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Short Communication

Cholangiocarcinoma with Multiple Organ Metastasis in a Captive Puma (*Puma concolor*)

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ABSTRACT

A 17-year-old captive male puma (*Puma concolor*) died after presenting anorexia, vomiting, weight loss and lethargy. At necropsy, the right middle lobe of the liver was severely affected by a tumor, and small tumor nodules were disseminated throughout the other lobes. The numerous tumor nodules were also found in the lung, stomach, kidney, heart and diaphragm, which were growing together, suspiciously metastatic, projecting, 5 to 40 mm in diameter and tawny to white in color. Histopathologically, the tumor was composed of prominent papillary-acinar structures and the cells had a resemblance to the biliary epithelium. Immunohistochemically, the tumor cells were strongly reactive for cytokeratin and CD10 and were negative for carcinoembryonic antigen, fetoprotein and hepatocyte paraffin-1. Taken together, the tumor was diagnosed as cholangiocarcinoma. This is the first case report of a cholangiocarcinoma in the puma.

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H-SC conducted lab experiments and case descriptions. YO wrote the paper with a concept as a whole.

Key words
Bile duct carcinoma, Cholangiocarcinoma, Metastasis, Puma, *Puma concolor.*

Cholangiocarcinoma (CCA) is a malignancy of the biliary duct system originating from the intra-or extrahepatic bile duct epithelium (Banales *et al*., 2016; Blechacz and Gores, 2008), and it can be classified as intrahepatic (iCCA), perihilar (pCCA) or distal CCA (dCCA) according to the anatomical location (Banales *et al*., 2016). CCAs are relatively uncommon in animals, although these tumors are the second most frequent type of primary liver cancer and comprise ~3% of all gastrointestinal neoplasias in humans. Infection with liver flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*) is known as a common risk factor in humans (Banales *et al*., 2016; Blechacz and Gores, 2008) as well as dogs and cats (Hou, 1964; Schmidt and Langham, 1967). Other causes include chronic bacterial infection of the bile duct in dogs (Ohta *et al*., 1991) and chemicals such as nitrofurans in laboratory rats (Maronpot *et al*., 1991), plutonium or americium induction in beagles (Taylor *et al*., 1991) and toxic chemical contaminants in lake whitefish (*Coregonus clupeaformis*) (Mikaelian *et al*., 2002).

Veterinary CCAs have mostly been reported in domestic animals such as dogs (Patnaik *et al*., 1981; Trigo, 1982), cats (Carpenter *et al*., 1987; Hou, 1964; Schmidt and Langham, 1967), horses (Sironi and Riccaboni, 1997), cattle (Anderson and Sandison, 1968; Bastianello, 1982), sheep (Anderson and Sandison, 1968) and goat (Domínguez *et al*., 2001). However, cases have been reported in other various species including polar bears (*Ursus arctos*) (Miller *et al*., 1985), ferrets (*Mustela putorius furo*) (García *et al*., 2002), an Adelie penguin (*Pygoscelis adeliae*) (Renner *et al*., 2001), a Florida sandhill crane (*Grus canadensis*) (Allen *et al*., 1985), a blue-fronted Amazon parrot (*Amazona estiva*) (Elangbam and Panciera, 1988), a double yellow-cheeked Amazon parrot (*Amazona autumnalis*) (Anderson, 1989), a peach-fronted conure (*Aratinga aurea*) (Gibbons *et al*., 2002), brown bullheads (*Ameiurus nebulosus*) (Pinkney *et al*., 2001), a bottlenose dolphin (*Tursiops truncatus*), a pygmy sperm whale (*Kogia breviceps*) (Martineau *et al*., 2002), lake whitefish (*Coregonus clupeaformis*) (Mikaelian *et al*., 2002) and a blue shark (*Prionace glauca*) (Borucinska *et al*., 2003). Histologically, CCAs are similar in all species, and are composed of cells that retain a resemblance to biliary epithelium lined by cuboidal or columnar cells that do not contain bile (Cullen and Popp, 2002; Stedman, 1982).

Materials and methods

This case study discusses a 17-year-old male puma (*Puma concolor*) that died after presenting anorexia, vomiting, weight loss and lethargy. At necropsy, the right middle lobe of the liver was severely affected by a

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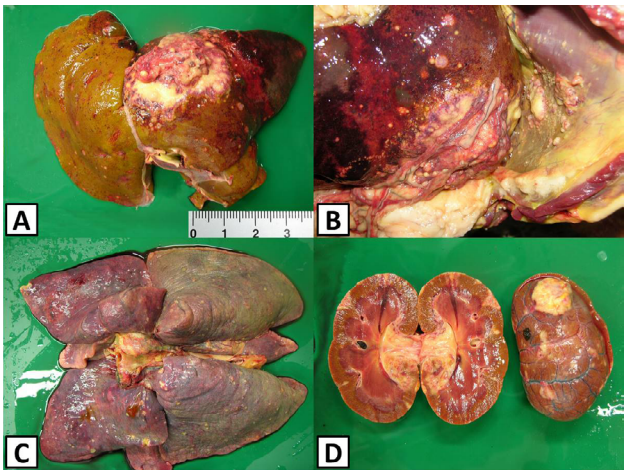

Fig. 1. Peritoneal organs of *Puma concola*. Gross pathological findings exhibited yellowish tumor masses of varying sizes in the liver (A), diaphragm (B), lungs (C), and kidneys (D).

massive tumor, and other lobes were affected by small multiple nodules as described in other literature (Fig. 1A) (Patnaik *et al*., 1981; Trigo *et al*., 1982). The largest tumor had a characteristic lobular pattern with an umbilicated appearance, sometimes protruding above the capsule of the liver. The cut surface colored white to gray-white and sometimes carried cystic nodules containing yellow-brown fluid. The border of the tumors was irregular but clearly delineated from the adjacent hepatic parenchyma. Palpation of the nodules revealed a firm texture as seen in connective tissue-abundant tumors. This firm texture is one of the diagnostic indexes which distinguish a cholangiocarcinoma from a hepatocellular carcinoma that generally have a soft and friable texture (Cullen and Popp, 2002). Numerous nodules that were growing together, suspiciously metastatic, projecting 5 to 40 mm in diameter and tawny to white in color, were scattered throughout the lung, stomach, kidney, heart and diaphragm (Fig. 1B, C, D). Tissue samples from the tumor nodule and representative tissue specimens were collected and fixed in

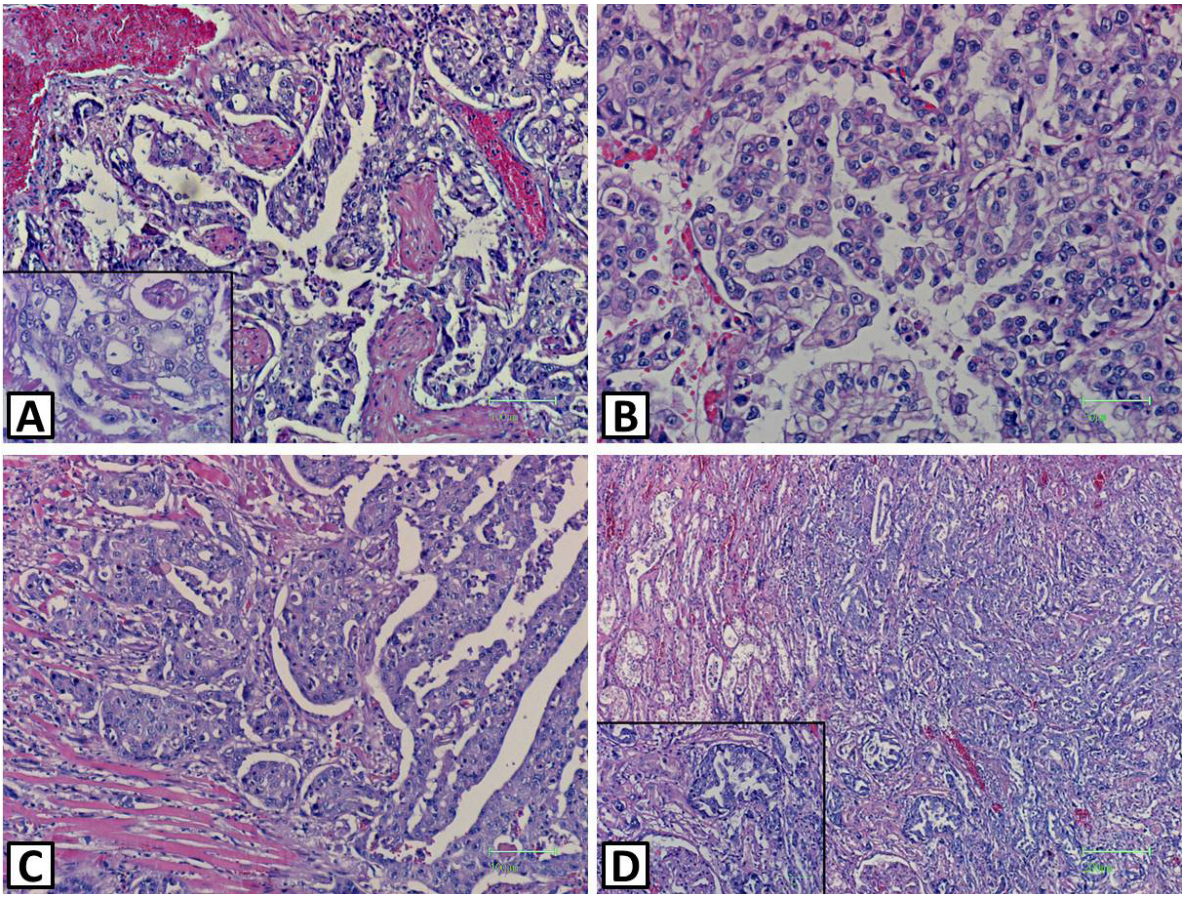

Fig. 2. *Puma concola.* Papillary growth pattern of the adenocarcinoma cells in the liver (A and B), heart (C) and kidney (D). H&E. Scale bar=25 um (A inset), 50 um (B, D inset), 100 um (A,C) and 200 um (D).

10% (w/v) neutral buffered formalin for 24-48h, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin (H&E) for light microscopy examination. Immunohistochemical (IHC) analyses were conducted as previously described (Oh *et al*., 2013) using six primary antibodies: anti-cytokeratin pan type II (AE3; 1:50, eBioscience™, San Diego, CA, USA), anti-cytokeratin pan type I (AE1; 1:50, eBioscience™, San Diego, CA, USA), anti-CD10 (56C6; 1:20, Abcam®, Cambridge, MA, USA), anti-carcinoembryonic antigen (COL-1; 1:500, eBioscience™, San Diego, CA, USA), anti-α-fetoprotein (1E8; 1:50, eBioscience™ , San Diego, CA, USA) and anti-hepatocyte paraffin-1 (OCH1E5; 1:20, NOVUS Biologicals, LLC, Denver, CO, USA). Briefly, endogenous peroxidase was quenched with 2% H_2O_2 solution for 2 min at 4°C and digested with proteinase K for 25 min at 37°C for antigen retrieval. After blocking with Power Block™ (BioGenex, USA) for 30 min at room temperature, primary antibodies were applied at 4°C overnight. After thorough washing in PBS containing 0.1% Tween 20, the VECTASTAIN® Universal ABC kit (Vector, CA, USA) was employed and then counterstained with Mayor's hematoxylin. Each antibody-treated slide had a corresponding mock-treated slide.

Results and discussion

Histopathological examination showed that tumor nodules were composed of cuboidal and columnar cells with round to oval nuclei, and mitotic figures were moderate to abundant. The border of the tumors was clearly delineated by fibrous connective tissue from the remaining apparently normal hepatic parenchyma. The lumen in some neoplastic tubules or acini contained copious amount of mucin that stained weakly basophilic by standard H&E stain (Fig. 2). Cells constituted prominent papillary-acinar structures and the epithelial components were separated by abundant fibrous connective tissue that yielded a firm consistency. The histomorphological characteristics of the tumor were highly indicative of cholangiocarcinoma. Immunohistochemically, the tumor cells were strongly reactive for the cholangiocyte marker cytokeratin pan type I rather than cytokeratin pan type II and were negative for the remaining hepatocyte markers, especially Hep Par 1, which is highly sensitive and specific for hepatocellular differentiation (Fig. 3) (Maitra *et al*., 2001).

The other tumor nodules in the lung, stomach, kidney and diaphragm also had very similar histological appearances to that of the liver nodules. There have been a large number of metastatic cases of CCA into the peritoneum, lungs, lymph nodes, diaphragm, spleen, kidneys, heart, adrenals and bone marrow (Cullen and Popp, 2002; Ilhan *et al*., 2008; Lepri *et al*., 2013; Mischke *et al*., 2003). This gives us the rationale to conclude that the liver nodules were the primary source of neoplastic cells that metastasized to the other organs. Cholangiocarcinomas in domestic cats are considered highly malignant with a high rate of metastasis to other organs (Carpenter *et al*., 1987). The puma belongs to the family Felidae and is presumably also susceptible to diseases and tumors affecting its domestic cousins. The exact cause of the cholangiocarcinoma is impossible to determine, but we speculate that the long life span of this animal (17 years) kept in captivity enabled enough time for the tumor to develop. Another factor that can be considered in the etiology of the tumor is the possible exposure to environmental chemical carcinogens during its long stay in captivity, especially from air pollutants in a crowded busy metropolis where the zoo is located and/or from the food given to the animal.

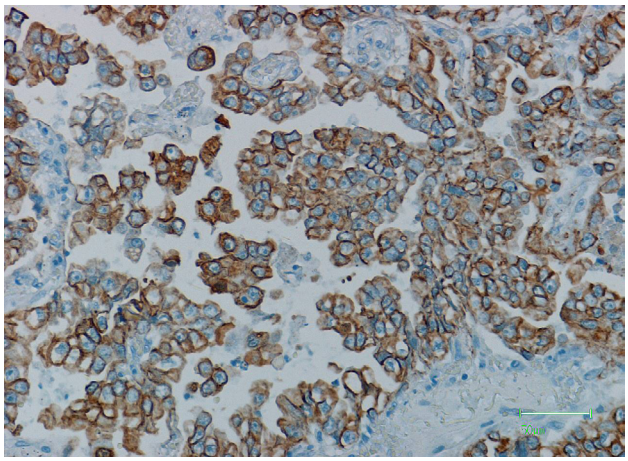

Fig. 3. *Puma concola.* Cytoplasmic immunoreactivity for cytokeratin pan type I (AE I). DAB chromogen, hematoxylin counterstain. Scale bar=50 um.

Conclusions

In conclusion, the captive puma (*Puma concolor*) had bile duct carcinoma seemingly due to the combined action of the internal as well as external etiologies, and to the best of authors' knowledge, this is the first study to describe the CCA and its metastasis and its metastasis into the various peritoneal organs in a captive puma.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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