

Cholangiocarcinoma in a Ring-Necked Pheasant (*Phasianus colchicus*): a case report

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ABSTRACT: A case of cholangiocarcinoma in a two-year-old Ring-Necked Pheasant (*Phasianus colchicus*) is reported. The liver was enlarged and numerous white foci were seen in the liver parenchyma. Histopathologically, channels with or without lumens and separated from one another by thin connective tissue septa were observed. Tumour cells were strongly positive for cytokeratin, Ki-67 and proliferative cell nuclear antigen. This is the first report of colangiocarcinoma in pheasants.

Keywords: cholangiocarcinoma; pheasant; histopathology; immunohistochemistry

Primary tumours of the liver may be of hepatocellular or bile duct origin (Schmidt et al. 2003). Cholangiocarcinomas (cholangiocellular carcinoma, bile duct carcinoma) originate from the bile duct epithelium and is the most frequent hepatic neoplasm reported in captive and free-ranging birds (Schmidt et al. 2003; Gesek et al. 2009). The most commonly affected birds are macaws, parrots and conures ((Schmidt et al. 2003).

Specific clinical signs are infrequent, although emaciation, weakness, hepatomegaly, ataxia, trembling, and neurologic signs are suggestive of hepatoencephalopathy. Grossly, bile duct tumours usually contain numerous, variably sized, firm, white-to-tan nodules and there may be multiple sites in one or more lobes of the liver which grow by expansion (Potter et al. 1983; Allen et al. 1985, Elangbam and Panciera 1988; Anderson et al. 1989; Schmidt et al. 2003).

Histopathologically, bile duct tumours consist of columnar-to-cuboidal epithelial cells arranged in ribbons, cords, tubules or ducts separated by minimal amounts of stroma. Infiltration of the hepatic parenchyma is apparent and a few mitotic figures may be observed. In some of these tumours, a scirrhous reaction may be present. Carcinoma cells are poorly differentiated, and metastasis to the lungs,

brain, kidney, pleura and serosa of the ventriculus is infrequently seen (Allen et al. 1985, Anderson et al. 1989; Elangbam and Panciera, 1988; Potter et al. 1983; Schmidt 1996).

Hepatobiliary neoplasias in avian species have been reported occasionally. The prevalence of neoplasms appears to be considerably greater in psittacines, especially budgerigars, than in other orders of birds (Allen et al. 1985, Anderson et al. 1989; Elangbam and Panciera 1988; Latimer 1994), but there are no reports of cholangiocarcinomas in pheasants and this case description of the occurrence of a cholangiocarcinoma in a two-year-old Ring-Necked Pheasant (*Phasianus colchicus*) is the first report of its kind.

Case description

A two-year-old, male, Ring-Necked Pheasant was initially evaluated for chronic depression and inappetance. The pheasant died two weeks after the presentation of the initial signs and the dead bird was presented by Antalya Zoo to the Department of Pathology for diagnosis. The pheasant had lived in the zoo from the time of hatching and no health problems had been reported until two weeks before



Figure 1. Gross appearance of the severely enlarged liver with numerous white foci completely filling the abdominal cavity of the pheasant

death. The bird was housed together with three female pheasants in a cage and was fed a diet consisting of a mixture of barley, wheat and corn as well as fruit and vegetables. The clinical findings were not suggestive of a liver tumour.

On gross examination the bird was diagnosed with severe hepatomegaly. At necropsy the liver totally filled the abdominal cavity. The liver contained numerous widely distributed firm pale, tan-coloured nodules (Figure 1). No other distinctive macroscopic lesions, such as icterus, were present at gross examination.

Tissues were fixed in 10% buffered formalin, routinely processed, sectioned at 5 µm, and stained with haematoxylin and eosin (HE). For immunohistochemical examination tissue sections

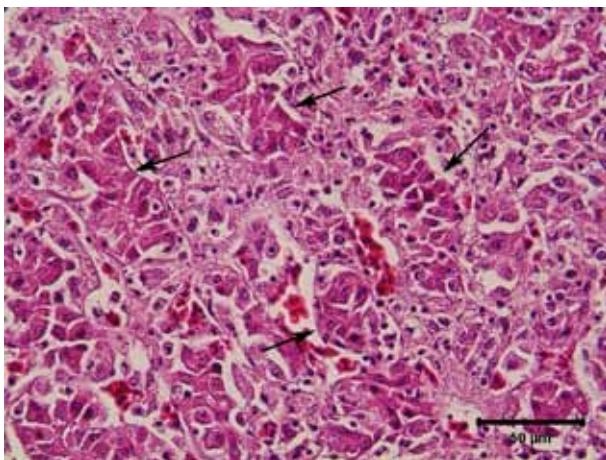


Figure 2. Histopathological appearance of the liver. Numerous pleomorphic cells and canal formations (arrows) are seen, HE

were stained with Ki67 (Rabbit Polyclonal Ki-67 Antibody, 250733, 1 : 100 dilution); proliferating cell nuclear antigen (PCNA) (Rabbit Polyclonal PCNA Antibody, 250812, 1 : 100 dilution), vimentin (Mouse Monoclonal Vimentin Antibody, 251809, 1 : 100 dilution); pancytokeratin (Mouse Monoclonal Pancytokeratin Antibody, 251788, 1 : 100 dilution); smooth muscle actin (SMA) (Mouse Monoclonal SMA, 251813, 1 : 200 dilution); and alpha fetoprotein (AFP) (Mouse Monoclonal Alpha-Fetoprotein Antibody, 251700, 1 : 100 dilution) using a routine streptavidin-biotin peroxidase technique, in order to identify the tumour. Commercial kits (Abbiotech, San Diego, CA) were used for immunohistochemical examination.

The microscopic examination revealed that most of the liver had been transformed into neoplastic tissue. Histopathological examination of the tissue revealed marked alteration of the hepatic architecture due to widespread infiltration by neoplastic biliary tissue. The hepatocellular architecture was characterised by an infiltrating pleomorphic population of cuboidal to low columnar epithelial cells forming irregularly arranged duct-like channels. Channels were with or without lumens and were separated from one another by thin connective tissue septa. These were also singly or multiply lined by tumour cells and were variably dilated. Nuclei were generally basally located and open, and they contained 1–3 nucleoli (Figure 2). Mitotic figures were not observed within the proliferating cells. In some areas, neoplastic tissue existed as masses of tubules supported by thin strands of stroma. Neoplastic tissue did not invade the capsular con-

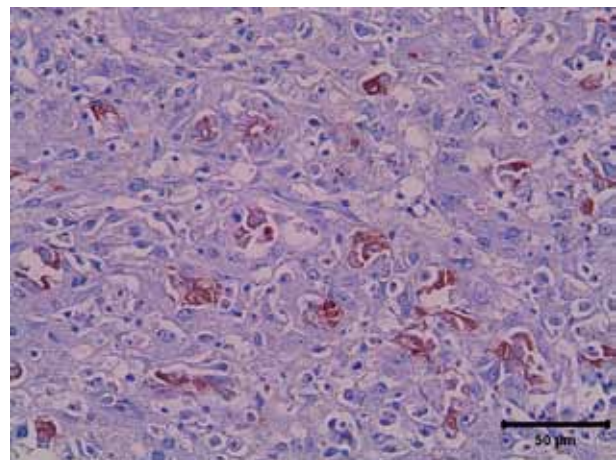


Figure 3. Cytokeratin immunoreactivity of the tumour mass, streptavidin-biotin peroxidase method with haematoxylin counterstain

nective tissue and was not seen in other organs and tissues.

To evaluate tumour cell origin and malignancy, tissues were immunostained with different markers. Immunohistochemical examination revealed positive staining for pancytokeratin (Figure 3), Ki67 and PCNA, but neoplastic cells were negative for vimentin, SMA and AFP.

DISCUSSION AND CONCLUSIONS

Primary hepatic cancers most frequently develop without any symptoms. Clinical symptoms appear late and they are not characteristic. Most often they concern the alimentary tract and include symptoms such as anorexia, flatulence and constipation. Enlargement of the liver and ascites are revealed at later stages (Mutinelli et al. 2009). In this case the bird showed clinically inappetence and depression that was not suggestive of hepatic carcinoma.

The macroscopic and microscopic examinations of the pheasant's liver were consistent with a diagnosis of cholangiocarcinoma. Microscopic examination revealed that most of the liver had been transformed into neoplastic tissue, and that normal tissue was preserved only in small fragments. The neoplastic infiltrations had the form of cuboidal to columnar cells, arranged in an acinar pattern, resembling biliary epithelium. No bile pigments were visible inside the ductules. Gross and microscopic features of the tumour were in agreement with previous reports (Potter et al. 1983; Allen et al. 1985; Elangbam and Panciera 1988; Anderson et al. 1989; Gesek et al. 2009).

The causes of cholangiocarcinoma include poisoning with aflatoxin and other chemicals, trematode infection, hormonal disorders, as well as infection with hepatitis virus in the body (Davies 2000; van Wettereab et al. 2010). In this case, due to the fact that the pheasant was kept in a zoo and usually fed a mixture of different seeds, as well as non-harmful fruit and vegetables, contamination of the seeds with aflatoxin is a possible cause of the cholangiocarcinoma. However, no mold was observed in the feed and it was not tested for mycotoxins. Another possibility for the etiology could be infection with a transforming virus such as papillomavirus or herpesvirus, but no inclusions were seen under light microscopy.

Cholangiocarcinoma in domesticated species and humans generally occurs in relatively older

individuals. The life span of pheasants in the wild is under 1 year but in zoos this life span may increase. The age of the pheasant in this study was high enough to support a notion of age-related tumour development.

The cytokeratins are a family of water-soluble proteins that form the cytoskeleton of epithelial cells (Battifora 1984). Ki-67 is a nuclear antigen associated with cell proliferation and is present throughout the active cell cycle but is absent in resting cells (Scholzen and Gerdes 2000). PCNA protein is one of the central molecules responsible for deciding the life and death of the cell (Warbrick 2000). SMA is useful for identifying tumours arising from smooth muscle and myoepithelial cells (Spector 2001). Vimentin is the main intermediate filament protein in mesenchymal cells, and therefore is of value in the differential diagnosis of undifferentiated neoplasms (Blain et al. 2006). The occurrence of neoplastic cells demonstrating biliary epithelium differentiation surrounded by desmoplastic stroma was shown to be diagnostic for cholangiocarcinoma (Warbrick 2000). Pancytokeratin immunohistochemistry demonstrated mild to intense cytoplasmic immunoreactivity in neoplastic cell-forming ducts. Immunoreactivity for pancytokeratin of the cells forming the glandular structure and tubules supported the diagnosis as did PCNA and Ki-67 immunoreactivity. This is the first report of gross, histological, and immunohistochemical characteristics of cholangiocarcinoma in a Ring-Necked Pheasant.

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Received: 2012–05–28

Accepted after corrections: 2012–06–13

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