

## HEPATIC DICROCOELIASIS IN BEEF CATTLE SLAUGHTERED AT ABU-SIMBEL ABATTOIR: FIRST SLAUGHTER HOUSE RECORD IN EGYPT

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### ABSTRACT

*Dicrocoelium dendriticum*, the small liver fluke, is capable of parasitizing the gall bladder and liver of various mammalian species, especially ruminants. In Egypt, there was no previous abattoir-based research concerned with the incidence of dicrocoeliasis. The objective of our study is to record hepatic dicrocoeliasis in imported beef cattle of Sudanese origin that were slaughtered at Abu-Simbel abattoir, Aswan Governorate, Egypt and describe the detailed hepatic gross as well as histopathological changes induced by it. During a period from December 2020 to October 2021, 1575 liver specimens were examined from which 172 cases showed gross pathological lesions. After collection, affected specimens were fixed in 10% neutral buffer formalin, then they were prepared for histopathological examination. The recorded incidence of hepatic dicrocoeliasis was 7.5% among the affected cases. The affected liver showed fibrosis and paleness with irregular yellowish foci on its surface. Fibrous tissue proliferation was seen surrounding bile ducts. Histopathologically, parasitic eggs were detected in affected liver tissues where they stimulated a granulomatous inflammatory reaction with hepatocellular necrosis and infiltrations of neutrophils, eosinophils and giant cells. Portal fibrosis resulted in the formation of pseudolobules. As a consequence, hepatic dicrocoeliasis is prevalent in imported Sudanese beef cattle and these animals should be protected from snail-infected regions and subjected to careful inspection by veterinarians in slaughterhouses.

**Keywords:** Liver, Dicrocoeliasis, Imported cattle.

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### INTRODUCTION

Parasitism accounts for one of the major problems that affect the livestock

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productivity. Although fascioliasis and cysticercosis were the most frequent causes (Borai *et al.*, 2013), *Dicrocoelium dendriticum*, the lancet liver fluke, was also incriminated in inducing parasitic hepatitis (Piegari *et al.*, 2021).

Dicrocoeliasis is a zoonotic parasitic disease affecting the bile ducts and gall bladder of various mammals, mainly ruminants, and

humans. The parasite had a complex life cycle that involved two intermediate hosts, various species of land molluscs were considered the first. However, ants were the second ones (Manga-González *et al.*, 2019). In ruminants, as a definitive host, *Dicrocoelium dendriticum* spreads directly within liver biliary passages without causing damage to other structures, such as the hepatic capsule or hepatic parenchyma, while fascioliasis could do so (Otranto and Traversa, 2002).

According to Chougar *et al.* (2019), the fluke's body was flattened, and translucent with a narrow anterior portion and a broad posterior end. The average body length was 3.69 mm. The parasite had a rounded oral sucker which was located at the anterior portion followed by a small rounded pharynx, fine esophagus and a rounded ventral sucker. Two globular testicles were arranged obliquely. The uterus was characterized by numerous convolutions and was located at the posterior two-thirds of the body.

Ghazani *et al.* (2008) said that infection with *Dicrocoelium dendriticum* in ruminants caused granulomas in the hepatic parenchyma beneath the parietal surface which were associated with fibrous tissue proliferation and inflammatory cellular infiltration of Glisson's capsule.

Slaughterhouses play an important role in the diagnosis of dicrocoeliasis either through postmortem examination of the liver or during coprological analysis for detection of eggs as animals seldom showed clinical manifestations which, when present, occurred in the form of anemia and emaciation (Cringoli and Rinaldi, 2014). Lesions induced by dicrocoeliasis were extensive fibrosis, calcification and thickened bile ducts which caused firmness of hepatic parenchyma (Otranto and Traversa 2002).

In Egypt, this disease had not been recorded or discussed in previous slaughterhouse studies, while dicrocoeliasis is considered a

well-known, worldwide parasitic condition of ruminants, so this study aimed at recording the first case of hepatic dicrocoeliasis in imported Sudanese beef cattle slaughtered at Abu-Simbel abattoir, Aswan Governorate, Egypt. Moreover, in our study, gross, as well as, histopathological changes of the liver in affected cases were described and discussed in detail.

## MATERIALS AND METHODS

**Study Area:** Our study was conducted at Abu-Simbel abattoir, Aswan governorate, Egypt in accordance with regulations of the Animal Ethical Committee for Veterinary Research of the Faculty of Veterinary Medicine, Assiut University, Assuit, Egypt.

**Animals:** Imported beef cattle of Sudanese origin. Their age group ranged from 2-3 years.

**Collection of specimens and gross monitoring:** Regular visits to **Abu-Simbel** abattoir during the period from December 2020 to October 2021 were performed. A total number of 1575 liver specimens were carefully inspected and examined, among them, 172 cases showed gross pathological changes. Affected liver cases were collected and imaged by a digital camera.

**Histopathological examination:** Liver specimens were fixed in 10% neutral buffered formalin immediately after slaughtering, and then processed as usual (Bancroft and Marilyn, 2002). After staining with Hematoxylin and Eosin, the microscopic examination was done using an Olympus CX-31 microscope and was photographed using a Sc30 Olympus camera.

## RESULTS

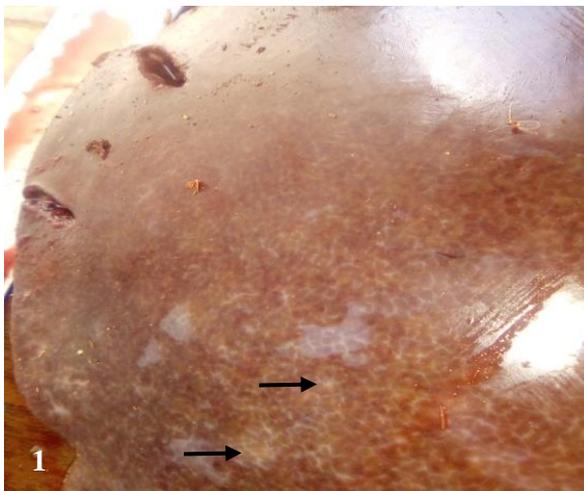
Among a total number of 172 liver specimens that showed gross lesions, the incidence of dicrocoeliasis was 7.5% (13\172). However, the recorded prevalence rate was 0.8% of the total examined cases (13\1575). Grossly,

affected livers were larger than their normal sizes with mild congestion and their surfaces exhibited numerous irregular foci that had white to pale yellow coloration (Fig. 1). Also, it appeared with firm consistency, thick hepatic capsule and irregular borders. Fibrosis was a constant finding in affected specimens which were sclerotic due to fibrous tissue proliferation. Bile ducts were dilated and inflamed and their walls were thick and fibrosed. Occlusion of bile ducts had occurred as a result of advanced stages of fibrosis (Fig. 2).

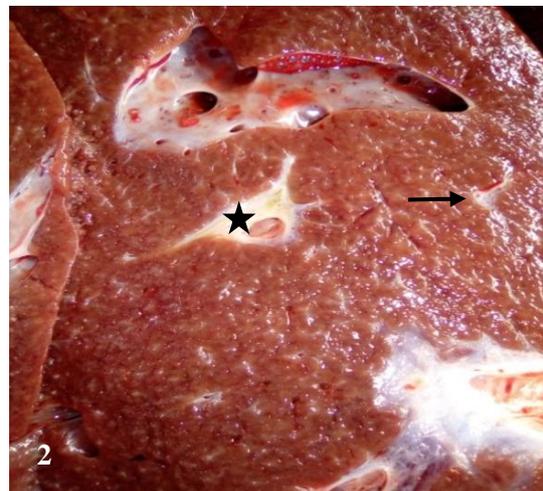
The most notable histopathological finding consisted of a granulomatous reaction with necrotic hepatocytes and neutrophilic cell reaction at the center (Fig<sup>s</sup>. 3&4). These granulomas were encapsulated with an outer layer of fibrous connective tissue. Also, at their site, numerous proliferative non-functional bile ductules were observed (Fig. 5). Peripheral to the fibrous layer, hepatic cells appeared with normal integrity and structure. Further examination of affected cases revealed portal fibrosis with variable

degrees of hyperplastic bile ducts (Fig. 6) that had papillary projections extending toward the ductular lumen. The massive proliferation of fibrous tissue at the portal areas resulted in pseudo-lobules formation (Fig. 7). At the center of the granulomatous lesion, Langhan's giant cells were detected and appeared with foamy cytoplasm and multiple nuclei which arranged as a ring or as a horseshoe shaped towards the cell wall (Fig. 8).

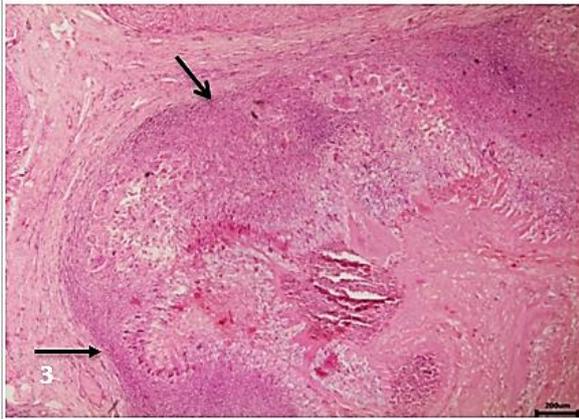
Ectopic eggs of *Dicrocoelium dendriticum* were noticed in sections of affected liver tissues at the site of the lesion along with Langhan's giant cells, degenerated and necrosed hepatocytes. These eggs were small in size, approximately, 30-40 microns in length and about 25 microns in width, oval in shape, with an operculum at one pole and a thick wall (Fig. 9). The detected parasitic eggs contained miracidium with germinal balls within its cavity (Fig. 10). At the site of aggregated eggs, there was inflammatory cellular reaction composed mainly of eosinophils and neutrophils (Fig<sup>s</sup>. 11&12).



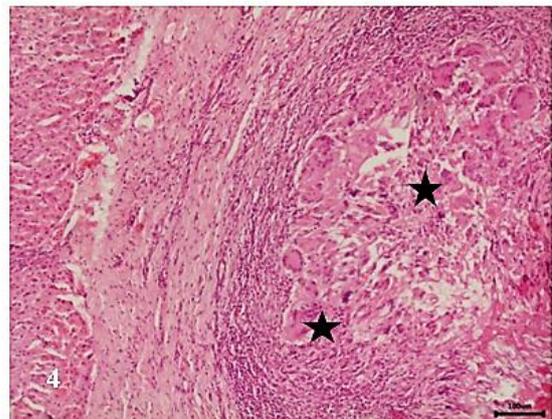
(Fig. 1): The liver of cattle infected with dicrocoeliasis showing irregular yellowish foci on its surface (arrows).



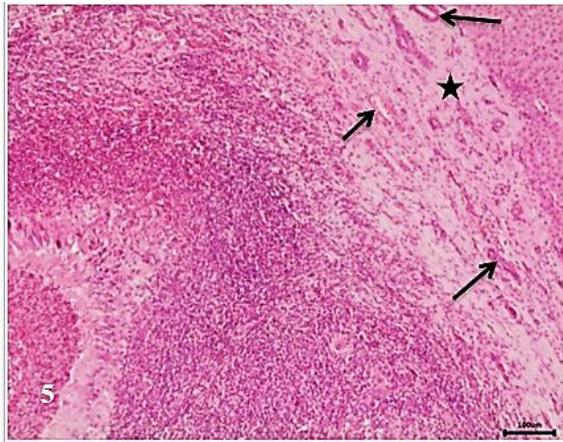
(Fig. 2): Cut section in cattle liver showing peri-ductal fibrous tissue proliferation (star) and occluded bile ducts (arrow).



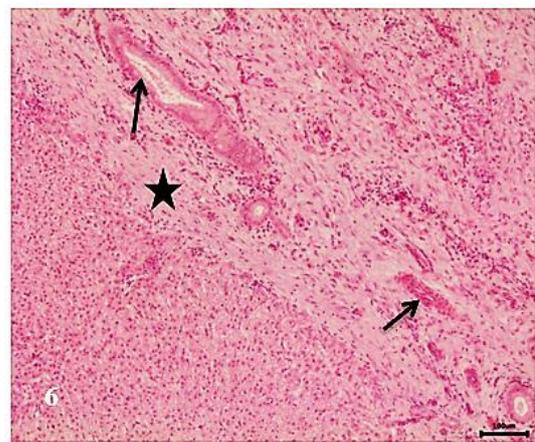
**(Fig. 3):** Liver of cattle infected with dicrocoeliasis showing parasitic granuloma (arrows) (Hx &E Scale bar =200µm).



**(Fig. 4):** Section of cattle liver with higher magnification showing parasitic granuloma (stars) (Hx &E. Scale bar =100µm).



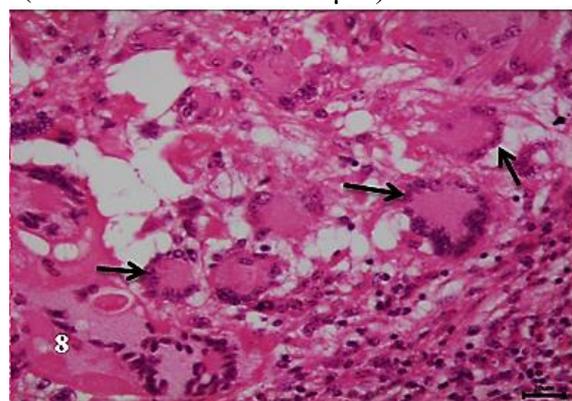
**(Fig. 5):** Thick connective tissue surrounding the parasitic granulomatous lesion (star) with numerous proliferated bile ducts (arrows) (Hx &E. Scale bar =100µm).



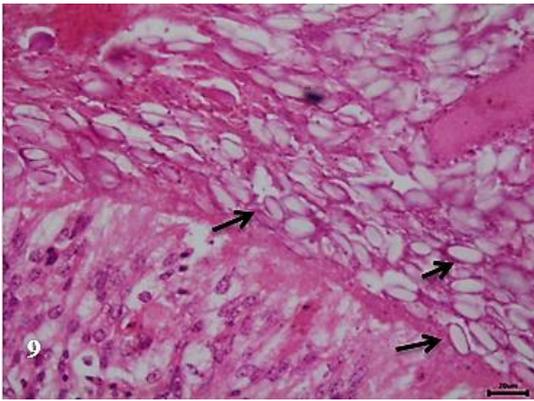
**(Fig. 6):** Liver of cattle infected with dicrocoeliasis showing portal fibrosis (star) with hyperplastic bile ducts (arrows) (Hx &E. Scale bar =100µm).



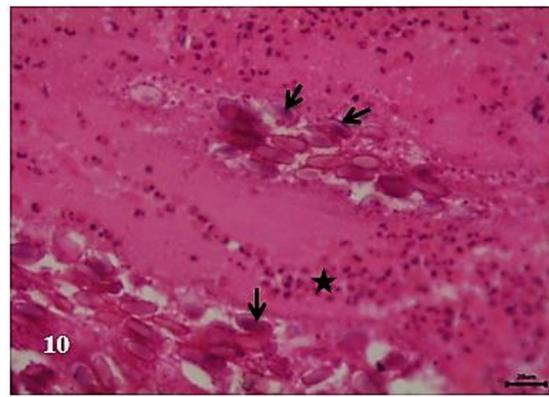
**(Fig. 7):** Liver of cattle showing pseudo-lobules formation (star) as a result of severe portal fibrosis (arrows) (Hx &E. Scale bar =100µm).



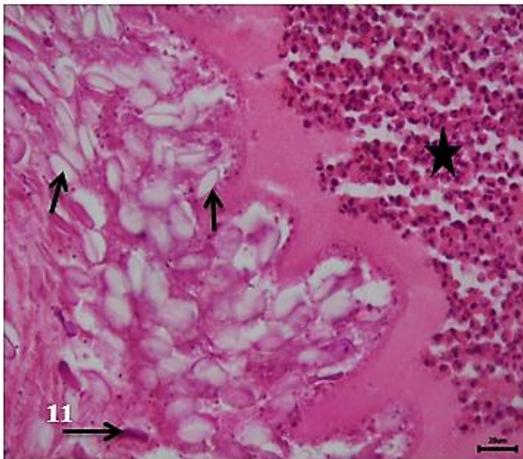
**(Fig. 8):** Giant cells at the center of parasitic granuloma with foamy cytoplasm and horse-shoe-shaped nuclei (arrows) (Hx &E. Scale bar =20µm).



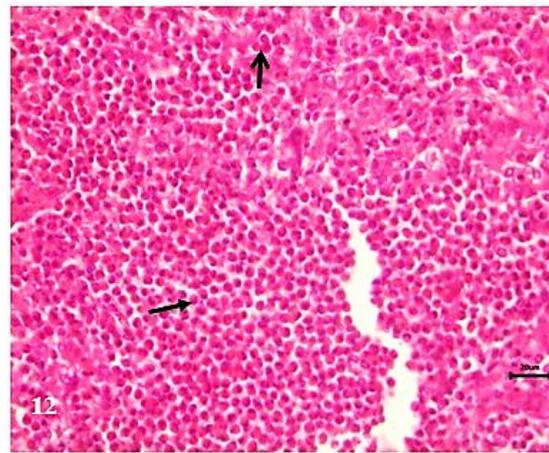
**(Fig. 9):** Cut section in cattle liver infected with dicrocoeliasis showing oval, small-sized parasitic eggs (arrows) (Hx & E. Scale bar =20 $\mu$ m).



**(Fig. 10):** Eggs of *Dicrocoelium dendriticum* in liver tissues containing miracidium (arrows) associated with inflammatory cells reaction (star) (Hx & E. Scale bar =20 $\mu$ m).



**(Fig. 11):** Liver of cattle infected with dicrocoeliasis showing parasitic eggs (arrows) with eosinophilic infiltration (star) (Hx & E. Scale bar =20 $\mu$ m).



**(Fig. 12):** Liver of cattle infected with dicrocoeliasis showing neutrophilic and eosinophilic aggregations (arrows) (Hx & E. Scale bar =20 $\mu$ m).

## DISCUSSION

During our study, we recorded the first cases of hepatic dicrocoeliasis among imported cattle slaughtered at Abu-Simbel abattoir, Aswan Governorate, Egypt with an incidence of 7.5% from the affected liver specimens, while the prevalence rate was 0.8% from the total examined cases. In similar studies, Nwosu and Srivastava (1993) examined 246 cattle in Nigeria to record the prevalence of liver fluke infestation and they reported that 18.3% were affected with dicrocoeliasis, while 3.3% were infected with fascioliasis and dicrocoeliasis simultaneously. In Algeria, Chaouadi *et al.* (2018) found that only one

liver from 1400 slaughtered cattle (0.07%) was positive for dicrocoeliasis, while Chougar *et al.* (2019) recorded the first case of hepatic dicrocoeliasis in northern slaughterhouses with a percent of 0.52% among slaughtered cattle.

Camara *et al.* (1996) thought that hepatic lesions might be caused by the attachment of numerous flukes to bile duct epithelium by their suckers, also it might be induced by toxic metabolites production. In accordance with our gross observations, Darzi *et al.* (2012) and Khajuria *et al.* (2013) stated that dicrocoeliasis-affected livers showed hepatomegaly, paleness, mild congestion,

firmness and scarring due to bile duct fibrosis.

Histopathologically, Camara *et al.* (1995); Ghazani *et al.* (2008) and Murshed *et al.* (2022) found varying degrees of bile duct hyperplasia. Heavy infiltration of lymphocytes, plasma cells, eosinophils and macrophages was observed in portal areas as well as on the ductal walls. Biliary fibrosis had resulted in bile duct occlusion. In regions of extensive portal fibrosis, calcification and desquamation of degenerated biliary epithelial cells were noticed with the formation of pseudo-lobules. Hepatic sinusoids showed blood engorgement and hepatocytes exhibited different levels of fatty changes. A similar histopathological picture was reported in our study, in addition, giant cells with foamy cytoplasm and multiple nuclei arranged as a horseshoe shaped were observed.

The most important histopathological observation in our results was the presence of granulomatous lesion which was stimulated by aggregation of ectopic parasitic eggs in the hepatic tissue. These eggs were infiltrated with eosinophils and neutrophils. Thick fibrous tissue was observed encapsulating the lesion. However, during histological examination, Chougar *et al.* (2019) noticed the adult parasite in affected liver tissue. Our description of parasitic eggs was in complete agreement with Chaouadi *et al.* (2018) who found it during the examination of bile samples at necropsy and they stated that eggs were oval in shape, dark brownish in color with thick walls. Their length was approximately 40 microns and their width was about 25 microns with miracidium and two germinal balls within their cavity. However, according to Khajuria *et al.* (2013) parasitic eggs with similar descriptions were found during microscopical examination of feces.

Darzi *et al.* (2012) thought that there was a correlation between carcinogenesis potential and hepatic dicrocoeliasis, especially in aged

animals. They observed tumorous growths in the affected livers. Histopathological examination of these lesions revealed cholangiocellular carcinoma which was characterized by protruding multilayered cuboidal cells into a proliferating bile duct lumens. Non-neoplastic bile ducts showed adenomatous hyperplasia that was heavily infiltrated with mast cells.

## CONCLUSION AND RECOMMENDATIONS

Based on the obtained data, *Dicrocoelium dendriticum* is prevalent in imported beef cattle slaughtered at Abu-Simbel Abattoir and, as a result, carcasses of imported Sudanese-origin beef cattle should be inspected and examined with great attention in slaughterhouses by the veterinary team work who had important role in preventing public health hazards.

It is recommended to prevent grazing of these imported cattle in snail-infected regions to reduce the infection rate with the subsequent economic losses caused by liver condemnation.

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## الإصابة الكبدية بطفيل ديكروسيليوم ديندريتيكوم في كبد الأبقار المذبوحة في مجزر أبو سمبل. أول حالة تسجل في المجازر في مصر

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تهدف هذه الدراسة الي فحص الإصابات الكبدية بطفيل ديكروسيليوم ديندريتيكوم (دودة الكبد الصغيرة) في الأبقار السودانية المستوردة والمذبوحة في مجزر أبو سمبل بمحافظة أسوان. تستطيع ديكروسيليوم ديندريتيكوم التطفل علي الكبد والحوصلات المرارية لمختلف سلالات الثدييات ومنها المجترات. وبالرغم من انتشار هذا الطفيل في جميع انحاء العالم، إلا انه لا توجد دراسات باثولوجية تسجل نسب حدوثه في الأبقار المستوردة وكذلك في المجازر المصرية. في هذه الدراسة قمنا بعمل زيارات منتظمة في الفترة من ديسمبر ٢٠٢٠ الي أكتوبر ٢٠٢١ الي مجزر أبو سمبل بمحافظة أسوان، تم خلالها الفحص الظاهري لكبد (١٥٧٥) حيوان وبلغ مجموع عدد الإصابات (١٧٢) حالة تم تصويرها وحفظها في الفورمالين تركيز ١٠% ثم صباغتها وتحضيرها للفحص الميكروسكوبي. اظهرت النتائج إصابة ١٣ حالة بطفيل ديكروسيليوم ديندريتيكوم والذي تسبب في حدوث تليفات وتغيرات في انسجة الكبد المصاب مع ظهور بقع صفراء غير منتظمة علي السطح، بينما اظهر الفحص الميكروسكوبي تجمعات من بويضات الطفيل في انسجة الكبد المصاب أدت الي التهابات تورمية مع تنكز في خلايا الكبد ونشاط لعدد كبير من الخلايا الالتهابية وكذلك تليف في القنوات المرارية والنسيج الكبدي. وعلي هذا فإن الإصابة بطفيل ديكروسيليوم ديندريتيكوم (دودة الكبد الصغيرة) منتشرة في الأبقار السودانية المستوردة ويجب إخضاعها لفحص دقيق من قبل الأطباء البيطريين في المسالخ.