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Cholangiocarcinoma in a male Labrador: A case report

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Abstract
A two-years-old male Labrador dog was presented to the Small Animal Multispecialty Hospital of the Department of Teaching Veterinary Clinic Complex, GADVASU, Ludhiana with a history of inappetance, vomition, lethargy and passing of dark colour urine. Physical examination revealed oedema of ventral abdomen. Blood smear examination showed mild anaemia, along with neutrophilic leucocytosis and mild left shift, whereas, serum chemistry analysis revealed high AST, ALT and ALKP level. On ultrasound examination, irregular margins along with multiple hypoechoic areas and hyperechoic liver parenchyma were observed. Ultrasound guided fine needle aspiration cytology from liver revealed presence of epithelial cells in clusters showing high cellularity with coarse chromatin and prominent nucleoli, whereas, ultrasound guided biopsy was suggestive of cholangiocarcinoma.

Keywords: Cholangiocarcinoma, Labrador, leucocytosis, ultrasound guided biopsy

Introduction
Cholangiocarcinoma is a rare primary hepatic tumour that arises from the epithelium of intrahepatic and extrahepatic bile ducts and may occur as a large single mass or as multiple lesions with poor prognosis (Jacobs and Snyder, 2007 [1] and Nagy *et al*., 2014) [2]. Hepatocellular adenoma, hepatocellular carcinoma, cholangiocellular adenoma, and cholangiocellular carcinoma account for 0.6–1.3 percent of all canine neoplasms (Javanbakht *et al*., 2013) [3]. Malignant biliary neoplasms are more common than benign neoplasms in dogs. Cholangiocellular carcinoma is aggressive and metastasis is common (Liptak, 2013) [4].
Cholangiocarcinoma has been reported in canine, felines, ovines, caprine and bovines. Cholangiocellular carcinoma is more common in old animals. Bile duct carcinoma in dogs is less prevalent and more aggressive with a higher rate of metastasis, despite less evident cellular pleomorphism and anaplasia than hepatocellular cancer (Javanbakht *et al*., 2013) [3]. Common clinical signs include lethargy, apathy, anorexia, weight loss, vomition and diarrhoea, whereas, ascites and neurological signs are rarely seen (Sharma *et al*., 2016 [5]; Aslan *et al*., 2014) [6]. The present communication deals with a rare case of cholangiocarcinoma in a two-year-old male Labrador dog.

Materials and Methods
The two-year-old male Labrador dog was presented with a history of inappetance, vomition, and lethargy with oedema of ventral abdomen. Clinical examination, hematobiochemical and radiological examinations of the dog were performed. Ultra sound guided (USG) fine needle aspiration cytology (FNAC) and biopsy was taken for cytological and histopathological examination. FNAC slides were stained by Leishman stain (Jain, 2006) [7]. Biopsy was collected and fixed in 10% neutral buffered formalin (NBF) for routine histopathological examination as per standard protocol (Luna, 1968) [8].

Results and Discussion
In the present case, anorexia, vomition, oedema and lethargy were the common clinical signs observed. On USG, margins of liver were irregular, nodular and liver parenchyma had multiple hypoechoic areas with hyperechoic parenchyma (Figure 1). Free fluid was seen in abdomen, whereas, spleen, kidney, urinary bladder were normal.

Haematological examination revealed mild anaemia and neutrophilic leucocytosis, whereas, other haematological parameters were normal (Table 1). Serum chemistry analysis revealed increased AST, ALT and ALKP (Table 2). Increase in these parameters were also reported by Mart´ınez-Sogues *et al*. (2019) [9] and Meuten *et al*. (2017) [10] which might be due to invasion of liver hepatocytes by neoplastic biliary epithelium. Cytosmear revealed clusters of the epithelial cells with indistinct cytoplasmic borders, anisocytosis, anisokaryosis and variable nuclear to cytoplasmic ratio (N:C) along with clumped chromatin patterns suggestive of carcinoma (Figure 2). Mart´ınez-Sogues *et al*. (2019) [9] also observed additional features of binucleated and multinucleated cells in their cytological findings of cholangiocarcinoma in the dog. Histopathological examination showed tubular structure of neoplastic biliary epithelium with moderate amount of stroma (Figure 3). Acinar arrangements of biliary epithelium in liver were also observed and separated by fibrous connective tissue (Figure 4). The cuboidal cells showed a moderate amount of clear to pale eosinophilic cytoplasm. Nuclei were round to oval, hyperchromatic and showing pleomorphism and mitotic figures were also observed. These findings were in accordance to previous findings. (Mart´ınez-Sogues *et al*., 2019 [9]; Javanbakh *et al*. 2013 [3]; Tanaka *et al*. 2021) [11]. Adenocarcinoma with a fibrous stroma is the most typical histological feature of cholangiocellular carcinoma in humans. In dogs, stromal fibrosis is linked to cholangiocellular carcinoma. Therefore, histological elements like fibrosis may have an impact on progressive enhancement. (Weber *et al*. 2015 [12]; Tanaka *et al*. 2021) [11].

On the basis of detailed cytological and histopathological findings, the case was diagnosed as cholangiocellular carcinoma in a young dog.

From the present study, it can be concluded the cholangiocellular carcinoma can be suspected on the basis of cytological examination and confirmed by histopathological findings.

Table 1: Haematological findings

Sr. No.	Parameters	Observed Value	Reference interval (Benjamin, 2010)
1.	Hb (g/dL)	9.0	12-18
2.	PCV (%)	27.0	37-55
3	TLC (cu mm)	41200	6-17000
4.	TEC (cu mm)	3.96	5.5-9.5
5.	Neutrophil (%)	94	60-72
6.	Absolute neutrophil count	38,728	3600-12240
7.	Lymphocyte (%)	06	12-30
8.	Absolute lymphocyte count	2472	720-5100

Table 2: Serum biochemical findings

Sr. No.	Parameters	Observed Value	Reference interval (Kaneko et al., 2008)
1.	SGOT/AST (U/L)	73	8.9-49
2.	SGPT/ALT (UL)	63	8.2-57
3.	ALKP (UL)	190	10.6-101
4.	Total protein (g/dL)	4.9	5.4-7.1
5.	Albumin (g/dL)	1.5	2.2-3.2
6.	GGT (U/L)	19	1.9-2.7
7.	BUN (mg/dL)	07	8.8-26
8.	Creatinine (mg/dL)	0.4	0.5-1.6

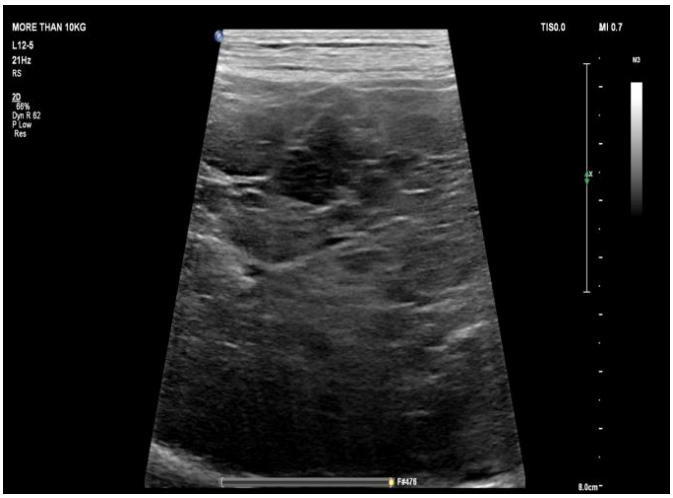

Fig 1: Liver parenchyma has multiple hypoechoic areas with hyperechoic parenchyma

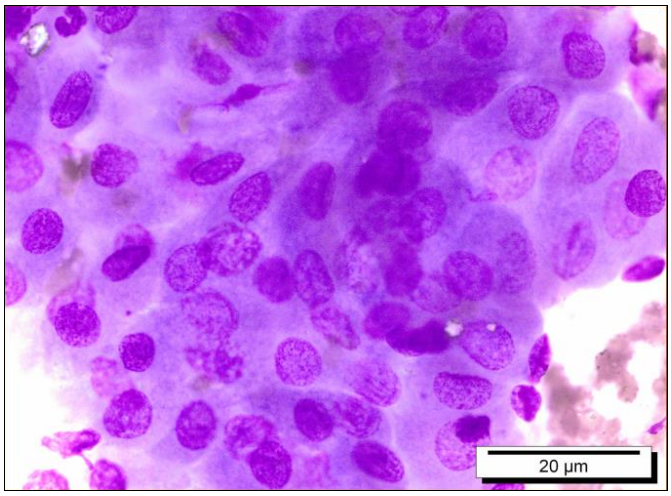

Fig 2: Clusters of the epithelial cells with indistinct cytoplasmic borders (Leishman x100)

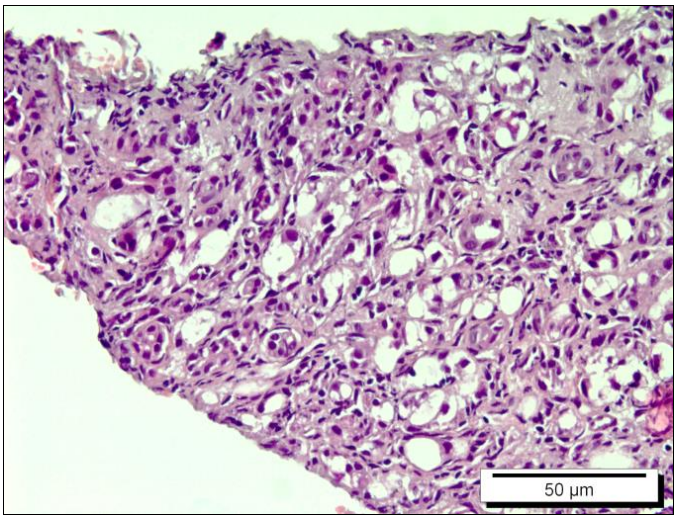

Fig 3: Acinar arrangement of well-differentiated cholangiocarcinoma (H&E x200)

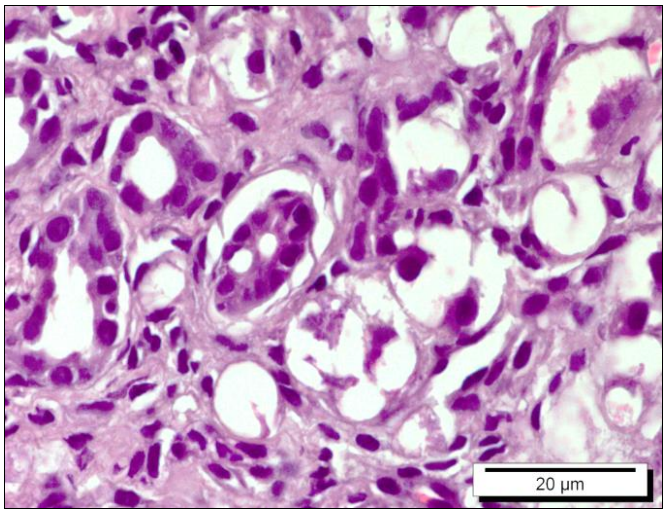

Fig 4: Cuboidal cells with diffuse fibrous stroma forming tubular structures (H&E x400)

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