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Pathology in Practice

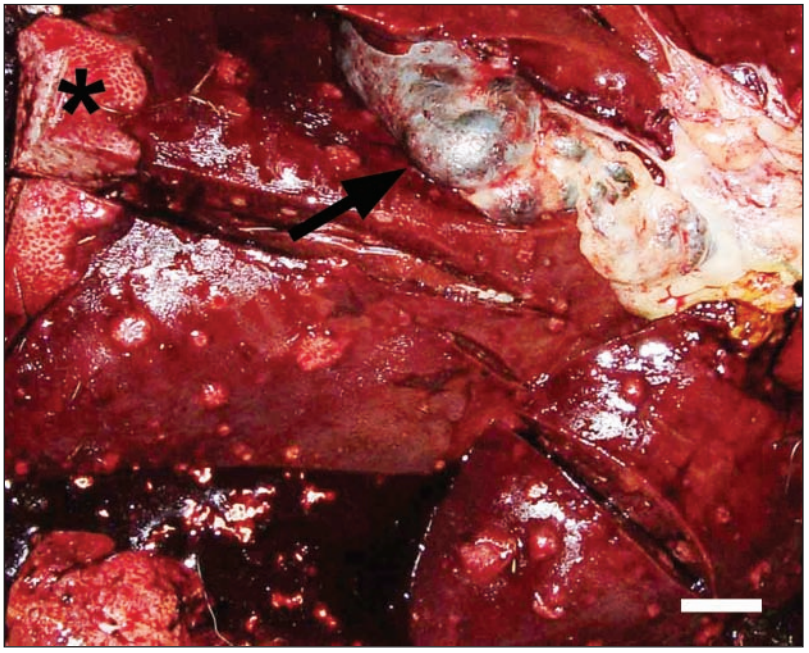

Figure 1—Photograph of the liver of an 11-year-old female lion that was evaluated because of signs of depression, lethargy, prostration, mild dehydration, loss of appetite, and vomiting of 9 days' duration. A liver biopsy procedure was performed, but the lion died following recovery from anesthesia. Notice the multiple variably sized, yellowish to white nodules that protrude from the liver capsule. Some of the nodules have coalesced to form ill-defined masses (asterisk). Hepatic lymph nodes are large (arrow). Bar = 3 cm.

History

An 11-year-old 110-kg (242.5-lb) sexually intact female lion (*Panthera leo*) from Senda Viva Zoologic Park in Arguedas, Spain, was evaluated because of signs of depression, lethargy, prostration, mild dehydration, loss of appetite, and vomiting of 9 days' duration.

Clinical and Gross Findings

A CBC and serum biochemical analyses were performed. Results of the CBC were unremarkable, but serum aspartate aminotransferase activity was high (288 U/L; reference range, 0 to 192 U/L). To obtain a hepatic wedge biopsy specimen, the lion was premedicated with medetomidine (10 µg/kg [4.5 µg/lb], IM) and midazolam (0.2 mg/kg [0.09 mg/lb], IM); an IM injection of morphine chlorhydrate (0.1 mg/kg [0.05 mg/lb]) was then administered, and anesthesia was maintained via continuous IV infusion of diazepam (0.2 mg/kg/h), medetomidine (2 µg/kg/h [0.9 µg/lb/h]), and morphine chlorhydrate (0.1 mg/kg). The lion initially recovered well from anesthesia, but its clinical condition suddenly worsened and it died after surgery.

Postmortem examination was performed immediately after the lion died. The hepatic tissue was distorted with multiple yellowish to white, ill-defined, irregularly shaped nodules that replaced most of the parenchyma and elevated the liver capsule (**Figure 1**). Many of these nodules had coalesced to form multilobulated, ill-demarcated masses. On cut section, the nodules and masses consisted of white to yellow firm tissue containing small necrotic foci. Hepatic lymph nodes were large, the gallbladder was dilated, and the common bile duct had an inflamed appearance.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

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Histopathologic Findings

Microscopic examination of imprints of cut surfaces of the lesions revealed only numerous degenerated neutrophils; therefore, suppurative cholangiohepatitis was first suspected. Hepatic tissue samples were collected, fixed in neutral-buffered 10% formalin, and submitted[a] for histologic examination. Microscopically, the nodules were composed of densely packed pleomorphic hepatocyte-like cells, which formed nests and trabeculae (3 to > 10 cells in thickness) that were separated by thin connective stroma, and vascular spaces packed with erythrocytes. The neoplastic cells had variable amounts of lightly eosinophilic, vacuolated cytoplasm with large and round to oval, occasionally irregular nuclei with prominent central nucleoli (**Figure 2**). Some areas were composed of anaplastic round or plump cells arranged in a diffuse, solid pattern without portal triads or central veins. The mitotic index was low (< 3 mitoses/hpf). An intense mixed inflammatory component including numerous neutrophils, hemosiderin-laden macrophages, lymphocytes, and plasma cells was also associated with the neoplastic nodules (**Figure 3**). Throughout some necrotic foci, there were numerous gram-positive, spore-forming bacterial rods. The neoplastic cells did not stain with Alcian blue stain (pH, 2.5), which detects acid mucopolysaccharides, or argirophyl Grimelius stain, which detects secretory granules. Immunohistochemically, the tumor cells were strongly positive for cytokeratin (CK) AE1/AE3 and moderately positive for CKs 8 and 18. Only a few scattered cells were weakly positive for CK 20. Results of immunolabeling for CK 7, chromogranin A, synaptophysin, and neuron-specific enolase were negative in neoplastic cells.

Morphologic Diagnosis

Hepatocellular carcinoma (HCC) with secondary inflammation.

Comments

For the female lion of this report, the neoplastic nature of the hepatic lesions was confirmed histologically. On the basis of gross findings alone, it may not be possible to discriminate between nodular hyperplasia and neoplasia. Nodular hyperplasia of hepatocytes is common in old dogs but rare in other species.[1,2] Hyperplastic nodules are usually multiple, spherical, and well circumscribed and vary in diameter from 2 mm to 3 cm, whereas the hepatic nodules detected in this lion were irregular and large. In addition, necrosis and hemorrhage were detected; these features are not typically associated with hyperplastic nodules.[1,2] The microscopic hallmark of hyperplastic nodules, in contrast to hepatic adenomas and HCCs, is that they largely retain normal

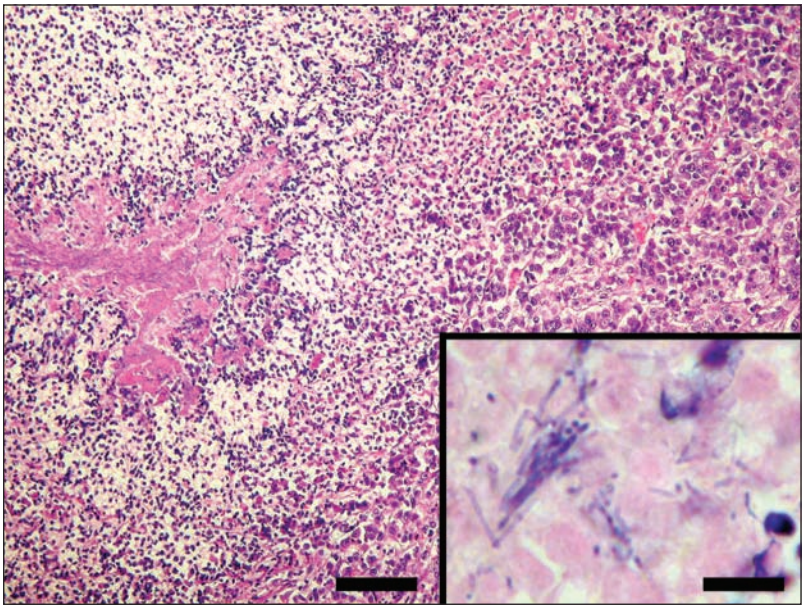

Figure 3—Photomicrographs of a section of the hepatic tumor of the lion in Figure 1. In the upper right portion of the main image, an area of necrosis with neutrophilic aggregation and neoplastic hepatocytes is present. H&E stain; bar = 250 µm. Inset—Higher magnification view in which large bacteria with a *Clostridium*-like appearance are visible. H&E stain; bar =10 µm.

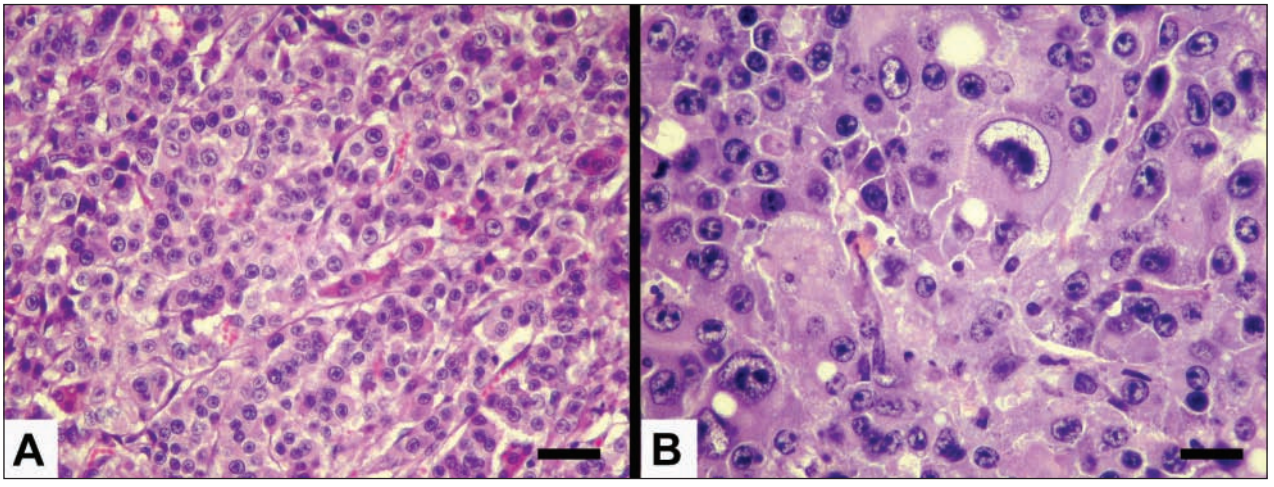

Figure 2—Photomicrographs of sections of nodules in the liver of the lion in Figure 1. A—Nests and trabeculae of densely packed pleomorphic neoplastic cells separated by a thin connective stroma and vascular spaces are visible. H&E stain; bar = 100 µm. B—In this view, anaplastic areas composed of round-shaped cells arranged in a solid pattern with some mononucleated bizarre giant cells containing intensely acidophilic cytoplasm, polymorphic nuclei, and prominent nucleoli are present. H&E stain; bar = 50 µm.

liver architecture, including a modified lobular structure with recognizable central veins and portal triads.[1,2] In the lion of this report, the tumor had cellular atypia and microscopic disarrangement, including loss of portal triads. Nodular hyperplasia was therefore eliminated from the list of differential diagnoses.

Hepatocellular adenoma or carcinoma, cholangiocarcinoma, and hepatic carcinoid were considered. Hepatocellular adenomas (also termed hepatomas) are usually single, smooth benign nodules, which are sometimes pedunculated, whereas HCCs may be single and massive, nodular, or diffuse. Absence of pedunculation or clear demarcation and the varied coloration of the cut surface produced by hemorrhage, necrosis, fatty change, and bile pigmentation are common features that suggest malignancy.[1,2] Cholangiocellular tumors can usually be distinguished from hepatocellular tumors by their firmness and white color (associated with more or less abundant stroma), and those that involve the capsule have a typical umbilicate appearance as a result of necrosis or cavitation associated with insufficient neovascularization of the central parts of the tumor.[1,2] Histologically, the typical acinar or tubular composition and the cuboidal to columnar lining epithelium of the cholangiocarcinomas are usually not difficult to distinguish from HCCs. The presence of desmoplasia and mucin production by biliary epithelium in cholangiocarcinomas are other features that can be used for this distinction.[1,2] No tubular growth, desmoplasia, or mucin was evident in the lion's tumor; most of the tumoral masses were poorly differentiated. Immunohistochemical analyses can facilitate the distinction of poorly differentiated hepatocellular neoplasms from cholangiocellular tumors by use of antibodies against CKs 7 and 19, which are expressed only by bile duct epithelium and are not present in hepatocytes.[3,4] In the lion of this report, CK 7 was only expressed by biliary epithelium both in neoplastic and nonneoplastic areas of the liver. Some scattered neoplastic cells were faintly CK 20 positive. Expression of CK 20 has been occasionally detected in hepatocellular carcinomas in cats.[3] These findings were consistent with a hepatocellular origin. Hepatocellular carcinoma must be also distinguished from primary hepatic carcinoid on the basis of histologic appearance and the use of silver impregnation or immunohistochemical stains that reveal neurosecretory granules in the cytoplasm of carcinoid cells.[1,2] Examination of Grimelius-stained sections of the lion's liver did not reveal granules in the neoplastic cells, and results of staining for chromogranin A, synaptophysin, and neuron-specific enolase—markers typically found in carcinoids—were negative.

The incidence of hepatic neoplastic disease in wild felids is not known.[2] The only reports[5–8] in English in the veterinary medical literature of which we are aware are of a hepatoma in a Bengal tiger (*Panthera tigris*), a cholangiocarcinoma in a leopard (*Panthera pardus*), and a biliary malignant lymphoma, 2 gallbladder adenocarcinomas, and a cholangiocarcinoma in lions. To the authors' knowledge, this is the first report of a malignant tumor of hepatocellular origin in a captive wild felid. Hepatocellular carcinomas are uncommon in domestic animals.[1] The incidence in dogs has been reported[9] to be < 1% of all neoplasms. In cats, primary hepatic tumors account for 1% to 3% of all tumors, with HCC representing 17% of all hepatic neoplasms.[10] Metastasis in domestic animals generally occurs late in the course of neoplastic development and most commonly in the lungs and hepatic lymph nodes. Despite the fact that the local lymph nodes may contain metastatic neoplastic cells, they rarely are massively enlarged.[1,2] In the lion of this report, hepatic lymphadenomegaly was evident but metastases were not confirmed because only liver samples were collected for histologic examination.

The clinical data and the physical examination findings in this case were nonspecific, in agreement with previous reports[2,7,11] of hepatic tumors in dogs, cats, and wild animal species. The inflammation associated with the neoplasm was attributed to secondary infection. Abscesses may develop in association with hepatic neoplasms as a result of progressive ischemia that causes a central area of necrosis, which provides a suitable environment for proliferation of bacteria, especially anaerobes.[11,12] Although we were unable to locate a publication listing the normal hepatic bacterial flora of felids, it has been suggested that *Clostridium* spp are normal inhabitants of the liver in dogs and that those organisms may proliferate when the portal blood supply is interrupted.[12] However, the high frequency of polymicrobial infections with enteric flora in dogs[12] and cats[11] suggests that, more often, the organisms may enter the damaged liver from the intestinal tract. In the lion of this report, organisms detected in liver tissue were not cultured and identified but there was a suspicion that they may have been *Clostridium* spp because they were gram-positive, spore-forming rods.

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References

1. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG, ed. *Jubb, Kennedy, Palmer's pathology of domestic animals*. Vol 2. 5th ed. Philadelphia: Saunders-Elsevier, 2007;382–386.
2. Cullen JM, Popp JA. Tumors of the liver and gall bladder. In: Meuten DJ, ed. *Tumors in domestic animals*. 4th ed. Ames, Iowa: Iowa State Press, 2002;483–504.
3. Espinosa de los Monteros A, Fernández A, Millán MY, et al. Coordinate expression of cytokeratins 7 and 20 in feline and canine carcinomas. *Vet Pathol* 1999;36:179–190.
4. Ramos-Vara JA, Miller MA, Johnson GC. Immunohistochemical characterization of canine hyperplastic hepatic lesions and hepatocellular and biliary neoplasm with monoclonal antibody hepatocyte paraffin 1 and a monoclonal antibody to cytokeratin 7. *Vet Pathol* 2001;38:636–643.
5. Owston MA, Ramsay EC, Rotstein DS. Neoplasia in felids at the Knoxville Zoological Gardens, 1979–2003. *J Zoo Wildl Med* 2008;39:608–613.
6. Douglass EM. Lymphosarcoma and blockage of the biliary duct in an African lion (*Panthera leo*). *Vet Med Small Anim Clin* 1979;74:1637–1641.
7. Sakai H, Yanai T, Yonemaru K, et al. Gallbladder adenocarcinomas in two captive African lions (*Panthera leo*). *J Zoo Wildl Med* 2003;34:302–306.
8. Smith RE, Boardman W, Turnbull PC. Hepatoma in a Bengal tiger. *J Am Vet Med Assoc* 1971;159:617–619.
9. Strombeck DR. Clinicopathologic features of primary and metastatic neoplastic disease of the liver in dogs. *J Am Vet Med Assoc* 1978;173:267–269.
10. Liptak JM, Dernell WS, Withrow SJ. Liver tumors in cats and dogs. *Compend Contin Educ Pract Vet* 2004;26:50–57.
11. Singh M, Krockenberger M, Martin P, et al. Hepatocellular carcinoma with secondary abscessation in a cat. *Vet J* 2005;83:736–739.
12. Grooters AM, Sherding RG, Jonhson SE. Hepatic abscesses in dogs. *Compend Contin Educ Pract Vet* 1995;17:833–838.