

## Histopathological Investigations of the Bovine Fascioliasis in Liver and Lymph Node

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### Abstract

Fascioliasis is not only an endemic infectious disease recorded in the most tropical and sub-tropical areas, but it also has been recognized as a significant disease attributed to appreciable animal mortalities, growth retardation, drops in livestock production, and condemnation of the infected livers attributed to hepatic damage. The cross-sectional study was conducted on slaughtered cows in between 2018 to 2022 in abattoirs belonging to Qena province, Egypt. Each selected carcass was macroscopically inspected for adult liver flukes by dissecting the livers and lymph nodes. For histopathological study, specimens from the livers and lymph nodes were taken and fixed in 10% neutral buffered formalin. Postmortem observation represented whitish pale and firm livers which indicated necrosis; notably bile ducts engorged with liver fluke and a thickened opaque wall. Concerning lymph nodes, darkish red collections obviously distributed because of suffered intense congestion and hemorrhages. For histopathological changes, infected cattle denoted hepatic cytoplasmic vacuolation of hepatocytes and necrobiotic changes of the liver. Thickening and hyperplasia of the bile duct wall was elucidated. Besides that, there was hemorrhagic inflammation of the lymph nodes and remarkable lymphoid depletion of the white pulp. We explored some evidence of a close relation between fasciola infection and damage in else critical organs other than liver fundamentally lymph nodes.

### Keywords:

Cattle, Fascioliasis, Histopathology, Liver, Lymph nodes

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## Introduction

Undoubtedly, helminths represent the major impediment in livestock productions in most tropical and sub-tropical countries (Raza et al., 2010). Many helminths including trematodes, infesting the bovine body and developed a harmful effect as toxic substances released in the course of their life (Postevoy et al., 2015). High prevalence rates of fascioliasis have been over and over recorded as endemic in many parts of the world from Asia, Africa, Europe, and Americas (Machicado et al., 2016). Fasciolosis globally causes enormous economic losses to animal breeding; it annually exceeds US\$ 3.2 billion which is probably attributed to reduction in the total body weight gain, draught capacity, reproductive potential, and milk production (Charlier et al., 2008), also increased susceptibility to secondary infections and expense due to control measures. *Fasciola hepatica* and *Fasciola gigantica* are two species of genus *Fasciola* that primarily infect the liver of herbivorous mammals such as cattle, sheep, and goats. Eventually responsible for deep pathoanatomic changes (Mas-Coma et al., 1999).

Matured fasciola in ruminants parasitize liver and gall bladder leading to severe irreversible pathological changes in such and other tissues. They frequently resulted in mortality of the animals at the acute stage of the painful process (Balqis et al., 2013).

In fasciolosis, destructive damage to the infected host occurs either mechanically or chemically by these parasites or by the host's inflammatory and immune responses. The mechanical injury frequently takes place when the infective metacercariae penetrate the hepatic tissue. This penetration in turn is correlated with trauma, hemorrhages and necrosis of the liver, subsequently hepatic granulation and liver cirrhosis developed (Ozer et al., 2003). Otherwise, several studies implied an association between fascioliasis and other

liver complications such as hepatic fibrosis, and possibly cancer (Almendras-Jaramillo et al., 1997; Machicado et al., 2016). Moreover, calcification of bile ducts, swollen gall bladder, and anomalous migration of the flukes is also frequent in infected animals (Badr & Nasr, 2009). Accordingly, most records regarding to the pathogenesis of fascioliasis only highlighted on the hepatic changes. Of note, several parasites also display as key immunomodulatory properties and often function in conjunction to subvert the host immune responses in favor of the liver fluke (Berasain et al., 2000; Cwiklinski et al., 2019).

Fasciolosis is accompanied by secondary immunodeficiency issues; manifested by the suppression of specific and nonspecific immune response of the body. The leading effect of the helminths pathogenesis is responsible for pathological conditions in the body including; immunopathological, toxic, and allergic reactions that upset the neurohumoral and enzymatic systems of the body. In consequence of metabolic disorders, resulted in a decrease in the productivity of the animals (Ruiz-Campillo et al., 2017). From here, published investigations related to fascioliasis injuries in targeted organs other than liver, notably immune system is kept limited (Matsuda et al., 2019; Lalor et al., 2021). Due to these gaps, the fundamental objective of this study is to shed light on the complications of *F. hepatica* on immune patho-morphology in bovine (cattle).

## Material and methods

### Ethical approval

This study was conducted in accordance with the rules and regulations of the Animal Ethical Committee for Veterinary Research of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

***Study of area and population:***

For this study, investigation was conducted within abattoirs belonging Qena Governorate, Egypt. Meanwhile, cattle of different age and sexes were conducted from the slaughtering houses from different areas during variable periods from 2018 to 2022.

***Tissue samples collection:***

Liver and lymph node samples of the control non-infected and infected cattle were randomly amassed. Collection of the samples was done with qualified veterinary team after the cattle have been slaughtered. Inspection occurred firstly by visual and palpation of the collected tissues.

***Gross monitoring:***

The gross changes were carefully noted. For better examination, inspection of livers performed firstly by visual and palpation of the tissue. Generalized liver fluke access was performed in different parts for detection the presence of the fasciola inside the parenchyma. Whereas, cleaving the liver and cutting through, making an incision using a sharp knife and pressed to squeeze out flukes from its tissue and smaller bile ducts. The bile ducts were opened first for chronic fasciolosis. A fluke was seen rushing out of the duct in heavily and slightly infected tissue. Concerning lymph nodes, it also dissected from control and infected cattle for any gross lesions.

***Histopathological examination:***

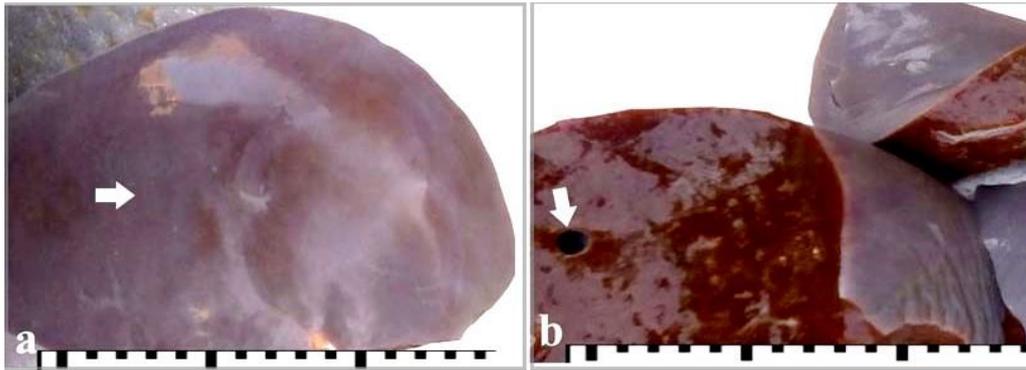
Samples were trimmed into small pieces and immediately fixed in 10% neutral buffered formalin. The fixed specimens were washed and dehydrated in

ascending grades of alcohol (absolute concentration, 95%, and 70%) according to several authors (Madkour & Mohammed, 2021; Madkour & Kandyl, 2022; Madkour et al., 2022), followed by clearing in xylene and finally embedded in paraffin wax. The sections (4-5  $\mu\text{m}$  thickness) were prepared using a rotary microtome. Routine staining with Haematoxylin and Eosin stain was performed for detailed histopathological examinations. The stained slides were carefully examined under Olympus microscope for accurate interpretation of results.

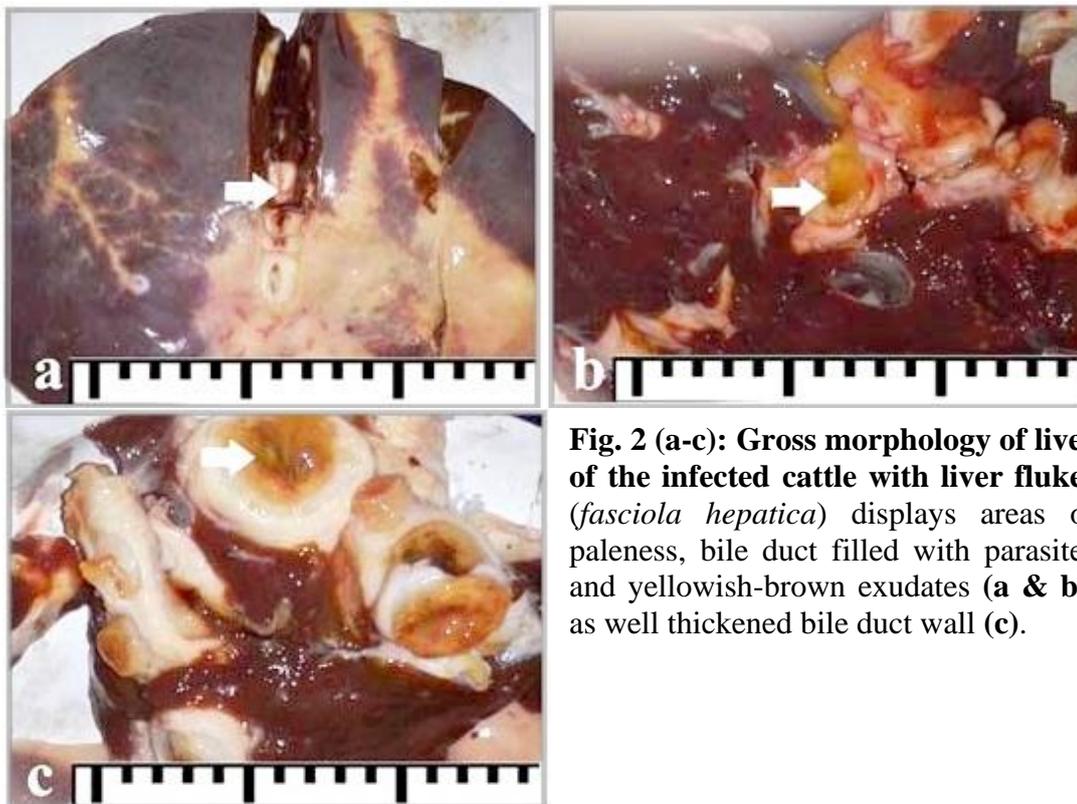
**Results*****Gross findings***

Grossly, healthy liver tissues from the non-infected cattle appeared bright brown and fluffy (Fig. 1a, 1b). Contrariwise, liver at an advanced stage of fascioliasis was greatly enlarged, whitish, and pale; and its texture is very firm. Additionally, there are several holes embedded on it due to necrosis or damage within the parenchyma regarded as fibrosis and calcification (Fig. 2a, 2b). Sectioning through hepatic parenchyma produced a gritty sound. At the same time in cut section, the bile ducts were distended with blackish brown exudates and identified by a dense texture. The swollen and thickened bile ducts wall was noticeable and blocked by live mature fasciola (Fig. 2c).

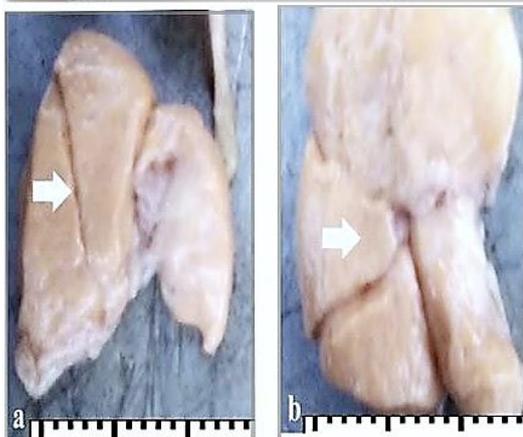
Regarding lymph nodes, the control non-infected one appeared as a white homogenous mass on the cut surface (Fig. 3 a, 3b). Conversely, lymph nodes of the infected cattle attended darkish red coloration because of detection of the continuous foci of congestion and hemorrhage (Fig. 4a, 4b).



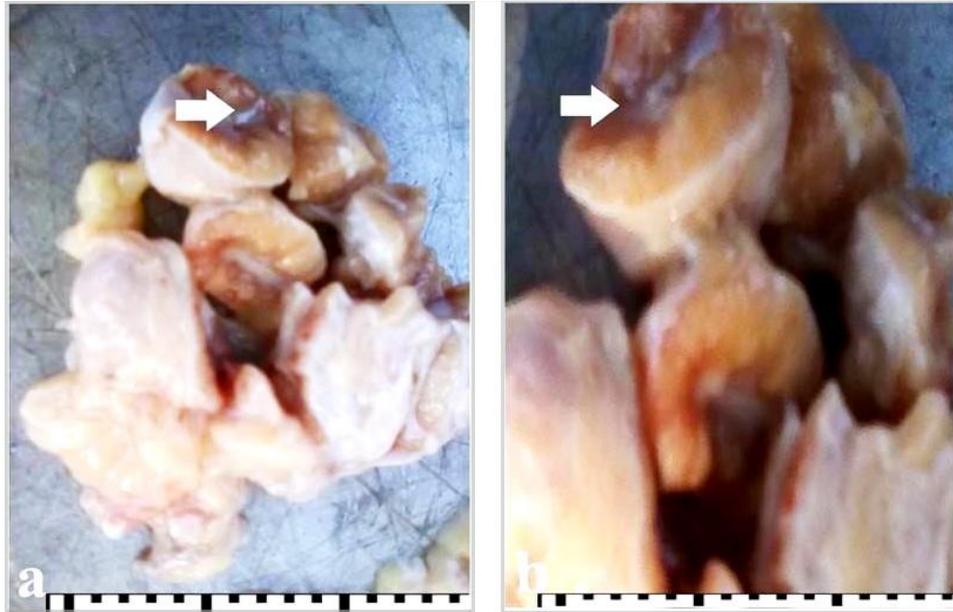
**Fig. 1. (a-b):** Gross morphology of liver of the control non-infected cattle appeared with a homogenous red-brown color within normal size, meanwhile normal lined bile duct wall on the cut surface.



**Fig. 2 (a-c):** Gross morphology of liver of the infected cattle with liver flukes (*fasciola hepatica*) displays areas of paleness, bile duct filled with parasites and yellowish-brown exudates (a & b), as well thickened bile duct wall (c).



**Fig. 3 (a-b):** Gross morphology of the control non-infected of lymph nodes displays homogenous white view on the cut surface.

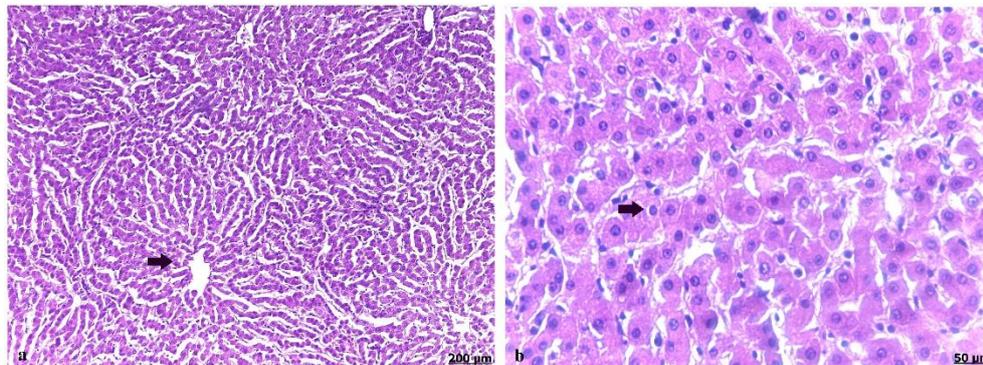


**Fig. 4 (a-b):** Gross morphology of the lymph nodes of the infected cattle of displays darkish red coloration on the cut surface.

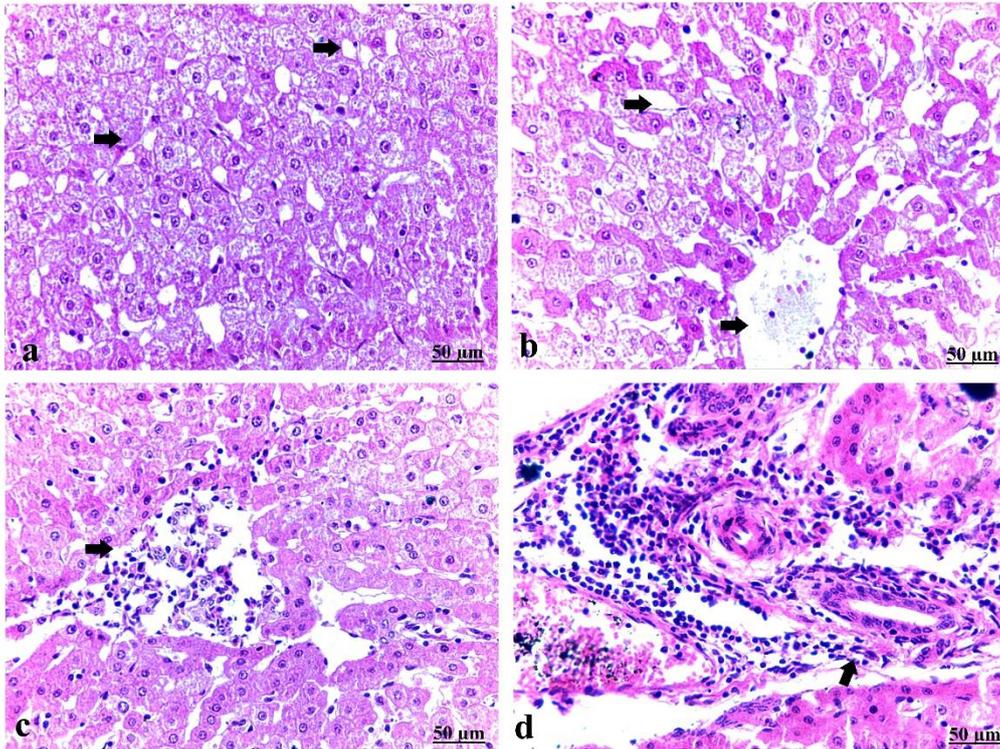
**Histopathological examinations**  
**Liver**

Histological investigations of control non-infected liver displayed arrangement of the hepatic cords in a good manner comprising intact hepatocytes with healthy vasculature mainly central vein and blood sinusoids (Fig. 5a, 5b). Contrary, liver of the infected cattle demonstrated severe damage to the hepatocytes resulting from parasitic migration through the hepatic parenchyma. Substantially, various histopathological changes were observed in the liver and bile duct sections of *Fasciola* infected cattle, changes were distinguished by hydropic degeneration undergone cytoplasmic vacuolation. In which their cell walls degenerated, the nuclei deformed, and

the cytoplasmic contents emptied into the sinusoids resulted in vacuolar degeneration of hepatocytes (Fig. 6a). Eventually, hepatocytes indicated clear vacuoles appeared in the cytoplasm with peripherally located nuclei. As well, there was infiltration of mononuclear leukocytes in the area attacked with liver fluke. Liver section also revealed severe dilatation of blood sinusoids and central vein and displayed an engorgement with stagnant red blood cells (Fig. 6b, 6c), besides the necrobiotic changes of central vein. Histopathological changes of the bile ducts were differentiated by thickening and hyperplasia of its wall, other than extensive aggregation of mononuclear cells mainly lymphocytes (Fig. 6d).



**Fig. 5 (a-b):** Light photomicrograph control non-infected liver stained with H &E., showing normally arranged hepatocytes with intact vasculature fundamentally central vein and blood sinusoids.

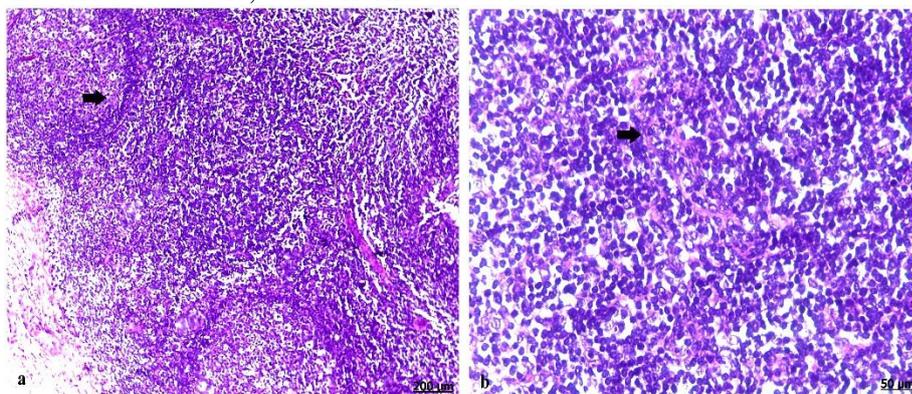


**Fig. 6 (a-d):** Light photomicrograph of liver of infected cattle with liver fluke stained with H &E., showing cytoplasmic vacuolation with clear vacuoles (a), severe dilatation of blood vessels comprising blood sinusoids and central vein (b), congestion of central vein and engorged with stagnant red blood cells (c), thickening and hyperplasia of the wall of the bile duct, besides prominent accumulation of lymphocytes (d).

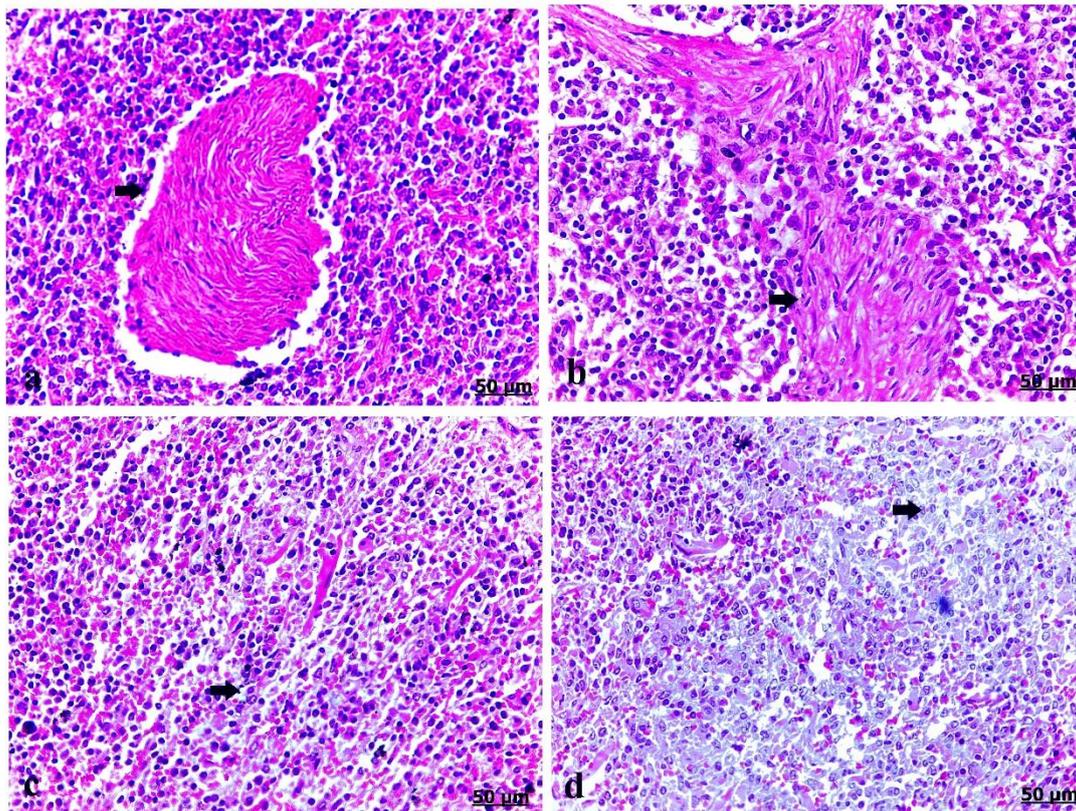
**Lymph nodes:**

Concerning lymph nodes, control non-infected cattle implied normal histological criteria of cortex and medulla, composed of intact populations of white and red pulps separated with connective tissues trabeculae and lined with healthy capsule (Fig. 7a, 7b). Conversely, infected lymph nodes were elucidated severe congestion of the blood vessels, and numerous blood

vessels expanded by excrement accumulation with stagnant erythrocytes (Fig. 8a). Further, thickening with protrusion of the trabeculae compensated for the necropsied lymphoid tissues (Fig. 8b). Hemorrhagic inflammation was characterized by erythrocytes, macrophages, and plasma cells infiltration (Fig. 8c) and prominent lymphoid depletion of the white pulp (Fig. 8d).



**Fig. 7(a-b):** Light photomicrograph of lymph nodes of control non-infected cattle stained with H &E., showing normal histological architecture of lymphoid follicles and connective tissues trabeculae.



**Fig. 8(a-d):** Light photomicrograph of lymph nodes of the infected cattle with liver fluke stained with H &E., showing severe congestion of the blood vessels (a), thickening with protrusion of the trabeculae compensated lymphoid tissues (b), hemorrhagic inflammation characterized by erythrocytes, macrophages and plasma cells infiltration (c), prominent lymphoid depletion of the white pulp (d).

## Discussion

Fascioliasis is a disease of ruminants including sheep, goats, and cattle. It recognized as an occasional zoonotic disease (Marcos et al., 2008). Fascioliasis has been shown to be a significant zoonotic disease (Ibironke et al., 2010); affecting a wide number of human populations, apart from its veterinary (Esteban et al., 2003). This disease in bovine is responsible for enormous economic losses in terms of condemnation of livers, reduced animal reproductive, weight gain, and general reduction in productivity (meat and milk) (Elshraway & Mahmoud, 2017). Lately, chronic fascioliasis causes a chronic hepatitis and cholangitis, accompanied by loss of condition, and disturbances in the digestive system (Rana et al., 2014). Studies

to determine the immune system complications correlated with bovine fascioliasis are still limited.

Infection by fascioliasis in ruminant takes place after ingestion of infected forage with metacercarial cyst, which excyst in the gut, and penetrate the intestinal wall, migrate via the peritoneal cavity on its way to the bile duct and liver parenchyma. Occasionally, some flukes promenade in the peritoneal cavity and developed destructive lesions in the other host tissues (Nappi, 2002).

Our gross pathological results of fascioliasis among the affected livers displayed enlargement, firmness, congestion and oozing blood when cut. These changes were in full agreement with those mentioned by Borai et al. (2013), and in disagreement with Badr and Nasr (2009)

who detected multiple soft abscesses on the liver capsules with viscous yellow material oozed during the cut section. Some infected livers revealed areas of cirrhosis and thickened with calcified bile duct wall. These results are in parallel with Borai et al. (2013) and Arjmand et al. (2015). The bile duct displayed yellowish brown concretions. Additionally, infected lymph nodes suffered intense congestion. Thickening and dilatation of the bile ducts was also found. These might be due to the immunological reaction of macrophages and lymphocytes that merges with fibrotic healing of the necrotic areas during the advanced stage of fasciolosis (El-Dakhly et al., 2007; Abo El Fetouh et al., 2010).

Attributing to microscopic findings in the current study, the infected liver suffered from cytoplasmic vacuolation and hepatic necrosis. Our results are consistent with MacGavin et al. (2001) who reported atrophy, necrosis, and fatty changes in the chronic inflammation due to release of proteases by the flukes which cause continuous irritation and migration through the liver parenchyma leading to hepatic damage (Okoye et al., 2015). The damage of hepatic cells which mainly was evident by cirrhosis perhaps is because of feeding habits of these premature parasites (Njoku-Tony & Okoli, 2011). Vacuolar degeneration of hepatocytes along the migratory route of the parasite was somewhat agreement with the studies of Adrien et al. (2013) who observed parenchymal and hepatic tissue destruction accompanied by haemorrhagic lesions. Other full agreement with the studies of Okoye et al. (2015) who discovered hepatocytic degeneration with periportal cellular infiltration fundamentally with eosinophils, macrophages and lymphocytes. These results were in compliance with Nabil et al. (2014) and Nourani et al. (2010) who also observed leucocytic infiltration in the hepatic parenchyma mainly with macrophages, lymphocytes and plasma cells.

In accordance with reports of Gabriela et al. (2010) the bile duct exhibited the hyperplasia accompanied by chronic fibrous cholangitis with abundant inflammatory infiltration predominant with mononuclear cells). However, Salmo et al. (2014) reported that invasion of the liver by immature liver fluke destruct the tissue through providing anaerobic condition, that permit the germination and proliferation of bacteria ultimately induce hepatocellular necrosis.

Regarding the infected lymph nodes, it announced depletion and atrophy in the lymphoid follicles, besides hemorrhagic inflammation with erythrocytes, macrophages, and plasma cells infiltration. In agreement with MacGavin et al. (2001), the atrophied tissues in chronic fascioliasis were in a consequence with lymphoid tissues may attributed to the digestion of the host components towards the flukes via the release of proteases, which facilitates their migration, feeding as well as the immune evasion.

### Conclusion

It could be concluded that a significant link between bovine fascioliasis and tissues damage not only pronounced in the liver but also recognized in other organs in a potential association of intense histopathological investigations.

### Recommendations

Depending on our conclusion, the following recommendations are bulleted as forwarded:

- Farmers should be aware of the zoonotic diseases of the economic impact through continuous training.
- Education of the farmers should be about the management system of the animals to diminish its risk of occurrence in their livestock.
- The grazing of cattle should be restricted to lesser snail infected sites to reduce the rate

of animal infection and the consequent economic losses.

- Control and management system should be on preventive and effective rather than treatment.
- Strategy of anthelmintic application with appropriate flukicidal drugs should be invested twice a year; to eliminate fluke burden to the host.
- Adequate attention is needed by veterinary team work to assure that heavily damaged livers are not for public consumption to avoid serious public health hazards.
- Public enlightenment particularly among the butchers and abattoir should be periodical on the public health significance of this parasite and how unwholesome abattoir practices can permit occurrence of accidental human infection.
- Cook water-grown vegetables thoroughly prior eating.

#### **Conflict of interest statement**

The authors declare that they have no conflict of interest.

#### **Availability of data and materials**

The datasets are available from the corresponding author on reasonable request.

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