











Case Report

Cholangiocarcinoma with splenic metastasis in a red-tailed amazon (*Amazona brasiliensis*)

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Abstract

Cholangiocarcinoma is a tumor originating from the epithelium of the biliary ducts that has been frequently reported in birds, particularly within the order Psittaciformes. A red-tailed amazon (*Amazona brasiliensis*) was voluntarily delivered to the Centro de Triagem e Recuperação de Animais Silvestres (CETRAS), in the Parque Ecológico do Tietê (São Paulo municipality, São Paulo state, Brazil), showing signs of prostration and respiratory distress. Despite supportive care, the bird died seven days after admission. Necropsy revealed cachexia and multiple small yellowish nodules scattered across the hepatic surface and extending into the parenchyma. Histopathological examination of the liver showed epithelial cell proliferation forming tubular structures within a moderately collagenous stroma, suggestive of cholangiocarcinoma. Immunohistochemical analysis of hepatic tissue was performed using a diagnostic panel including AE1/AE3, CK7, and HepPar-1. Only AE1/AE3 yielded a positive result, confirming the diagnosis of cholangiocarcinoma. This is the first report of cholangiocarcinoma in a red-tailed amazon. We highlight the importance of proper nutritional management to reduce the likelihood of neoplastic disease development, thus improving quality of life and increasing life expectancy in captive birds.

Keywords: Avian pathology, neoplasm, immunohistochemistry.

Introduction

Cholangiocarcinoma originates from the biliary duct epithelium and exhibits aggressive behavior (9). Grossly, it often manifests as discrete whitish nodules of varying sizes (1). Clinical signs are nonspecific and may include prostration, anorexia, regurgitating, and progressive weight loss. Affected birds usually show poor response to treatment and rapidly progress to death (5, 19, 28).

Cholangiocarcinoma is a malignant hepatic tumor commonly reported in birds (25, 12), particularly within the

order Psittaciformes, with a higher prevalence in the genus *Amazona* (12). Documented cases include the blue-fronted Amazon [*Amazona aestiva*; (12)], yellow-crowned amazon [*Amazona ochrocephala*; (16)], red-lore Amazon [*Amazona autumnalis*; (1)], orange-winged amazon [*Amazona amazonica*; (7)], and yellow-faced parrot [*Alipiopsitta xanthops*; (20)]. These tumors have also been described in birds from other orders, such as the ring-necked pheasant [*Phasianus colchicus*, Galliformes; (19)], greater rhea [*Rhea americana*, Rheiformes; (23)], Adélie penguin [*Pygoscelis adeliae*, Sphenisciformes; (22)], and sandhill crane [*Grus canadensis*, Gruiformes; (15)].

In dogs and cats, cholangiocarcinoma accounts for less than 1% of all diagnosed neoplasms, with an estimated incidence of 0.36% in dogs (26). In domestic animals, this tumor may manifest as a single large mass that replaces an entire hepatic lobe or, more commonly, as multifocal nodular lesions affecting multiple or all lobes of the liver (29). In birds, the multifocal nodular pattern is also predominant, with lesions varying in size and exhibiting a whitish to brownish coloration (12, 28, 30).

This case report describes the gross, histological, and immunohistochemical findings of cholangiocarcinoma with splenic metastasis in a captive red-tailed amazon (*Amazona brasiliensis*). This species is classified as Vulnerable on the List of Threatened Species of the State of São Paulo (24) and as Near Threatened on the International Union for Conservation of Nature (IUCN) Red List (4). Therefore, studies investigating diseases affecting this species—whether in the wild or in captivity—are essential for its conservation.

Case description

On August 23, 2023, a female red-tailed amazon (*Amazona brasiliensis*), approximately four years old and kept as a pet, was voluntarily delivered by its caretaker to the Centro de Triagem e Recuperação de Animais Silvestres

(CETRAS SP), in São Paulo State, Brazil. Upon admission, the bird showed severe prostration, weakness, and respiratory distress. After one week of treatment without any clinical improvement, the bird died.

A necropsy was performed, and all organs were examined macroscopically. Tissue samples were collected, fixed in 10% phosphate-buffered formalin (pH 7.4), processed using standard paraffin-embedding techniques, cut at 5 μ m, and stained with hematoxylin and eosin. Liver fragments containing the tumor lesion were subjected to immunohistochemical analysis. Tissue sections were placed on silanized slides, incubated at 80 °C, deparaffinized in xylene-alcohol baths, rinsed in running water, and treated with an endogenous peroxidase blocker. Antigen retrieval was performed in a pressure cooker, followed by washing in running water and immersion in phosphate-buffered saline (PBS). Slides were incubated with primary antibodies, and detection was performed using 3,3-diaminobenzidine (DAB) with Harris hematoxylin counterstaining. The antibodies used were pancytokeratin (AE1/AE3, dilution 1:100), HepPar-1 (1:200), and CK7 (1:200).

External examination revealed cachexia and onychogryphosis (Fig. 1A, 1B). Upon opening the coelomic cavity, the liver appeared mildly enlarged, firm in consistency, and contained multiple yellowish nodules (1–5 mm) with a multifocal to coalescing distribution extending from the surface into the hepatic parenchyma (Fig. 1C, 1D).

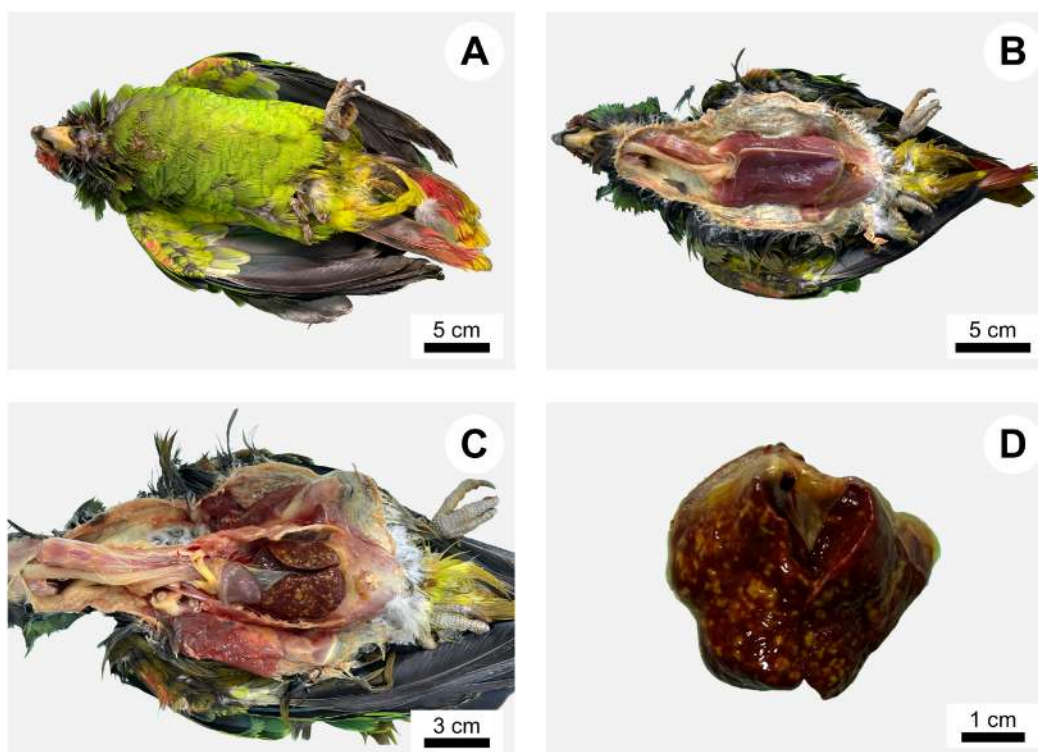


Figure 1. Postmortem examination of a red-tailed amazon (*Amazona brasiliensis*). A. Bird positioned for necropsy. B. Bird showing severe atrophy of the pectoral muscles, with exposure of the keel bone (cachexia). C and D. Liver with multifocal to coalescing yellowish nodules, measuring between 1 and 5 mm.

Microscopically, the liver showed marked structural disruption, with a proliferation of malignant epithelial cells exhibiting high cellularity. The tumor was poorly defined, unencapsulated, and showed an infiltrative growth pattern. Neoplastic cells were arranged in acinar or tubular structures embedded within a moderately fibrous stroma. The cells were cuboidal, with indistinct shapes and borders, and had moderate eosinophilic cytoplasm. The ducts were small, composed of non-mucin-producing cuboidal cells resembling biliary duct epithelium. The nuclei were round to oval, with finely clumped chromatin and prominent nucleoli. Moderate anisocytosis and anisokaryosis were observed; however, no mitotic figures were identified in 10 high-power fields (400 \times magnification) (Fig 2A, 2B). Extensive areas of multifocal necrosis were observed (Fig. 2A), accompanied by infiltration of heterophils and lymphocytes.

Immunohistochemical analysis of the hepatic tissue revealed positive staining for AE1/AE3 and negative staining for HepPar-1 and CK7 (Fig. 2C). This immunoprofile, along with the histopathological findings, supported a diagnosis of cholangiocarcinoma. In the liver, neoplastic cells were arranged in ductal and tubular patterns, whereas in the spleen they predominantly formed solid aggregates, with tubular

structures also occasionally observed. Metastatic involvement of the spleen was characterized by significant disruption of splenic structure, hemosiderosis, and heterophilic inflammation (Fig. 2D).

Discussion

Neoplasms are frequently reported in birds, particularly among Psittaciformes (12), and cholangiocarcinoma is the most common hepatic tumor in captive individuals (17). Despite this, cholangiocarcinoma has not been reported in *Amazona brasiliensis*. This case report describes, for the first time, a cholangiocarcinoma with splenic metastasis in an individual of this endangered psittacid species. This report expands knowledge of neoplastic diseases affecting wild and captive birds, providing valuable insights into diagnosis, management, and conservation.

Metastasis in birds with cholangiocarcinoma is has been previously reported in an Adélie penguin (*Pygoscelis adeliae*), with tumor cells found in the pancreas, lungs, mesentery, and cloaca (22), and in a red-tailed hawk (*Buteo jamaicensis*), with neoplastic cells identified in the lungs,

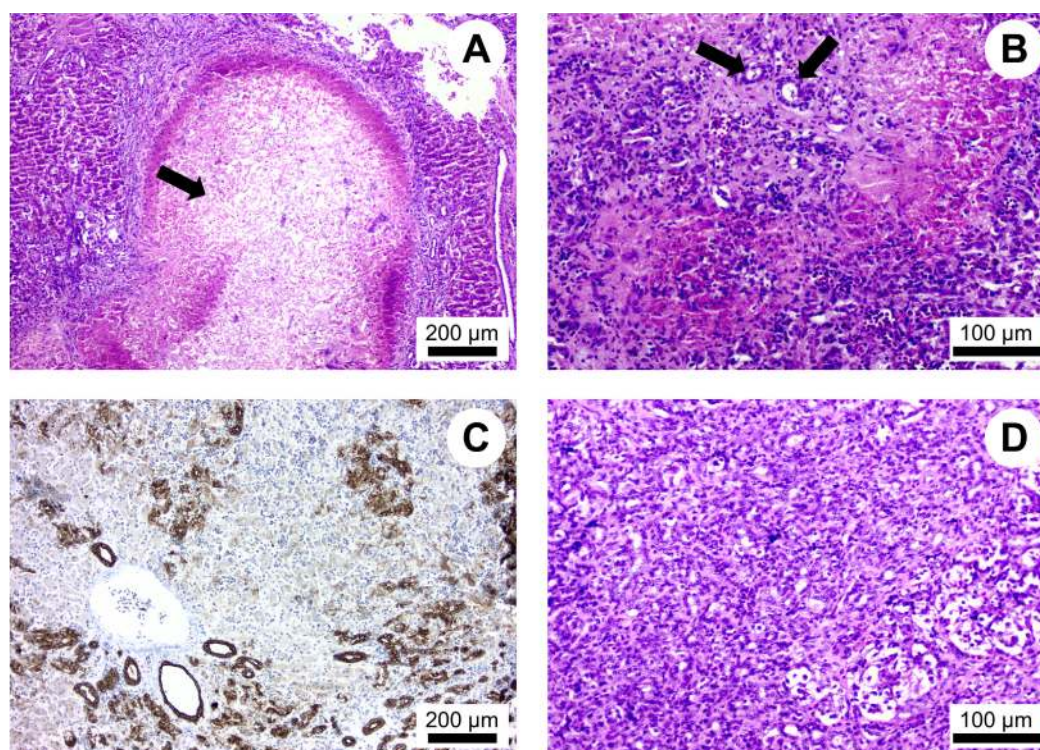


Figure 2. Histopathological evaluation of the liver and spleen of a red-tailed Amazon (*Amazona brasiliensis*). A.

Extensive area of necrosis (black arrow) associated with neoplastic biliary epithelial cells arranged in tubules. Hematoxylin and eosin, scale bar = 200 μ m. B. Presence of cuboidal to columnar neoplastic cells forming tubules (black arrow) within a collagenous stroma. Moderate anisocytosis and anisokaryosis. Hematoxylin and eosin, scale bar = 100 μ m. C. Immunohistochemical analysis of cholangiocarcinoma. Neoplastic cells exhibit positive staining for AE1/AE3. Scale bar = 200 μ m. D. Spleen with metastatic cholangiocarcinoma. Neoplastic cells are arranged in tubules. Nuclei are round to oval with prominent nucleoli. Hematoxylin and eosin, scale bar = 100 μ m.

adrenal glands, and femoral medullary canal (14). In contrast, a case without metastasis was documented in a greater rhea (*Rhea americana*), suggesting that the absence of dissemination may reflect an early-stage tumor or limited exposure to carcinogens (23). In the present report, splenic metastasis was accompanied by heterophilic inflammation.

The etiology of cholangiocarcinoma in birds is poorly understood. However, similar to mammals, hepatic neoplasms have been linked to chronic liver injury caused by mycotoxins, parasitic trematode infections, the hepatitis B virus, avian leukosis virus, exposure to heavy metals, or poor nutrition (3, 8, 10). Mycotoxins are chemical metabolites produced by fungi that grow on food items such as grains and seeds. Aflatoxins, in particular, are produced by *Aspergillus flavus* and *Aspergillus parasiticus* and are directly associated with hepatic lesions in birds. Experimental studies have shown that the ingestion of aflatoxins by turkeys and chicks induces hepatic alterations such as fatty degeneration, bile duct hyperplasia, fibrosis, and tissue necrosis (2, 18). In psittacids, severe bile duct hyperplasia may precede the development of hepatic neoplasms (6, 13).

In this case, we were unable to determine the cause of the hepatic neoplasm. However, the anamnesis revealed that the bird had been exclusively fed sunflower seeds for four years, with minimal fruit variety. These food items were not tested for fungal contamination or mycotoxins, and the origin of the seeds is unknown. Nevertheless, we consider chronic exposure to mycotoxins a plausible hypothesis due to their carcinogenic potential and documented association with hepatic tumors in birds (12, 21). Another possible cause for tumor development is infections by herpesvirus or papillomavirus (11, 27). However, we observed no viral inclusions in histological sections and did not perform diagnostic tests such as PCR or electron microscopy. Regarding papillomavirus infection, we found no papillomatous lesions on pathological examination.

As observed in other studies involving cholangiocarcinoma in birds (5, 19, 28), the red-tailed amazon in this case showed nonspecific and late clinical signs, dying a few days after the onset of symptoms. This finding highlights the importance of preventive medicine and early diagnosis. We emphasize that dietary management of captive birds must be conducted appropriately to ensure their quality of life and reduce the risk of disease. Finally, this case underscores the importance of veterinary pathology as a diagnostic tool for wildlife species. Further studies—such as toxicological analyses of seeds and liver samples from psittacid birds to detect aflatoxins—are needed to clarify the etiology of neoplasms and their relationship with environmental factors.

Conflict of Interest

The authors declare no competing interests

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