Pathology in Practice

History

A 26-year-old sexually intact male sloth bear (Melursus ursinus) weighing 97 kg (213.4 lb) with a 2-week history of lethargy was evaluated at the Kansas State University College of Veterinary Medicine’s Zoological Medicine Service. Several days prior to the initial evaluation, the bear had become partially anorexic. The bear had also reportedly been losing weight over the past year.

Clinical and Cytologic Findings

The bear was chemically restrained and anesthetized. Physical examination revealed moderate loss of body condition, with a decrease of 13 kg (28.6 lb) from the weight recorded a year earlier. Abdominal palpation revealed mild distension with a noticeable fluid wave. Abdominal ultrasonography revealed a large amount of free peritoneal fluid, a moderately thickened urinary bladder wall, and irregular, nodular areas of increased echogenicity within the liver. A sample of the free fluid was collected via abdominocentesis and submitted for cytologic evaluation; a blood sample was collected for a CBC and serum biochemical analysis.

Clinicopathologic findings were assessed with respect to data obtained from a database of overtly healthy sloth bears (all ages and both sexes) at various other institutions and compared with the sloth bear’s own historical values (obtained at previous examinations). The CBC revealed mature neutrophilia, mild lymphopenia, and monocytosis, which were most consistent with a stress leukogram. No abnormalities of the erythron were detected.

Serum biochemical analysis revealed high activities of alanine aminotransferase and alkaline phosphatase and a high concentration of total bilirubin. Serum bile acids concentration was 179 µmol/L (no reported value from which to determine a range for apparently healthy animals). Electrolyte abnormalities included hyponatremia, hypochloremia, and hyperkalemia.

The collected sample of peritoneal fluid was yellow and cloudy and had an estimated total nucleated cell concentration of 15,000 to 20,000 nucleated cells/µL and a total protein concentration of 3.6 g/dL. A concentrated cytocentrifuge preparation was examined microscopically (Figure 1) and was found to be highly cellular; 2 populations of cells were identified. The inflammatory cell population included mildly degenerate neutrophils and fewer macrophages. The other cell population was composed of epithelial cells found in small cohesive clusters. These cells had round nuclei, coarsely granular chromatin, prominent nucleoli, and variable amounts of basophilic cytoplasm that often contained abundant foamy vacuoles. There was moderate to marked anisocytosis and anisokaryosis, and occasional mitotic figures were found.

Figure 1—Photomicrograph of a cytopreparation of peritoneal fluid obtained from a sloth bear (Melursus ursinus) that was evaluated because of lethargy, partial anorexia, and weight loss. Notice the cluster of vacuolated epithelial cells and the large atypical cell with marked anisocytosis, marked anisokaryosis, and prominent nucleoli. Numerous erythrocytes, neutrophils, and occasional macrophages are also visible. Wright stain; bar = 10 µm.

In cooperation with

This report was submitted by Daniel V. Fredholm, MS, DVM, James W. Carpenter, MS, DVM, DACZM; Don J. Petersen, VMD, DACVP; and Chanran K. Ganta, BVSc, PhD, DACVP; from the Departments of Clinical Sciences (Fredholm, Carpenter) and Diagnostic Medicine and Pathobiology (Petersen, Ganta), College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506. Dr. Petersen’s present address is Abaxis Veterinary Reference Laboratories, 14830 West 117th St, Olathe, KS 66062. Dr. Fredholm’s present address is Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608. The authors thank Dr. Rodney Schnellbacher for technical assistance. Address correspondence to Dr. Fredholm (dfredholm@ufl.edu).
One week after the initial examination, the bear was euthanized because of the persistence of anorexia and lethargy and the development of depressed mentation. A postmortem examination was performed. On gross examination, the serosal surface of the diaphragm, urinary bladder, parietal tunic of the testicles, and omentum contained numerous pinpoint to 1-cm, pale white nodules. The diaphragmatic surface of the liver was diffusely adhered to the diaphragm. Approximately 50% to 60% of the liver (all lobes) was affected (Figure 2). There were multifocal to coalescing, pale white, firm nodules measuring 0.1 to 3 cm in diameter with centrally depressed dark areas of necrosis. On cut section, these nodules extended deep into the parenchyma, with distinct borders. The surrounding liver parenchyma was grossly normal. Additional gross findings included 3 enlarged mesenteric lymph nodes measuring 7 × 4 × 3 cm, 3 × 2 × 2 cm, and 2 × 1 × 1.5 cm. On cut section, the lymph nodes were slightly bulging and diffusely pale; there was loss of corticomedullary distinction but evidence of central liquefactive necrosis.

**Histopathologic Findings**

Sections of the liver were examined histologically. The hepatic parenchyma was infiltrated and replaced by multiple coalescing nodules of a well-delineated to locally infiltrative, unencapsulated neoplasm composed of polygonal cells that formed tubules, which were supported by abundant fibrous connective tissue admixed with basophilic mucinous matrix (Figure 3). The neoplastic cells had a moderate amount of eosinophilic cytoplasm with indistinct cell borders; each cell contained a round to oval nucleus with finely stippled chromatin and 1 to 2 prominent large nucleoli. There was marked anisocytosis and anisokaryosis with frequent mitoses. Multifocally extensive areas of necrosis were evident. The liver parenchyma adjacent to the neoplasm appeared normal. The neoplasm also infiltrated the mesenteric lymph nodes and the serosal surface of the diaphragm, parietal tunic of the testicles, and mesentery.

**Morphologic Diagnosis and Case Summary**

Morphologic diagnosis: cholangiocellular carcinoma (CCC) with abdominal carcinomatosis and metastasis to the mesenteric lymph nodes.

Case summary: CCC in a captive sloth bear presumptively diagnosed on the basis of results of peritoneal fluid analysis.
have been reports3–12 of hepatobiliary neoplasia in captive bears are considerably less extensive. Despite this, there documentation of neoplasia in captive and free-ranging with data available for domestic animals, studies and concerns involve management of feeding schedules. Many presence in a kibble-based bear diet as well. Another dietary terus, and dyspnea.14 Similarly, results of baseline laboratory and may include vomiting, lethargy, anorexia, weight loss, icteric agents within bile.

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environments do not provide the intermittent feeding regimen and do not feed on a continuous basis. Conversely, zoo practices in captivity may expose captive bears to higher amounts of carcinogens than they would encounter in the wild. This could be a particular concern for sloth bears, which are primarily insectivorous and frugivorous in the wild. In captivity, however, sloth bears are often fed a mixed diet, of which omni-vore kibble is a large component. Diethylnitrosamine, a potent carcinogen and mutagen, has experimentally induced CCC in dogs and can be found in some kibble-based animal diets.13 If this or any other carcinogen is found in animal food, which is often kibble based, then there exist at least a possibility of its presence in a kibble-based bear diet as well. Another dietary concern involves management of feeding schedules. Many bears in the wild spend a substantial amount of time hibernating and do not feed on a continuous basis. Conversely, zoo environments do not provide the intermittent feeding regimen that hibernation would force. As a result, the biliary apparatus in captive bears functions on a more continuous basis, possibly overwhelming the hepatobiliary cells with exposure to cholecyt agents within bile.

In dogs and cats, clinical signs of CCC are nonspecific and may include vomiting, lethargy, anorexia, weight loss, icterus, and dyspnea.14 Similarly, results of baseline laboratory diagnostic testing such as CBCs and serum biochemical analyses are often vague, with abnormalities that indicate liver damage or cholestasis but that are not specific for neoplastic disease.15 As such, it is not surprising that the sloth bear of this report had somewhat nonspecific, yet concerning clinical signs. The initial clinicopathologic findings were helpful but were insufficient for a definitive diagnosis of the disease. The electrolyte imbalances identified via serum biochemical analysis were likely attributable to loss of sodium and chloride into the peritoneal exudate and decreased renal excretion of potassium. A hypoadrenal state was unlikely, consid-

ering that metabolic acidosis was not detected, whereas as a stress leukogram and adrenal gland hyperplasia were evident. Similar electrolyte changes have been reported for cats with peritoneal effusions, including a cat with carcinomatosis.15

For the sloth bear of this report, abdominal ultrasonography was helpful in identification of nodular abnormalities within the liver but did not reveal the many areas of metastasis within the abdomen and provided no definitive diagnostic evidence of neoplasia. Other imaging techniques, such as CT and MRI, were not used in the case described in this report, but results of those procedures could have been useful in diagnosis and staging of this disease. Evaluation of a peritoneal fluid sample proved to be the most valuable test for diagnosis and prognosis assessment. Cytologic examination of the sample revealed the presence of a neoplastic effusion with features consistent with a carcinoma. When combined with the other test results and the animals overall clinical condition, the diagnosis of hepatobiliary carcinoma was almost certain.

Considering the apparent frequency of biliary neoplasia in captive bears, CCC should be strongly suspected in any captive ursid with clinical signs of hepatic disease. The disease in the sloth bear of this report had reached a very advanced state, thus leading to development of neoplastic effusion. In animals in which the disease has not yet reached this level of severity, effusion may not be present and a definitive diagnosis of neoplasia may not be obvious, especially if clinical signs and results of laboratory diagnostic testing are nonspecific.

**Comments**

In domestic species, CCCs, or bile duct carcinomas, are relatively rare. It has been estimated that CCCs account for < 0.36% of all neoplasms in domestic animals, although specific estimates are quite varied.1,2 Compared with data available for domestic animals, studies and documentation of neoplasia in captive and free-ranging bears are considerably less extensive. Despite this, there have been reports3–12 of hepatobiliary neoplasia in captive ursids. These case reports have described CCCs either of intrahepatic or extrahepatic origin, with those of extrahepatic biliary origin being more common. In most affected animals, including the sloth bear of the present report, multiple areas of metastasis were identified.4–8,10

The etiology of hepatobiliary neoplastic disease in bears remains unclear, although many possible causes have been suggested. Predisposing factors in other species include cholestasis, parasitism (infection with Ancylostomidae, Trichu-

nidae, or Clonorchis spp.), and viral disease. However, none of these factors have been implicated in any of the previous reports of CCC in bears, and none were detected in the sloth bear of this report. Genetic predisposition leading to a high rate of cellular mutation is a possibility but has not been studied, to our knowledge. Ingestion of toxic substances (ie, possible carcinogenic contaminants within an enclosure) should always be considered in cases of liver disease. However, because of the frequency of neoplasia specifically in captive bears and the inherent variety (eg, geographic and temporal) in the reported cases, environmental intoxication seems unlikely to be a major underlying cause. On the other hand, routine dietary practices in captivity may expose captive bears to higher amounts of carcinogens than they would encounter in the wild. This could be a particular concern for sloth bears, which are primarily insectivorous and frugivorous in the wild. In captivity, however, sloth bears are often fed a mixed diet, of which omnivore kibble is a large component. Diethylnitrosamine, a potent carcinogen and mutagen, has experimentally induced CCC in dogs and can be found in some kibble-based animal diets.13 If this or any other carcinogen is found in animal food, which is often kibble based, then there exist at least a possibility of its presence in a kibble-based bear diet as well. Another dietary concern involves management of feeding schedules. Many bears in the wild spend a substantial amount of time hibernating and do not feed on a continuous basis. Conversely, zoo environments do not provide the intermittent feeding regimen that hibernation would force. As a result, the biliary apparatus in captive bears functions on a more continuous basis, possibly overwhelming the hepatobiliary cells with exposure to cholecyt agents within bile.

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**References**