

Cholangiocarcinoma in a Free-ranging Eurasian Brown Bear (*Ursus arctos arctos*) from Northern Spain

Ana Balseiro,^{1,3} Luis José Royo,² Elena Gayo,¹ and Juan Francisco García Marín¹ ¹Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain; ²SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Centro de Biotecnología Animal, 33394 Gijón, Asturias, Spain; ³Corresponding author (email: abalm@unileon.es)

ABSTRACT: A hepatic cholangiocarcinoma with metastases in the gallbladder, left elbow joint, adrenal glands, and lungs was observed in a female 21-yr-old free-ranging Eurasian brown bear (*Ursus arctos arctos*) found in the Principality of Asturias (northern Spain). Gross and histopathologic findings are described.

Cholangiocarcinomas are reported in domestic animals and may replace the majority of the hepatic parenchyma or be multinodular (Cullen 2017). Metastases to the regional lymph nodes, lungs, and peritoneal cavity are commonly seen in animals with these tumors. There are few reported cases of cholangiocarcinomas in bears: one in a 35-yr-old polar bear (*Ursus maritimus*), one in a Himalayan brown bear (*Ursus arctos isabellinus*), and two additional cases in sloth bears (*Melursus ursinus*; Moulton 1961; Black et al. 1991; Darzi et al. 2007; Fredholm et al. 2013); older captive animals are overrepresented. To the best of the authors' knowledge, cholangiocarcinomas have not been previously reported in free-ranging bears. This report describes the gross and histopathologic features of a cholangiocarcinoma in the liver and other tissues of a free-ranging Eurasian brown bear (*Ursus arctos arctos*), locally known as the Cantabrian brown bear.

A geriatric free-ranging female Eurasian brown bear was found in very poor health and body condition in April 2015 in the Principality of Asturias (northern Spain). The animal died the next day. On postmortem examination, the animal weighed 44 kg, and had a rough hair coat, generalized muscle atrophy, and was extremely thin. There was generalized edema in the subcutaneous tissue, ascites, hydrothorax, and hydropericardium. The total protein concentration measured in a

sample of ascites was 2.1 g/dL. Two large hepatic masses were adhered to the peritoneum. Additionally, disrupting approximately 60% of the hepatic parenchyma were 2–40-mm masses that extended into all lobes (Fig. 1a). The masses were white to yellow-brown, firm, well-delineated, and protruded above the capsule of the liver with multiple areas of central and soft necrosis (Fig. 1a, inset). Multinodular masses were also observed in the gallbladder with an umbilicated appearance (Fig. 1b), the joint of the left elbow (Fig. 1c), and in the adrenal glands and lungs throughout the parenchyma. In the elbow joint, there was tumor infiltration and necrosis of the olecranon bone (Fig. 1c). This animal also had mitral valvular endocardiosis, ovarian atrophy, and old fractures in two right ribs and one thoracic vertebra.

Tissues were evaluated histologically using routine H&E stain. A dental histologic study of the first molar (Klevezal 1996) was performed to determine the age of the bear. Microscopically, tumors observed in the liver, gallbladder, left elbow joint, adrenal glands, and lungs retained characteristics of biliary epithelium (Fig. 2). Neoplastic cells were arranged in trabeculae of tubules and acini surrounding central areas of necrosis. The cells were cuboidal to columnar, the nuclei hyperchromatic, and the cytoplasm slightly granular. Nucleoli were not prominent. Anisocytosis and anisokaryosis were marked and there were numerous mitotic figures.

Hepatic cholangiocarcinoma with metastases to the gallbladder, left elbow joint, adrenal glands, and lungs was diagnosed in this brown bear. Cholangiocarcinomas have a highly invasive pattern of growth and usually metastasize in lymph nodes, lungs, and peritoneal

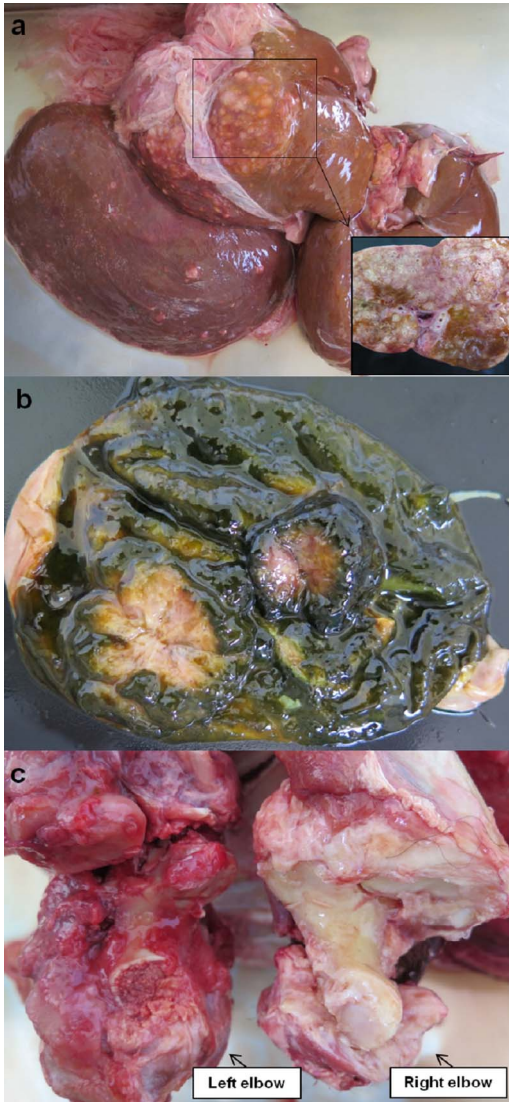


FIGURE 1. Cholangiocarcinoma in tissues of a free-ranging Eurasian brown bear (*Ursus arctos arctos*). (a) Liver. Two large masses with adherence to the peritoneum. Disrupting approximately 60% of the parenchyma were multiple 2–40-mm masses that extended into all lobes. The masses were white to yellow-brown, firm, well delineated, and protruded above the capsule of the liver. Inset: Section of the liver tumor with central necrosis characterized by friable, yellow, and caviated foci. (b) Multiple 2–30-mm umbilicated masses in the gallbladder. (c) Elbows. The right elbow is normal. The left elbow joint is disrupted by the neoplasia with infiltration and necrosis of the olecranon bone.

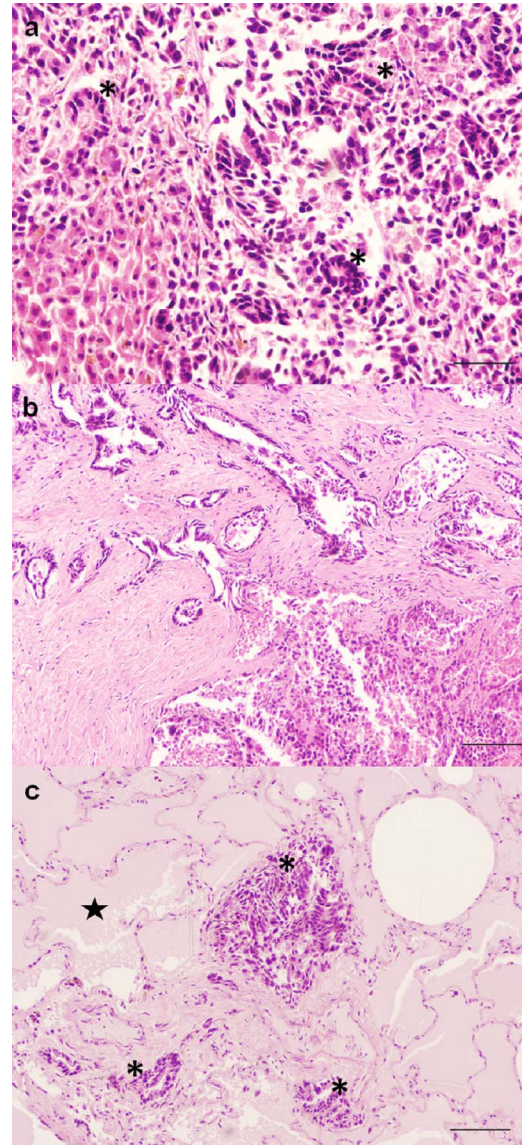


FIGURE 2. Histopathologic features of cholangiocarcinoma in a free-ranging Eurasian brown bear (*Ursus arctos arctos*). (a) Liver. Neoplastic biliary epithelial cells organized in trabeculae of tubules and acini (asterisks). H&E. Bar=50 μ m. (b) Joint of the left elbow. Neoplastic infiltration is observed. H&E. Bar=100 μ m. (c) Lung. Metastases in the parenchyma (asterisks) admixed with edema (star). H&E. Bar=100 μ m.

cavity, although any organ can be affected (Cullen 2017). Bone metastases from cholangiocarcinoma had not been previously described in bears and are in general rare

compared with other tumors. Bone metastases have been reported in a dog in the iliac crest and femur (Mischke et al. 2003) and in humans in bones including mandible, humerus, and scapula (Federico et al. 2013; Chindaprasirt et al. 2018). Cholangiocarcinomas should be considered as a differential diagnosis in patients who present with an infiltrative and nodular bone lesion and a liver or gallbladder mass.

Cholangiocarcinomas are usually found in old animals and the incidence increases with age (Cullen 2017). The etiology of this neoplasm, however, remains unclear. Many causes have been suggested, including parasitism related to chronic infestation with opisthorcid flukes, especially *Opisthorchis viverrini* and *Clonorchis sinensis* associated with hyperplastic or adenomatoid proliferation of the epithelium, *Ancylostoma* sp. and *Trichurus vulpis*, viral infection, genetic predisposition, ingestion of toxic substances, or even dietary habits (Hayes et al. 1983; Cullen 2017). The origin of the tumor in this brown bear is unknown, although hepatobiliary neoplasia is frequent in captive bears and usually affects old animals (Dorn 1964; Ramsay 2003). Endocardiosis, a gross lesion found in this bear, is also associated with aging (Maxie and Robinson 2016). Generalized edema in subcutaneous tissue as well as ascites, hydrothorax, and hydropericardium were likely a result of the chronic hepatic damage, hypoproteinemia, and cachexia.

This report confirms a potential cause of mortality in bears of advanced age unrelated to human activity (i.e., illegal hunting), infectious disease, or other causes of mortality in this species. The study also provides interesting data regarding the age of the wild bears affected by these problems. The dental histologic study showed that this animal was 21 yr old at the time of death, reaching the greatest longevity previously reported in wild brown bears from the Iberian Peninsula (Palomero 2007). This case shows evidence of aging bears that may be a result of the steady recovery of the Eurasian brown bear population in the Cantabrian Range in recent decades.

We thank the Consejería de Fomento, Ordenación del Territorio y Medio Ambiente, Oviedo, Principado de Asturias, Spain. We thank Kevin P. Dalton for critically reviewing the manuscript. This study was funded by Plan de Ciencia, Tecnología e Innovación de Asturias 2018–2020 (GRUPIN: IDI2018-000237) and Fondo Europeo de Desarrollo Regional.

LITERATURE CITED

- Black S, Mainka S, Cooper R, Lowes N. 1991. Electron microscopic, flow cytometry, and histologic features of two anaplastic hepatic tumors in bears. 1991. In: *Proceedings of the annual meeting of the American Association of Zoo Veterinarians*, Calgary, Alberta, Canada, 28 September–3 October, pp. 305–307.
- Chindaprasirt P, Promsorn J, Ungareewittaya P, Twinprai N, Chindaprasirt J. 2018. Bone metastasis from cholangiocarcinoma mimicking osteosarcoma: A case report and review literature. *Mol Clin Oncol* 9:532–534.
- Cullen JM. 2017. Tumors of the liver and gallbladder. In: *Tumors in domestic animals*, 5th Ed., Meuten DJ, editor. Wiley Blackwell, Ames, Iowa, pp. 602–631.
- Darzi MM, Mir MS, Ganai TAS, Nashiruddin N, Mansoor M, Kamil SA. 2007. Mixed hepatocellular and cholangiocarcinoma in a Himalayan brown bear (*Ursus arctos isabellinus*). *Indian J Vet Pathol* 31: 163–165.
- Dorn CR. 1964. Biliary and hepatic carcinomas in bears at the San Diego Zoological Gardens. *Nature* 202:513–514.
- Federico A, Addeo R, Cerbone D, Iodice P, Cimmino G, Bucci L. 2013. Humerus metastasis from cholangiocarcinoma: A case report. *Gastroenterol Res* 6:39–41.
- Fredholm DV, Carpenter JW, Petersen DJ, Gata CK. 2013. Pathology in practice. *J Am Vet Med Assoc* 243: 497–499.
- Hayes HM Jr, Morin MM, Rubenstein DA. 1983. Canine biliary carcinoma: Epidemiological comparisons with man. *J Comp Pathol* 93:99–107.
- Klevezal GA. 1996. *Recording structures of mammals: Determination of age and reconstruction of life history*. 1st Ed. AA Balkema Publishers, Rotterdam, the Netherlands, 448 pp.
- Maxie MG, Robinson WF. 2016. Cardiovascular system. In: *Jubb, Kennedy and Palmer's pathology of domestic animals*, 6th Ed., Maxie MG, editor. Elsevier, London, UK, pp. 1–101.
- Mischke R, Höinghaus R, Lütkefels E, Buhl K, Gerhardt A, Hewicker-Trautwein M. 2003. Immunocytological confirmation of bone marrow metastases in a dog with cholangiocarcinoma. *J Small Anim Pract* 44:411–414.
- Moulton JE. 1961. Bile duct carcinomas in two bears. *Cornell Vet* 51:285–293.

Palomero G. 2007. *Ursus arctos* L., 1758. In: *Atlas y libro rojo de los mamíferos terrestres de España*, Palomero LJ, Gisbert J, Blanco JC, editors. Dirección General para la Biodiversidad, SECEM-SECEMU, Madrid, Spain, pp. 321–326.

Ramsay EC. 2003. Ursidae and Hyainidae. In: *Zoo and wild animal medicine*, 5th Ed., Fowler ME, Miller

RE, editors. Saunders (Elsevier Science), St. Louis, Missouri, pp. 523–538.

Submitted for publication 1 March 2019.

Accepted 13 April 2019.