai *et al.* (2013). On the other hand, Okoye *et al.* (2015) observed that the formation of granuloma around flukes formed from the eosinophil granulocytes, along with inflammatory molecules. In the current study the chronic fascioliasis, the livers were mostly small in size and firm in consistency with corrugated capsule. Mature liver flukes were occasionally observed within the lumen of the thickened bile ducts. Black minute granules (hematoporphyrin pigment) of gritty sensation were found in the bile duct. However, some normal sized liver showed areas of cirrhosis and thickened calcified bile duct wall. This result was similar with that reported by Borai *et al.* (2013) and Arjmand *et al.* (2015). Microscopically, Liver characterized by chronic cholangitis and hyperplasia in bile duct. Dead and calcified parasite can be seen. Which is similar to that mentioned by Okaiyeto *et al.* (2012). On other hand, Salmo *et al.* (2014) reported that invasion of the liver by migrating immature liver fluke damages the tissue and provide anaerobic condition, that allowed the germination and proliferation of bacteria that induce hepatocellular necrosis and abscess formation. However gross findings of lung revealed that the pulmonary parenchyma was firm in texture and a few numbers of immature flukes were encapsulated and can be seen randomly. Similar results, mentioned by Hashemnia *et al.* (2015). Similarly, the unusual migration to lung may be the result of aberrant migration of parasite to other organs specially lung (Eslami 2006). The results of histopathological studies agreement with Hashemnia *et al.* (2015) who indicated that the immature flukes encapsulated by connective tissue. Multiple areas of necrosis and hemorrhage caused by young flukes which led to hemosiderosis.

CONCLUSIONS

Slaughterhouse survey may be a good tool to monitor the status of infectious disease especially those of public health importance. The present study revealed a moderate fascioliasis infection in cattle, in El-Kharga, New Valley Governorate with unusual migration of *Fasciola* spp. into the lung and recommended that:- (awareness should be taken to increase the public health) Veterinary services are important to advice farmers to maintain their animals free from parasites by keeping the animals under hygienic conditions away from grazing in the infected pasture and provide them with safe drinking water source that is free from snails. Fascioliasis control at farm to diminish the economic losses due to infection.

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معدل الاصابة والتغيرات النسيجية المرضية لداء المتورقات (مثقبيات الكبد) البقري مع الهجرة غير العادية إلى الرئة بمحافظة الوادي الجديد

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الهدف الرئيسي من هذه الدراسة هو تحديد معدل الاصابة بالمتورقات الكبدية في الماشية ووصف المتغيرات الهستوباثولوجية (الفحوصات النسيجية) في الكبد والرئة. في هذه الدراسة تم فحص ٦٠٠ ماشية (بقر) ≤٢ سنة، ظاهريا للتحقق من الاصابة بالدودة الكبدية (الفاشيولا). خلال الفترة من مايو ٢٠١٧ الى نوفمبر ٢٠١٨ في مجازرالخارجة محافظة الوادي الجديد. وقد بلغت نسبة الاصابة في الكبد ٢٠% منهم ٣,٣% في الرئة ناتج من هجرة الدودة الكبدية. تشير النتائج الى ان نسبة الاصابة بالفاشيولا هيباتيكا (٥٨,٣ %) اعلي من باقي المتورقات التي تشمل الفاشيولا جيجانتيكا والفاشيولا هيباتوجيجانتيكا (١٢,٥%) و (٢٩,٢%) على التوالي. اوضحت الفحوص النسيجية وجود اصابات حادة بالدودة الكبدية (٦٦,٧%) واصابات مزمنة (٣٣,٣%) واصابات خارجية (غير طبيعية). من النتائج السابقة يمكن استخلاص ان داء المتورقات (مثقبيات الكبد) متوطن في محافظة الوادي الجديد ويشكل سبب رئيسي للخسائرالاقتصادية وتهديد الصحة العامة.

الكلمات الارشادية: الاصابة بالديدان الكبدية (الفاشيولا) ، ثور، تغيرات الهستوباثولوجية (الفحوصات النسيجية) اصابة خارجية (غير طبيعية)، محافظة الوادي الجديد.