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PATHOLOGICAL STUDIES ON LIVER AND BILE DUCT TUMORS IN SUDANESE CATTLE

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ABSTRACT

Due to their rare occurrence in bovine species, neoplasms of the liver and bile ducts were reported in few available researches. In Egypt, their prevalence in domestic animals was documented in a few records. An abattoir-based survey was conducted at Abu-Simbel slaughterhouse, Aswan Governorate, from December 2020 to October 2021. The study aimed to report the incidence rate of these tumors in imported Sudanese cattle. The percentage was 4 % and affected specimens revealed lesions ranging from single compact to multiple, nodular, metastatic masses with umbilicated appearance and necrotic centers. Diagnosis was confirmed histologically and tumorous lesions were identified as cholangioma, in which bile ducts showed marked proliferation and formed tubular structures lined with hyperplastic cuboidal cells with fibrous tissue proliferation and inflammatory cellular reaction, cholangiocarcinoma with its acinar pattern, mucin production and the fibrous stroma and mixed hepatocholangiocarcinoma that was characterized by simultaneous presence of hepatocellular and cholangiocellular carcinomas' features. In conclusion, neoplasms are prevalent in Sudanese cattle, which could be exposed to carcinogenic chemicals or aflatoxins that cause tumor induction. Careful veterinary inspection at slaughterhouses and more studies with larger sample sizes are recommended.

Keywords: Liver, neoplasms, Sudanese cattle, slaughterhouse.

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INTRODUCTION

The most recorded neoplasms in bovine species are lymphoma and squamous cell carcinoma, which can occur in different parts of the body, including the alimentary tract (Lucena *et al.,* 2011). However, tumors of hepatocellular and cholangiocellular origin are rarely reported, and represent less than 10% of all diagnosed neoplasms (Cullen and Stalker, 2016).

Primary hepatic neoplasms may appear in the form of focal tumors in which a single nodule or mass is present in the liver, multifocal that is characterized by a random distribution of multiple nodules in the liver parenchyma, or diffuse neoplasms that can affect the majority of the liver and accompanied by changes in color and consistency (Vielmo *et al.*, 2020)

Tumors of hepatocytic origin may be classified as adenomas, hepatocellular carcinomas, and hepatoblastomas. However, those possessing biliary epithelial cell origin include biliary cystadenomas (cholangioma) and cholangiocarcinomas (Head *et al.,* 2003). Cholangioma is a benign tumor of the bile duct that appears as well-circumscribed, multiple nodular lesions with cavernous aspect and small amounts of bile, and sometimes it is represented as compact nodules (Baba and Câtoi, 2007). It has not been mentioned as a predisposing cause of cholangiocellular carcinoma without complete exclusion of this possibility (Lendavi *et al.,* 2020). Cholangiocellular carcinoma is a malignant tumor that originates from bile duct epithelial cells. Dogs and cats are more frequently affected than cattle and other ruminants (Cullen and Popp, 2002). Combined hepatocellular cholangio-cellular carcinoma is sporadically reported in cattle and has neoplastic characteristics of both hepatocytic and biliary epithelial cell origins (Meuten, 2016).

These tumors have a high metastatic tendency, especially towards regional lymph nodes and lungs. Recognition of tumor emboli in pulmonary blood vessels indicates that hematogenous spread plays an important role in metastasis (MacLachlan and Cullen, 1995). Spontaneous cases may arise with an unknown cause (Kato *et al.*, 2004). Whereas, concerning etiological agents, chronic parasitic infestation, different strains of bacteria and viruses, and ingestion of various carcinogenic chemicals such as nitrosamines, aflatoxins, and pyrrolizidines constitute an important role in tumor development (Gholami *et al.,* 2006).

Diagnosis of liver neoplasms, as well as distinguishing benign from malignant types, have relied on both gross examination and histological features of lesions (Meuten, 2016). Histological evaluation can be performed according to the following criteria: cellular arrangement, stromal pattern, cell pleomorphism, vascular or lymphatic invasion, necrosis, hemorrhage, intratumoral inflammatory infiltrate, intratumoral vascular spaces, and the degree of mitoses (Vielmo *et al.,* 2020). The objective of this study is to record the incidence and prevalence rates of hepatic and bile duct tumors in imported Sudanese beef cattle, which are supposed to be a major source of animal protein for Egyptians, in addition to characterization of gross and histopathological picture of the detected tumors.

MATERIALS AND METHODS

1. Ethical approval:
The study protocol was approved by the Animal Ethical Committee for Veterinary Research of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. Under the No. 06/2023/0098. Used samples were collected postmortem and no animals were killed for the study.

2. Animals and study area:
Imported Sudanese cattle were quarantined for up to 21 days under the supervision of Egyptian Quarantine Veterinarians in Wadi Halfa, a Sudanese city bordering Egypt. Then they were transported to Abu Simbel city, Aswan Governorate, Egypt, where they were slaughtered at Abu Simbel abattoir. All animals were males and aged from two to three years. The period of study extended from December 2020 to October 2021.

3. Sampling and visual examination:
1575 slaughtered cattle were inspected and examined with particular attention to liver affections. Of these, 172 (10.9%) cases showed gross lesions. Specimens suspected to have tumors were discarded, collected, and photographed by a digital camera. Small sections of cubic shapes were taken from lesions and fixed in 10% neutral buffer formalin for preservation.

4. Histopathological examination:
Fixed specimens were processed routinely, according to the procedure of Bancroft *et al.* (1996). Tissue sections of 3-5 µm thickness were stained with Hematoxylin and Eosin for microscopic examination by light microscope, and were photographed using a Sc30 Olympus camera.

RESULTS

1. Incidence rate
Among the affected liver specimens, the incidence of tumors was 4% (7/172) (Fig. 1). Following histopathological examination, 57.1% of liver neoplasms were identified as benign, and 42.9% showed features of malignancy. Cholangioma, as a benign form, was detected in 4 cases with an incidence of 2.3%. Intrahepatic cholangiocarcinoma was present in 2 cases with an incidence of 1.1%, and mixed hepatocellular cholangiocellular carcinoma was diagnosed in one case with an incidence of 0.5%, which were categorized as malignant forms (Table 1 & Fig. 2).

2. Types of neoplasms

A. Cholangioma

Gross morphology:
Affected livers were enlarged and slightly congested, with rounded borders and irregular surfaces. An intrahepatic pale whitish-colored compact mass with approximately 5 cm diameter was detected and protruded from the liver surface. This mass was pedunculated and attached to hepatic tissue through dense pale yellowish fibrous tissue. In addition, varying degrees of biliary fibrosis and necrosis were noticed in affected cases (Fig. 3a).

Histopathology
Normal hepatic parenchyma was replaced with the tumorous mass, which was characterized by large numbers of variably sized tubular to papillary structures lined by a single layer, occasionally hyperplastic, low cuboidal or columnar cells with basally arranged round to oval nuclei and pale eosinophilic cytoplasm. There is no evidence of

cellular pleomorphism, mitosis, necrosis, desmoplasia, or vascular invasion. These glandular structures contained minimal amounts of eosinophilic mucinous secretions and were infiltrated with inflammatory cells, especially lymphocytes and neutrophils (Fig. 3b). Massive fibrous connective tissue proliferation was detected, separating papillary structures from each other, as well as surrounding the benign tumorous lesion. The occasional presence of epithelial cell nests in the stroma suggests progression toward malignancy (Figs. 3c, d).

B. Intrahepatic cholangiocarcinoma

Gross morphology:

The liver was paler than normal, and friable with an irregular surface. It exhibited multiple large nodular lesions with firm consistency, pale yellow color, and approximately 5 cm diameter distributed randomly throughout the parenchyma and attached to the surface. These lesions had central raised necrotic areas with grayish coloration, giving them somewhat an umbilicated appearance and on cut section, spreading to the biliary tree was observed (Fig. 4a). In association with large nodules, multiple smaller ones were present and scattered within affected hepatic tissues (Fig. 4b). Excision of nodular lesions had resulted in depressed areas or cavitation with dark red necrotic patches (Fig. 4c).

Histopathology:

The neoplasm was formed of anaplastic, large, cuboidal to rounded cells that were highly pleomorphic and disorganized with moderate to minimal amounts of cytoplasm, large nuclei with varying shapes, and prominent one or more nucleoli. These cells formed isolated gland-like structures. Cellular atypia was an important feature and occurred in the form of cytoplasmic basophilia, nuclear hyperchromasia, high nuclear to cytoplasmic ratio, loss of nuclear polarity, and abundant mitotic figures. However, many cells were binucleated (Fig. 5a). Cholangiocarcinoma was characterized by a poorly differentiated acinar histological pattern in which variably sized acini or tubules that were lined by a single layer of neoplastic biliary epithelial cells were detected. Also, they were filled with acidophilic mucin and distributed in the fibrous stroma (Fig. 5b). Adjacent non-neoplastic hepatic cells showed variable degrees of hemorrhage, degenerative and necrotic changes as a result of compression (Fig. 5c).

C. Mixed hepatocellular cholangiocellular carcinoma

Gross morphology:

The liver was congested with dark coloration and sharp edges. Its surface was irregular and showed a diffuse large yellowish-white circumscribed lesion with a firm texture, measuring approximately 15 cm and replacing most of the hepatic parenchyma. At the site of the junction, minute hemorrhagic zones with yellowish necrotic patches were detected. A tumorous mass was attached to the liver surface by dense whitish collagenous fibers. Generally, the lesion was unencapsulated with evidence of hematogenous invasion and the possibility of metastasis (Fig. 6a).

Histopathology:

Cellular features of both hepatocellular and cholangiocellular carcinomas were detected. In areas of hepatocellular carcinoma, hepatocytes showed abnormal arrangement in nearly parallel cords, with the formation of thick irregular trabeculae in stromal fibrosis. Neoplastic

hepatocytes were large, rounded, or polygonal in shape with finely granular, acidophilic cytoplasm. Also, they contained large vesicular nuclei with obvious nucleoli. However, mitotic figures were visible (Fig. 6b). Along with neoplastic hepatocytes, hemorrhages, and the characteristic neoplastic clear cells with clear cytoplasm that contained variable amounts of glycogen, fat vesicles and non-uniformly round to oval nuclei were observed. Sinusoidal capillaries suffered from compression, which was caused by large neoplastic cells (Figs. 6b, c). In Cholangiocarcinoma and combined areas, neoplastic bile duct epithelial cells were cuboidal to columnar with pale basophilic cytoplasm, round to oval one or more nuclei whose chromatin was condensed and nucleoli were prominent. These cells formed tubules distributed in a desmoplastic scirrhous stroma (Fig. 6d).

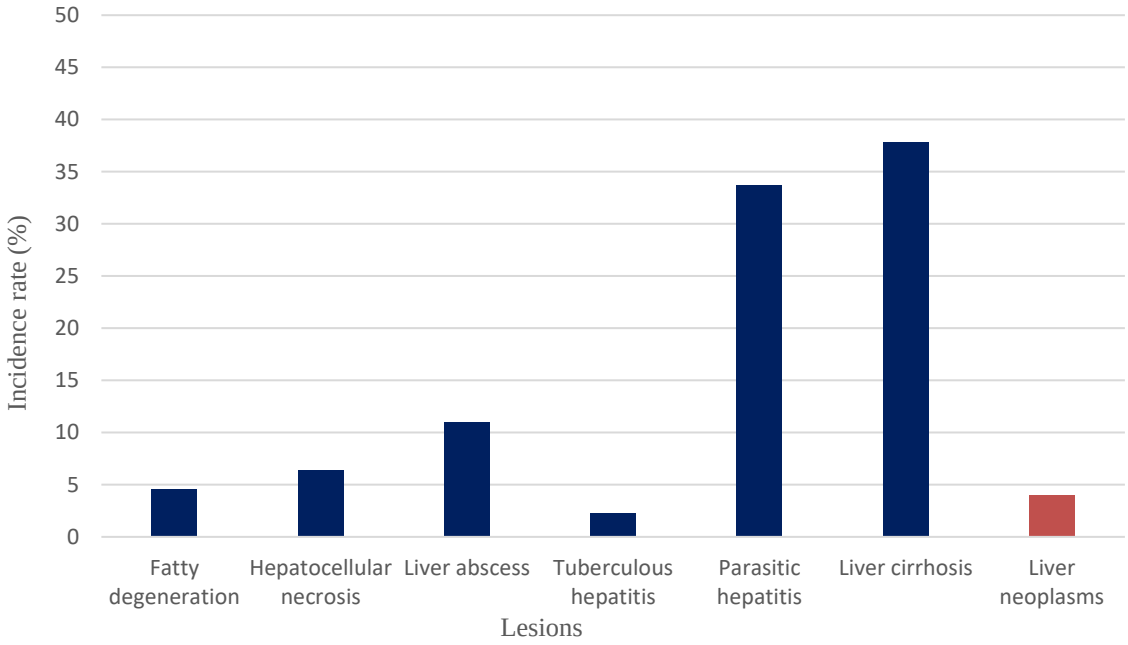


Figure (1): The overall incidence of tumors among the diagnosed liver lesions.

Table 1: Numbers and incidence rates of different types of liver neoplasms.

Type	Specimens affected	Incidence rate
Cholangioma	4	2.3%
Intrahepatic cholangiocarcinoma	2	1.1%
Hepatocholangiocarcinoma	1	0.5%
Total	7	4%

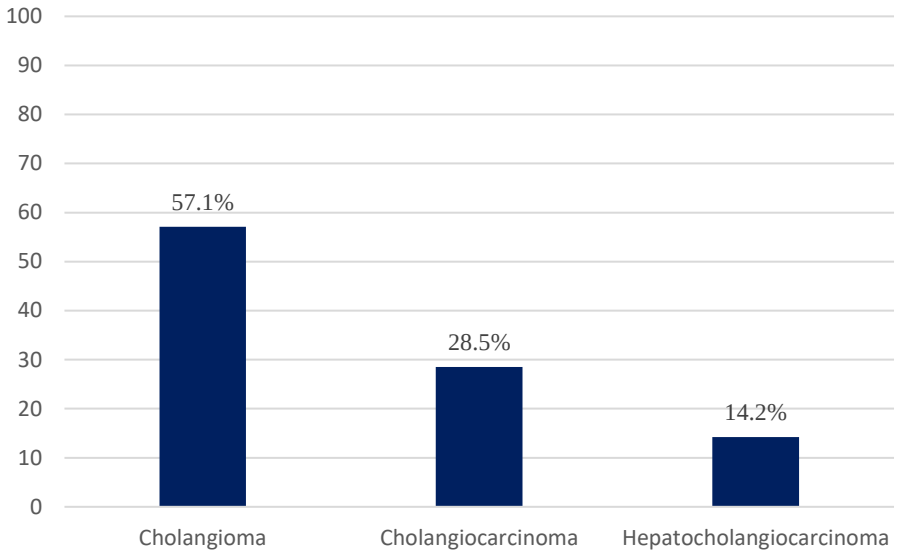
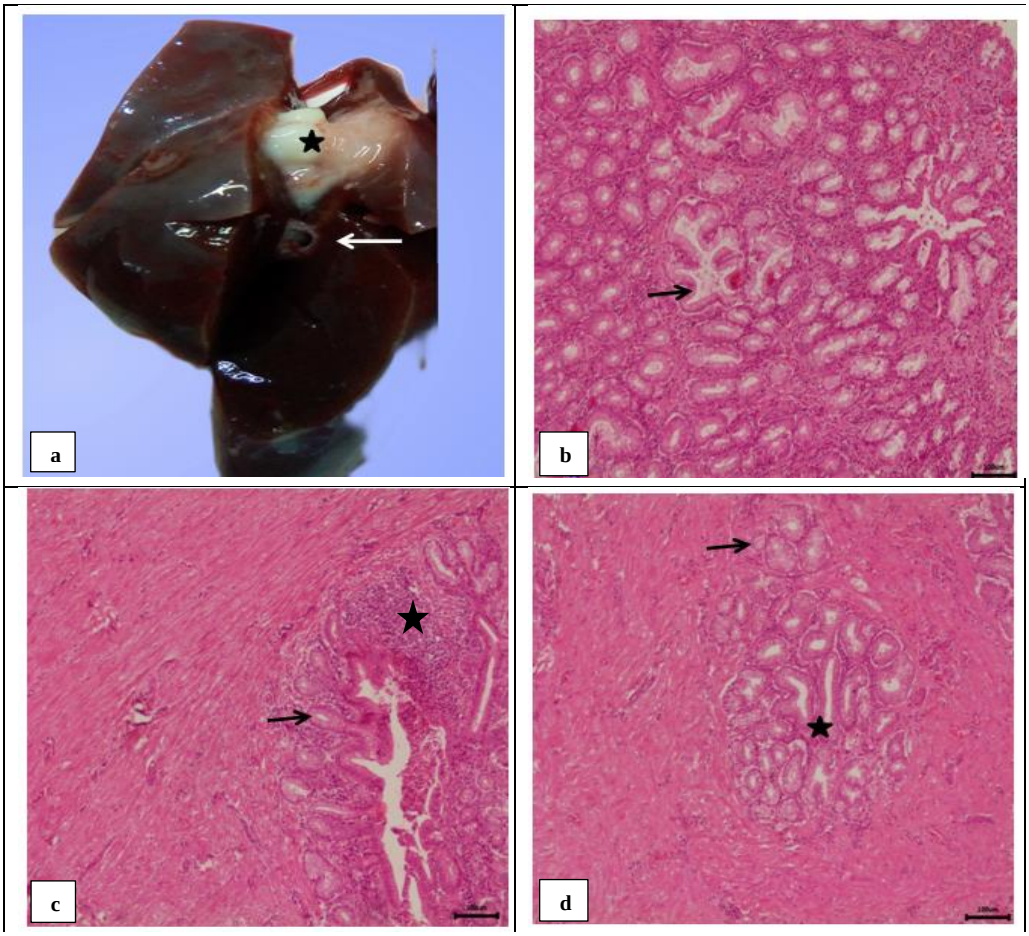
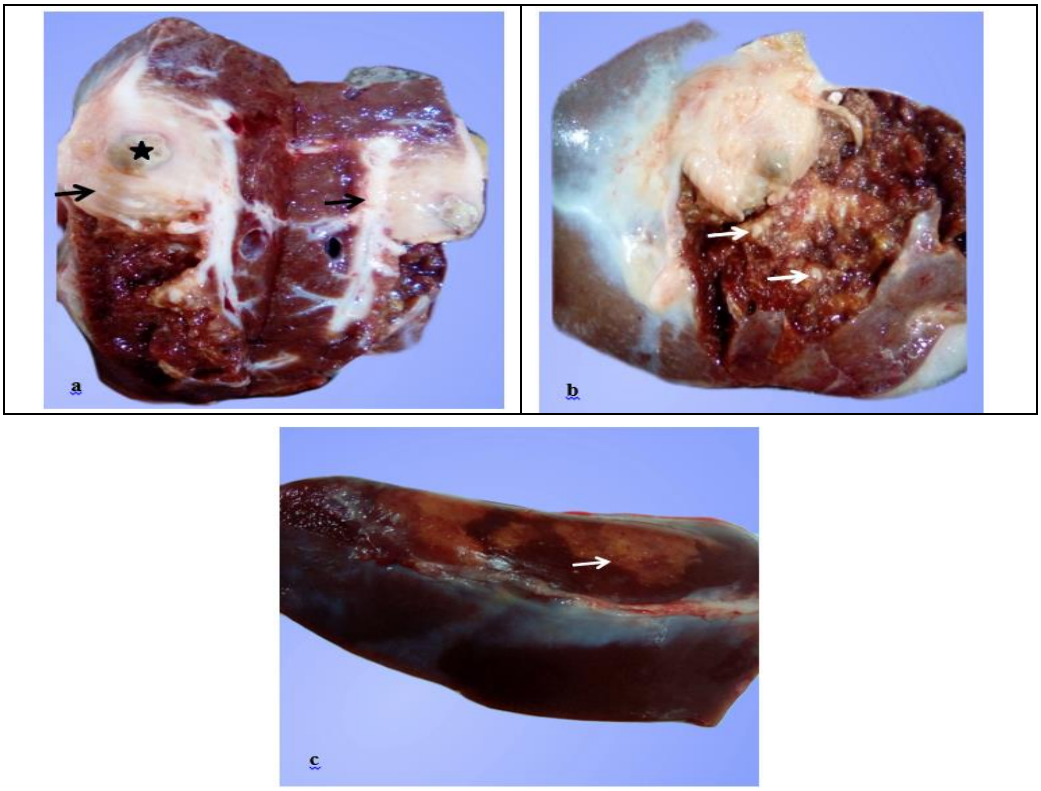


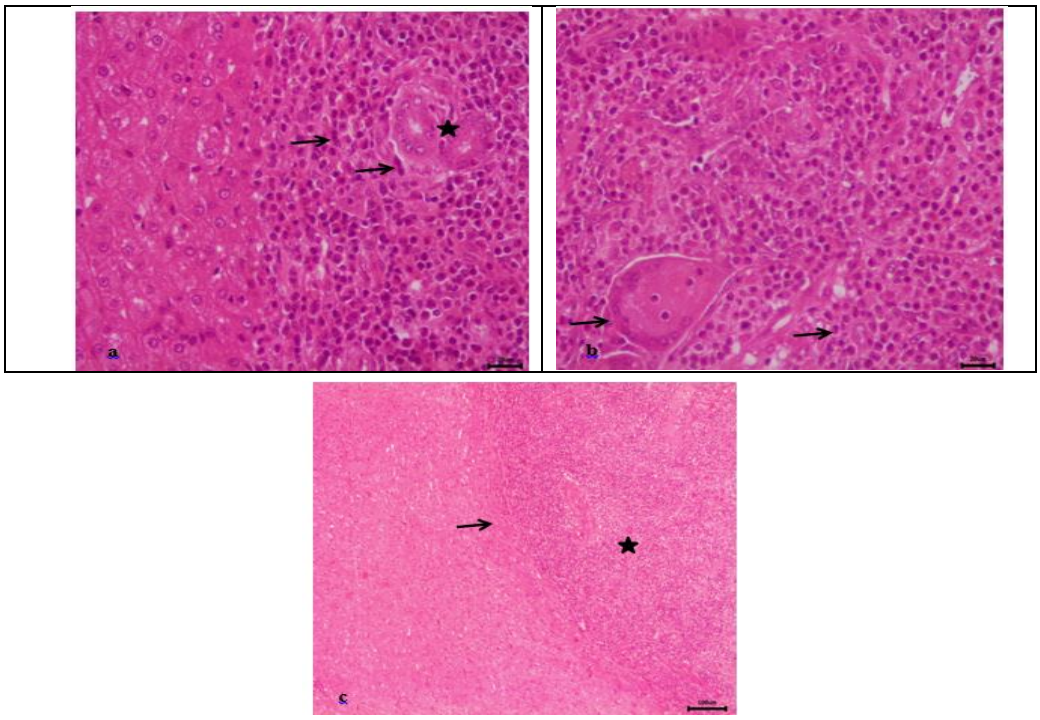
Figure (2): Percentage of each type of liver tumor in relation to their overall incidence



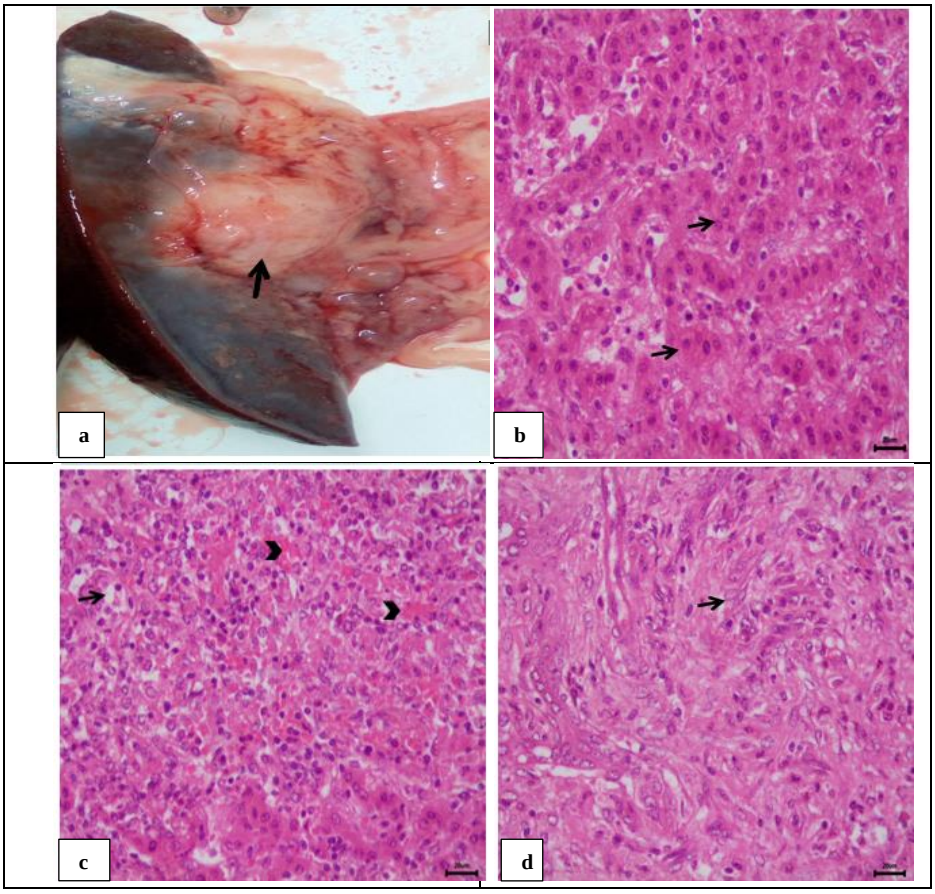
(Figure 3): Photomicrograph of liver affected with cholangioma. (a): Liver with pale whitish colored compact mass (star) embedded in hepatic tissue and protruded from the surface. Notice the associated biliary cirrhosis (arrow). (b): Section in cattle liver exhibiting large numbers of tubule-like structures lined with cuboidal to columnar cells (arrow). The stroma contains inflammatory cells, but not desmoplastic. (c): Section in liver showing marked papillary projections (arrow) associated with inflammatory cellular reaction (star). (d): Well-circumscribed tumor consists of tightly packed glands (star). The lumen is obstructed by hyperplastic cuboidal cells (arrow) (b – d: H & E.)



(Figure 4): Photomicrograph of condemned cattle livers showing: (a): Multiple, large, nodular lesions attached to the surface and extending through the biliary tree (arrows) with grayish raised necrotic centers (star). (b): Numerous, small, pin-head-sized yellowish nodules embedded in liver tissues (arrows) (c): Dark red necrotic patches and depressed area with cavitation (arrow) resulted following removal of the tumorous lesion.



(Figure 5): Photomicrograph of the liver showing the histological pattern of intrahepatic cholangiocarcinoma. (a): Poorly differentiated neoplasm consists of anaplastic cells with marked cellular pleomorphism, mitotic figure abundance (arrows), and the formation of isolated gland-like structures (star). (b): Neoplastic cells form acini of different sizes, filled with acidophilic mucinous secretion (arrows) and embedded in the fibrous stroma. (c): Neoplasm with lower magnification (star) is surrounded by hemorrhagic, degenerative, and necrotic hepatocytes (arrow) (a – c: H & E.)



(Figure 6): Photomicrograph of liver with mixed hepatocholangiocarcinoma. (a): Liver with a diffuse large yellowish-white mass attached to the surface (arrow). (b): Section in cattle liver showing the trabecular arrangement of neoplastic hepatocytes, which appear rounded or polygonal in shape (arrows). Stroma contains fibrous tissue. (c): Neoplastic hepatocytes are associated with hemorrhages (arrowheads) and neoplastic clear cells with clear cytoplasm (arrow). (d): Cholangiocarcinoma affected area showing neoplastic cholangiocytes (arrow) distributed in the desmoplastic scirrhous stroma (b – d: H & E.)

DISCUSSION

In Egypt, few researchers report the incidence rate of liver neoplasms in cattle. So the present study aimed to record their incidence and prevalence in slaughtered cattle imported from Sudan, as well as describe their detailed gross and histopathological picture. The incidence rate was 4% among the affected specimens. A lower incidence was recorded by Mohammed *et al.* (2012), who reported only 0.1% in slaughtered cows. This may be attributed to different study areas and older age groups in our study. Also, Mathewos, (2021) examined 60 liver specimens in an abattoir-based study and found that (0.6%) of the examined livers showed neoplasm, which was classified histologically as hepatocellular carcinoma. In accordance with our results, Braun *et al.* (2005) and Jeong *et al.* (2005) mentioned that cattle of similar age, approximately 3 years old, were more susceptible to several types of hepatic neoplasms.

Concerning cholangioma, in complete agreement with our findings, Puette and Hafner (1995) observed an encapsulated 5-10 cm growth on the liver surface with no evidence of metastasis. Histopathological examination revealed numerous glandular structures that had variable sizes and were lined by cuboidal cells with abundant

eosinophilic cytoplasm and basally oriented nucleus. These structures were distributed in a collagenous stroma. Also, aggregation of lymphocytes was noticed at the site of the lesion with minimal compression to the adjacent liver parenchyma. However, according to Martin (2014), biliary cystadenomas were formed from bile ducts with anarchic arrangement, atrophied or prismatic epithelial cells. They were filled with bile and seromucous fluid. In addition, the general picture of fibrosis was observed.

Jubb *et al.* (2007) stated that cholangiocellular carcinomas differ from hepatocellular variety by their tendency to multiply, firmness consistency, pale beige coloration caused by more or less abundant stroma, and the typical umbilicated appearance that produced by central depressed necrotic areas or cavitation-associated collapse of tumor vessels. Among different gross forms of cholangiocarcinoma, Vielmo *et al.* (2020) detected only the multinodular form, which appeared as firm nodules, occasionally umbilicated with irregular shape and approximately 6 cm diameter. These nodules took whitish-yellow coloration and were randomly distributed throughout the hepatic parenchyma. These previous gross pictures were in agreement with those recorded in our study. However, Ilha *et al.* (2005) observed two cauliflower-like masses whose diameter was 8-14 cm obstructing the lumen of the common bile duct, which appeared enlarged and tightly adhered to the liver capsule. Cullen *et al.* (2016) mentioned that cholangiocarcinoma rarely becomes restricted to one hepatic lobe, and usually occurs as a massive lesion in the form of multiple umbilicated whitish masses of variable sizes protruding from the liver surface. According to Darzi *et al.* (2012), this condition was mostly related to parasitic infestation, especially with *Dicrocoelium dendriticum*. Such a conclusion was based on observing tumor-like growths in parasitized livers which were confirmed histologically as cholangiocellular carcinomas with protruding multilayered cuboidal cells into the lumen of proliferating bile ducts. Elbert *et al.* (2021) mentioned that lesions of cholangiocarcinoma in cattle were similar to those caused by tuberculosis and occurred in the form of numerous coalescing pale yellowish nodules that were not only present in the liver, but also in the omentum and ruminal serosa.

The most characteristic histological features of cholangiocarcinoma are mucin production and the fibrous stroma, which were regarded as valuable findings for tumor recognition (Head *et al.,* 2003). Meuten (2016) stated that cholangiocarcinomas might be either well differentiated with an acinar or tubular histopathological pattern or undifferentiated with a solid pattern. However, in agreement with our observed acinar form, Vielmo *et al.* (2020) reported that it was formed of undifferentiated cells that displayed high mitotic index, marked cellular pleomorphism, and vascular invasion. In their study, the solid pattern was the most frequent, in which large neoplastic cells with prominent, multiple nuclei mostly replaced the hepatic parenchyma. Jubb *et al.* (2007) and Ohfuj (2012) mentioned that cholangiocellular carcinomas form acini, ductules, or papillary projections within the lumen of neoplastic ducts. Cells were cuboidal or columnar with minimal amounts of clear cytoplasm. Mitotic figures were abundant. Tubules were filled with mucinous secretions and didn't contain bile. In contrast, Azizi *et al.* (2016) detected mucin-free acinar structures or solitary islands located mostly in periportal areas and were embedded in excessive fibrous tissue stroma. Cuboidal to columnar neoplastic cells appeared with variable-sized, round-to-oval nuclei and basophilic cytoplasm. According to Elbert *et al.* (2021), neoplastic epithelial cells formed ductules surrounded by dense or edematous areas of

desmoplasia, necrosis, and dark basophilic clumps of calcium.

Honda *et al.* (2020) recorded a case of hepatocholangiocarcinoma in an aged cow. During the post-mortem examination, the liver showed some degrees of adhesion with various internal organs, its external surface was irregular and nodular. Some hepatic lobes were mostly replaced by whitish connective tissue. The cut section revealed a pus-like fluid filling distended bile ducts and orange to yellow-colored liver tissue. Also, they observed a severely enlarged gall bladder that contained bile with blackish stones. However, a nearly similar gross picture was reported in our study, with the presence of a large diffuse yellowish-white lesion measuring about (15) cm and replacing most of the hepatic parenchyma. This was associated with a narrow hemorrhagic zone and necrotic areas at the site of its attachment. Aishma *et al.* (2010) and Yeh, (2010) mentioned that the characteristic histopathological feature of hepatocellular carcinoma was trabecular or pseudo glandular arrangement of neoplastic hepatic cells that appeared to resemble hepatocytes with fat, while cholangiocellular carcinoma was characterized histologically by glandular structure, production of mucin and the desmoplastic stroma. Honda *et al.* (2020) observed the collision of both features of hepatocellular and cholangiocellular carcinomas, which resulted in the development of combined hepatocellular cholangiocellular carcinoma. In hepatocellular carcinoma-affected areas, neoplastic hepatocytes were polygonal in shape, with round to oval nuclei, abundant pale acidophilic cytoplasm, and were arranged in solid nests or trabeculae, while in areas of cholangiocellular carcinoma, neoplastic cholangiocytes were cuboidal to columnar, with round to oval nuclei and mucin on its apical surface or in the cytoplasm. Neoplastic cholangiocytes formed tubular-like structures embedded in stromal fibrosis. This picture was in complete agreement with our histopathological findings.

CONCLUSION AND RECOMMENDATION

Liver and bile duct tumors are prevalent in beef cattle imported from Sudan. These animals might be exposed to certain hepatocarcinogens as aflatoxins or certain drugs administered for treatment purposes. Strict veterinary inspection at slaughterhouses should be applied to these animals, as they are considered a major source of meat in our country. In addition, a larger sample size and further studies concerning lesions in other organs are required to be conducted.

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دراسات باثولوجية على أورام الكبد والقنوات المرارية فى العجول السودانية

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تهدف هذه الدراسة الى تسجيل نسب حدوث أورام الكبد و القنوات المرارية فى العجول السودانية المستوردة، وكذلك وصف التغيّرات الظاهرية والمجهرية الخاصة بهم. فى مجزر أبو سمبل بمحافظة أسوان تم فحص ١٥٧٥ كبد من هذه العجول المستوردة حيث سجلت أورام الكبد والقنوات المرارية عدد ٧ حالات بنسبة ٤% من إجمالى الإصابات. أظهرت العينات المصابة وجود كُتل تورمية فى أنسجة الكبد مع حدوث درجات مختلفة من التليف الكبدى. تم إستبعاد العينات المصابة وتصويرها وأخذ عينات منها وحفظها فى محلول فورمالين متعادل، وبعد إجراء الفحص الهستوباثولوجى تم تصنيفها الى ثلاثة أنواع : ورم حميد فى القنوات المرارية بعدد ٤ حالات، ورم خبيث فى القنوات المرارية بعدد حالتان، و ورم خبيث مختلط فى الكبد والقنوات المرارية بعدد حالة واحدة.

وخلُصت هذه الدراسة الى احتمال تعرض هذه الحيوانات الى مواد مسرطنة قبل الذبح مثل بعض السموم الفطرية التى قد تختلط بالأعلاف أو بعض المواد الكيميائية التى تستخدم فى الأغراض العلاجية، لذلك لابد أن تخضع هذه الحيوانات للكشف البيطرى الدقيق بعد الذبح فى المجازر للتأكد من صلاحيتها للاستهلاك الآدمى.