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Case Report

Metastatic gallbladder adenocarcinoma in a cat

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Abstract
Biliary neoplasms are uncommon in cats and affect older animals. A 12-year-old female crossbreed cat showed prostration, lethargy, apathy, and severe jaundice. Ultrasonography showed distention of the gallbladder associated with severe obstruction of the bile ducts with thickening of the biliary wall, forming amorphous masses of irregular contour and heterogeneous appearance directed to the lumen measuring up to 2 cm. Necropsy showed a gallbladder with a yellowish and soft nodule measuring 3 × 3 cm, compressing the extrahepatic bile duct, occluding the passage of bile. There were also firm, yellowish multifocal to coalescing nodules in the liver, ranging from 0.5 to 1 cm, affecting 10% of the organ, in addition to lungs with firm, yellowish multifocal nodules ranging from 0.2 to 0.5 cm, affecting 20% of the organ. Histologically, gallbladder and bile ducts had malignant epithelial neoplastic proliferation, which was organized into multiple papillary and ductal projections, separated by moderate fibrovascular stroma compatible with gallbladder adenocarcinoma. The liver and lungs also contained neoplastic structures with a ductal appearance and papilliform projections identical to those observed in the gallbladder. The immunohistochemical examination (IHC) showed intense positive staining of epithelial neoplastic cells for pan-cytokeratin (AE1/AE3) and no staining for vimentin (Clone V9). The diagnosis of gallbladder adenocarcinoma with metastasis in the liver and lungs was established based on the clinical, macroscopic, histopathological, and immunohistochemical findings.

Keywords: adenocarcinoma, hepatobiliary system, immunohistochemistry.

Introduction

Primary tumors that affect the hepatobiliary system represent 1% to 3% of all neoplasms in cats, originating from hepatocytes, intra and extrahepatic bile duct cells, mesenchymal cells, and neuroendocrine cells, in which the gallbladder is considered a rare site to develop neoplasms, with carcinomas being reported more frequently than adenomas (1, 15, 26, 28).

In cats, neoplasms originating in bile duct cells occur more frequently than those originating in hepatocytes, and extrahepatic bile duct obstruction is a differential for gallbladder adenocarcinoma, especially in cases in which there has been no response to clinical treatment (2, 10). There are reports of several animal species affected by adenomas and carcinomas of the biliary tract, such as cats (5, 10, 19, 22), dogs (2, 14, 21), cattle (13, 16), bears (20), and African lions (25).

In many cases, the diagnosis is late due to the presentation of nonspecific clinical signs. The most common signs are loss of appetite and weight, lethargy, and vomiting, in addition to jaundice, which is an important manifestation of biliary diseases (10). Blood count and abdominal ultrasound serve as screening tests for diseases of the hepatobiliary system. On the other hand, cytology, histopathology, and immunohistochemistry are mandatory tools for confirmation, treatment, and prognosis of the neoplasm (4, 22, 24). The prognosis in cats with malignant hepatobiliary tumors is unfavorable due to the high frequency of metastases and invasive degree, making surgical resection often unfeasible (9). The study aimed to report an unusual case of gallbladder adenocarcinoma with liver and lung metastasis in a cat.

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Case Description

A 12-year-old female crossbreed cat with prostration, lethargy, apathy, and intense jaundice was treated at the Hospital de Clínicas Veterinárias Professor Lauro Ribas Zimmer no Centro de Ciências Agroveterinárias da Universidade do Estado de Santa Catarina (HCV, CAV/UDESC), in Lages/SC, Brazil. Abdominal ultrasound, blood count, and biochemical profile were requested after the physical examination.

The ultrasound evaluation showed a distention of the gallbladder with anechoic content associated with severe dilation of bile ducts and canaliculi ranging from 0.35 to 3.1 cm. There was also an area of greater biliary distension in the medial epigastric region, close to the duodenal papilla, with severe focal thickening of the wall, forming amorphous masses of irregular contour and heterogeneous appearance directed to the lumen, measuring up to 2 cm in diameter. Thus, ultrasound findings suggested cholangiohepatitis associated with extrahepatic biliary obstruction due to a bile duct mass, suggesting neoplastic disease or chronic cholangitis.

Blood count alterations included intense leukocytosis (74,690 cells/µL; reference: 5,500–19,500 cells/µL/L) with neutrophilia (70,958 cells/µL; reference: 2,500–13,000 cells/µL/L), and the serum biochemical profile showed an increase of liver enzymes alkaline phosphatase (496 IU/L; reference: 5–24 IU/L) and gamma-glutamyl transferase (21 IU/L; reference: 1-10 IU/L). Tests were performed to detect feline leukemia virus (FeLV) antigen and feline immunodeficiency virus (FIV) antibody by enzyme immunoassay (ELISA) using the SNAP Combo FeLV/Ag FIV/Ac test (IDEXX Laboratories®), obtaining negative results for both viruses. The patient was submitted to euthanasia 11 days after the first clinical consultation due to the failure to respond to medications and the progressive worsening of the clinical condition.

At necropsy, the mucosa, as well as the subcutaneous tissue, were intensely icteric (Fig. 1-A). Abdominal cavity showed moderate ascites and the organs had their serosae diffusely icteric. The gallbladder accommodated a yellowish and soft mass measuring 3 x 3 cm, which compressed the extrahepatic bile duct, occluding the passage of bile. The vesicle in the initial portion was distended with bilious content with a gelatinous appearance (Fig. 1-B). The liver contained multifocal to coalescing firm, yellowish nodules ranging from 0.5 to 1 cm, affecting 10% of the organ. In addition, a dilatation was observed in the intrahepatic bile ducts, from which gelatinous bile flowed on the cut. The lungs had moderately multifocal firm yellowish nodules, which ranged from 0.2 to 0.5 cm, affecting 10% of the organ.

Fragments of all organs were collected and stored in 10% buffered formalin. After fixation, the fragments were routinely processed for histopathology, embedded in paraffin, stained with hematoxylin-eosin, and slides read under light microscopy.

The histopathological evaluation of the gallbladder and adjacent ducts showed a non-delimited malignant epithelial neoplastic proliferation, which was organized in multiple papillary and ductal projections, separated by moderate fibrovascular stroma with moderate desmoplasia (Fig. 1-C). The nuclei of these cells varied from rounded to oval, predominantly apical, with 1 to 2 evident nucleoli. The cytoplasm was elongated polygonal, eosinophilic, and moderately delimited. In addition, moderate to marked anisocytosis and anisokaryosis were observed and two mitotic figures per 2.37 mm^2. There was also a moderate multifocal lymphoplasmocytic infiltrate, in addition to a large amount of eosinophilic amorphous material, fibrin bundles, and cellular debris. The liver had a neoplastic infiltrate of the same appearance as that observed in the gallbladder and bile duct, which infiltrated the portal spaces and promoted fibrosis. The lungs also had neoplastic structures with a ductal appearance and papilliform projections in multiple foci.

The histological sections were placed on silanized glass slides and submitted to antigenic recovery by moist heat in a pressure cooker (pan-cytokeratin and vimentin) and water bath (anti-FeLV and anti-FIV), using citrate buffers (pH 6) and tris-EDTA (pH 9), respectively. Blocking of endogenous peroxidase was performed in 10% hydrogen peroxide diluted in methanol whereas blocking of nonspecific reactions was performed with skimmed milk powder (Molico®). The primary antibodies used in the neoplasm sections consisted of pan-cytokeratin (1:100, AE1/AE3 clone cocktail, Biocare Medical, Concord, California, USA) and vimentin (1:200, V9 clone, Biocare Medical, Concord, California, USA), while anti-FeLV (1:500, gp70, Biocare Medical, Concord, California, USA) and anti-FIV (1:500, p24, Biocare Medical, Concord, California, USA) were used in lymph node and bone marrow sections, all diluted in phosphate-buffered saline (PBS). Antibody signaling was performed using the polymer technique linked to endogenous peroxidase (Kit MACH 4 HRP, Biocare Medical, Concord, California, USA), revealed with the chromogen 3,3′-diaminobenzidine (DAB, Dako, Glostrup, Denmark), and counterstained with hematoxylin. Positive and negative controls were also inserted in the tested slides.

There was a diffuse, moderate to marked positive staining for pan-cytokeratin in the cytoplasm of neoplastic cells (Fig. 1-D) and positive for vimentin (Clone V9) only in cells that make up the neoplasm stroma. IHC evaluations of anti-FeLV and anti-FIV lymph node and bone marrow were negative. Thus, the macroscopic, histopathological, and immunohistochemical findings allowed for determining the diagnosis of gallbladder adenocarcinoma with metastasis in the liver and lungs.

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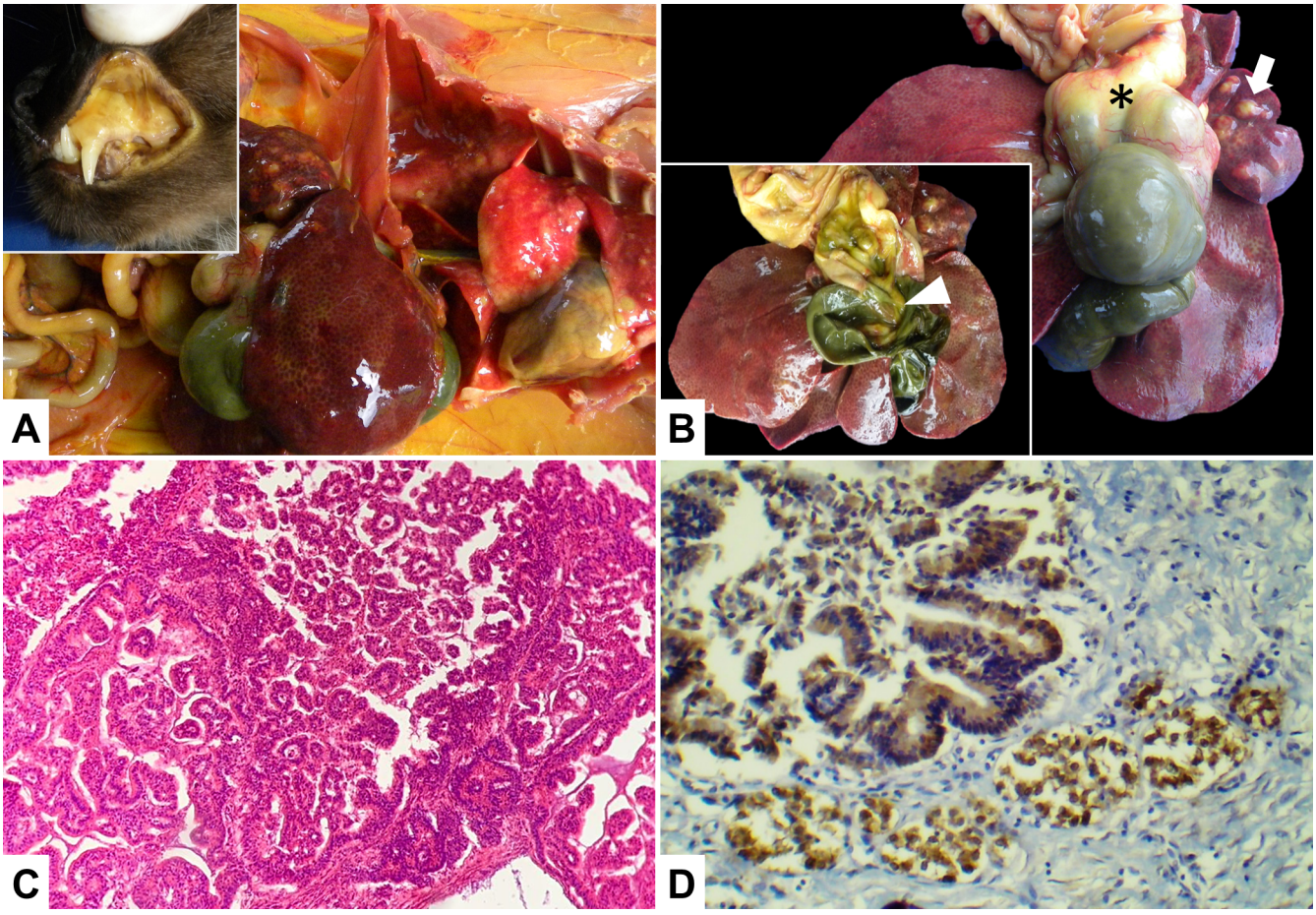

Figure 1. Gallbladder adenocarcinoma in a 12-year-old female crossbreed cat. A. The oral mucosa, subcutaneous and organs of the thoracic and abdominal cavities with marked jaundice. B. Gallbladder with a soft, yellowish nodule in its final portion, compressing the extrahepatic bile duct associated with bile accumulation and organ distention (*), in the section shown by the arrowhead. Liver with multifocal to coalescing metastatic nodules of the same appearance (arrow). C. Malignant epithelial neoplasm, organized into multiple papillary and ductal projections supported by a moderate fibrovascular stroma. (HE, x20). D. Intense immunostaining for pan-cytokeratin (Cocktail AE1/AE3) in neoplastic epithelial cells of papillary projections (Polymer linked to endogenous peroxidase [MACH 4 HRP], DAB 3,3'-diaminobenzidine chromogen) (IHC, x300).

Discussion

Neoplasms that affect the hepatobiliary system are uncommon, usually affecting elderly animals with a mean age of 10 years (1, 17), with no predisposition for breed and gender (1, 18). The advanced age of the feline in the present report was a predisposing factor, but the development of this neoplasm in young cats has already been described (10).

Clinical signs are nonspecific and similar to those of liver or gastrointestinal diseases, which include apathy, anorexia, and weight loss (4, 26). The location of the primary nodule resulted in the obstruction of the flow of bile to the intestine, generating a marked picture of post-hepatic jaundice. Biliary stasis stimulates other gastrointestinal alterations presented by the patient, such as weight loss, vomiting, and apathy, as bile is an important component in the digestion process, and this accumulation of bile acids has a hepatocellular cytotoxic effect, corroborating the worsening of the clinical picture of the animal (23, 27). Gallbladder tumors are commonly incidental findings in surgeries or necropsies, a factor that is often favored by the non-specificity of clinical signs or even by the absence of hepatic alterations in the evaluation of serum biochemistry (27).

Hepatobiliary tumors may reflect a nonspecific hematologic examination, and cats diagnosed with biliary adenocarcinomas are asymptomatic in up to 38% of cases, with liver enzymes within reference values (3, 27). However, there are cases in which the increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) may be correlated with malignant hepatobiliary tumors, but they are not specific enzymes for neoplasms since they only indicate degeneration of liver cells and bile stasis (4, 18). In this case, the patient had

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AST and ALT values within the reference parameters. However, alkaline phosphatase (AF) and gamma-glutamyl transferase (GGT) values were increased because these enzymes indicate cholestasis. The increase in AF is present in 78% of the cases but can be explained by the site of the neoplasm, which compresses the gallbladder and causes bile stasis (19).

Abdominal ultrasound is a widely used exam to determine the presence of masses or nodules, as well as visualize their adjacent structures (4). The ultrasound findings of biliary distention associated with biliary wall thickening and dilated bile ducts due to extrahepatic biliary obstruction suggestive of gallbladder neoplasia found in the present report are consistent with those described by other authors (5, 10).

There are few reports in the literature related to gallbladder adenocarcinoma in cats. The lesions found at macroscopic examination corroborate the findings in other reports, with thickening and dilatation of the gallbladder with gelatinous bile content and formation of nodules, leading to obstruction of the bile ducts (10, 22). The lesions in other species may show a cauliflower-like appearance, necrotic, and sometimes present rupture (16, 21, 25), which were not observed in this case.

The gallbladder adenocarcinoma of the present case showed a ductal and papillary pattern (10, 25) compared to other cases in which the cell arrangement may be acinar (8, 16, 22). Furthermore, it can be supported by discrete fibrovascular stroma and there may be areas of desmoplasia marked by abundant fibrous tissue due to the cirrhotic response caused in the liver. Neoplastic epithelial cells commonly present a polygonal to columnar shape with moderate pleomorphism, in addition to a low mitosis count despite the malignant character of the neoplasm and the high invasive potential to the liver parenchyma. Mucin production by the biliary epithelium in varying amounts is also observed (8, 16, 22).

The gallbladder adenocarcinoma presented metastasis in the lung and liver. It is a malignant, invasive, and metastatic tumor. Tumors originating from the biliary system in cats are more malignant than those with hepatocellular origin and that metastases occur in 80% of cases (22). The lungs are the most common site of dissemination, with hepatic lymph nodes, abdominal serosa, and distant lymph nodes also involved (8).

Cytokeratins are immunohistochemical markers of epithelial cells, expressed in pancreatic duct and bile duct neoplasms in humans and animals (1, 11, 24). This study showed a positive expression for cytokeratin in the cytoplasm of neoplastic cells of the gallbladder (10). The use of cytokeratins for bile duct epithelial cells, aiding in the diagnosis of cholangiocarcinoma, a neoplasm that originates from the bile ducts (1). ELISA and IHC tests were performed for FeLV and FIV, with negative results for both. Diseases of neoplastic origin, especially lymphomas and leukemias, have a high association with FeLV (6, 7), but there are no reports in which gallbladder adenocarcinoma is associated with FeLV and FIV. A report of a feline affected by a gallbladder adenoma showed no association with FeLV and FIV viruses (5).

In conclusion, this report provides clinical, pathological, and immunohistochemical description of a gallbladder adenocarcinoma with metastasis in the lungs and liver in an elderly cat, consisting of a differential diagnosis for cases of bile duct obstruction, especially cases without response to medical treatment.

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