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## Biliary Adenocarcinoma in a Stranded Northern Elephant Seal (*Mirounga angustirostris*)

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**ABSTRACT:** A stranded adult female northern elephant seal (*Mirounga angustirostris*) was admitted to a rehabilitation hospital grossly underweight and lethargic in April 2001. The animal was icteric, had severe bilirubinemia, and elevated serum gamma glutamyl transferase concentrations. Laparoscopy under anesthesia revealed multiple masses up to 3 cm diameter throughout the liver and spleen and the animal was euthanized. Abnormal gross postmortem findings included green serous fluid in the abdominal cavity and 0.5 to 3 cm diameter yellow nodules scattered throughout the liver, spleen, and lymph nodes. Histopathology revealed a biliary adenocarcinoma with metastases to the liver, spleen, lymph nodes, adrenal gland, and pancreas. This is believed to be the first reported case of neoplasia in a northern elephant seal.

**Key words:** Adenocarcinoma, anesthesia, bile duct, laparoscopy, northern elephant seal, neoplasia.

Neoplasia has been reported in many species of marine mammals (Gulland et al., 2001), with high prevalences observed in belugas (*Delphinapterus leucas*; De Guise et al., 1994) and California sea lions (*Zalophus californianus*; Gulland et al., 1996). Adenocarcinomas of the small intestine, lung, and uterus have recently been reported in a Steller sea lion (*Eumatopias jubatus*), a beluga, and a ringed seal (*Phoca hispida*) (Lair et al., 1998; Sato et al., 1998; Mikaelian et al., 2001). Tumors in elephant seals appear to be rare, with only one neoplasm, a granulosa cell tumor, reported in a southern elephant seal (*Mirounga leonina*; Mawdesley-Thomas, 1971). Reports of tumors in northern elephant seals have not been published to our knowledge. This report describes the clinical and pathologic characteristics of biliary adenocarcinoma in a northern elephant seal (*Mirounga angustirostris*).

On 26 April 2001, an adult female northern elephant seal was admitted to a rehabilitation hospital (The Marine Mammal Center, Sausalito, California, USA). The animal had stranded in Monterey County, California (36°45'N, 121°90'W), was underweight, and lethargic. On physical examination the northern elephant seal was icteric, dehydrated, and weighed 196 kg; maximum adult female weight for this species is 600 kg (Reeves et al., 2002). Serum biochemistry analysis on blood collected from the epidural sinus revealed bilirubinemia (28 mg/dl), elevations of serum alanine aminotransferase (ALT; 196 U/l), and gamma glutamyl transferase (GGT; 1070 U/l) concentrations suggestive of liver disease. Reference ranges for these values in this species range from 0.1–0.6 mg/dl for bilirubin, 18–65 U/l for ALT, and 22–136 U/l for GGT (Bossart et al., 2001). Red and white cell parameters were within normal limits for this species (Bossart et al., 2001). On fecal examination *Otostrongylus circumlitus* larvae were found. The animal was treated with antibiotics including an initial 4 day course of injectable cefotiofur sodium (Naxcel®, Pharmacia Upjohn Company, Michigan, USA) at 5 mg/kg intramuscularly followed by oral sulfamethoxazole/trimethoprim (Tribrissen®, Mutual Pharmaceutical Company, Pennsylvania, USA) at 12 mg/kg for 6 days. The animal was also treated with corticosteroids, antihelmintics, and supportive care but continued to be inappetent and lethargic.

Sixteen days after presentation, the animal was anesthetized with 140 mg of a 1:1 solution of zolazepam with tiletamine (Telazol®, Fort Dodge Animal Health, Iowa, USA) intravenously into the epidural

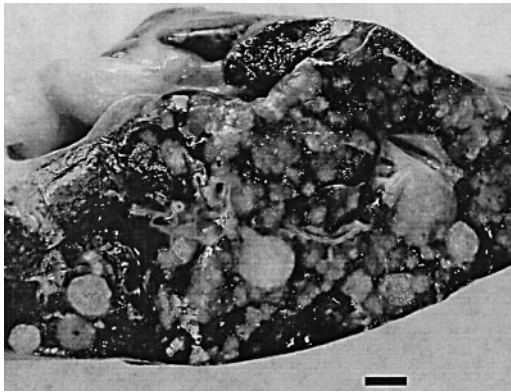


FIGURE 1. Photograph of cut-section of liver of an adult elephant seal. Note yellow neoplastic masses. Bar=2 cm.

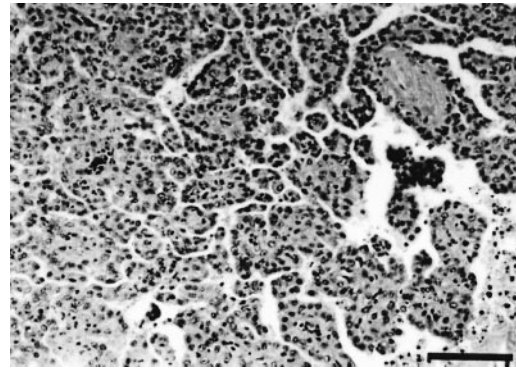


FIGURE 2. Photomicrograph of bile duct carcinoma of the liver in an adult elephant seal. Note the papillary projections and the tubular structures. H&E. Bar=666  $\mu$ m.

sinus after premedication with 3.5 mg atropine sulfate (Radix Laboratories Inc., Eau Claire, Wisconsin, USA) intramuscular into the gluteal muscle. The animal was maintained on 0.5 to 1.5% isoflurane (Aerane®, Fort Dodge Animal Health) with an oxygen flow of 6 l/min.

Laparoscopic examination revealed moderate splenomegaly with thickened and rounded margins. The spleen and liver had generalized, multifocal, raised, pale yellow, nodular masses of up to 3 cm, and several of the large nodules were umbilicated. Dark green serous fluid was present in the abdominal cavity. The peritoneum and serosa of the visible gastrointestinal tract was green-tinged. Due to the extent of the lesions in the affected organs and poor prognosis for recovery, the elephant seal was euthanized with pentobarbital (Beuthanasia®, Schering-Plough Animal Health Corporation, New Jersey, USA) intravenously in the epidural sinus.

Gross postmortem examination revealed 300 ml of green serous fluid in the abdominal cavity and multifocal, 0.5 to 3 cm diameter yellow nodules scattered throughout the liver (Fig. 1) and spleen. Inguinal, sublumbar, gastric, pancreatic, hilar, and tracheobronchial lymph nodes were totally effaced with tumor. One 0.5 cm nodule was found on the surface of the pancreas, but the parenchyma of the pancreas was

unremarkable. All other major organs were free of neoplastic masses. The masses located in all affected tissues except the spleen were firm on cut section and several of the larger hepatic masses (>2 cm diameter) were umbilicated. The majority of the masses in the spleen contained yellow purulent material. Three small ulcers 0.5 cm diameter were found in the mucosa of the pylorus. The broad ligament of the ovary and uterus had five cysts (0.5 cm diameter), a corpus luteum was present on the left ovary, and the right uterine horn was thickened.

Tissue samples from multiple organs were fixed in 10% neutral buffered formalin, routinely processed for paraffin embedding, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. The seal was aged by counting the annual growth layers of dentin in sagittal sections of the upper canine tooth (Payne, 1978); the minimum age of this animal was 15 yr. Average life span for an adult female elephant seal is 18–20 yr (Syderman and Nur, 1994).

Microscopically the masses in the liver, spleen, lymph nodes, and pancreas were composed of cords and gland-like structures of neoplastic cells (Figs. 2, 3). These neoplastic cells were large cuboidal cells with round to oval nuclei and moderate cytoplasm, in some areas the cytoplasm was foamy. One to two mitotic figures

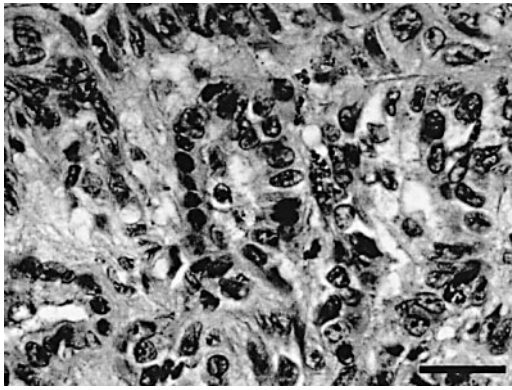


FIGURE 3. Photomicrograph of a bile duct carcinoma of the liver in an adult elephant seal. Note the cuboidal epithelial cells forming glandular to tubular structures. H&E. Bar=133  $\mu$ m.

were found per high-powered field. Extensive desmoplasia was found around some edges of the masses, several masses had necrotic centers, and neoplastic emboli were present in portal veins and some smaller vessels in the liver and spleen. In-spissated bile within biliary canaliculi was extensive throughout the liver. Multiple lymph nodes were effaced by the neoplasm; tumor cells within lymph nodes and lymphatics were similar to those in the liver. The right adrenal gland had a large metastatic focus identical to the tumor in the liver. The tumor in the adrenal gland occupied all three layers of the cortex and a portion of the medulla and the central portion of the tumor was necrotic. There was a neoplastic nodule attached to the pancreas, although the pancreatic parenchyma was normal. The stomach had several ulcerated areas but the ulcers did not penetrate deeply into the submucosa and there was only mild inflammation. An acid-fast stain and stains for fungi of the liver were negative. Based on its histologic appearance, this tumor was diagnosed as an adenocarcinoma of bile duct origin with metastases to the liver, spleen, lymph nodes, right adrenal gland, and capsule of the pancreas. Histologic sections of the tumor were deposited in the Registry of Veterinary Pathology, Armed Forces Institute of Pathology (Washington D.C., USA; ac-

cession number 283229600). The only other histologic lesion was mild degeneration and mineralization of the aorta. This incidental lesion may have been due to a previous *Otostrongylus* sp. infection.

The clinical signs of jaundice with elevation of serum ALT and GGT levels were likely a result of leakage of these enzymes from cells of the bile canaliculi and neoplastic cells in the blood stream (Bossart et al., 2001). The amount of peritoneal fluid was elevated for an elephant seal and was probably a consequence of decreased lymphatic drainage from the peritoneal cavity. Gastric ulcers are common in pinnipeds, and to date have been attributed to gastric nematodes and stress (Gulland et al., 2001). Recently *Helicobacter* sp. have been identified in a number of marine mammal species, but their role in the pathogenesis of ulcers is unknown (Harper et al., 2002).

The etiologies of biliary adenocarcinomas in most species are unknown, although bile duct tumors have been associated with infection with opisthorcid flukes in humans (Watanapa and Watanapa, 2002). Flukes (*Zalophotrema hepaticum*) are common in the bile ducts of California sea lions (*Zalophus californianus*), in which the prevalence increases with age (Dailey, 2001). However, these flukes were not observed in the bile ducts or gall bladder of this seal. In the present case, the etiology of the tumor was not determined.

Although over 2,000 northern elephant seals, mostly weaned pups and yearlings, have been treated and over 800 elephant seals have been necropsied at The Marine Mammal Center since 1975, this is the first observed case of neoplasia in this species. However, less than 10 postmortem examinations have been performed on adult elephant seals and the prevalence of neoplasia in older animals of this species is unknown. There has been an increase in the number of reports of neoplasia in marine mammals in recent years (Gulland et al., 2001; Mikaelian et al., 2001). This increase may be due to a real increase in the



incidence of neoplasia, it may be due to the increase in the number of pathologists examining marine mammals, or a combination of both. Thorough postmortem examinations, coupled with long-term monitoring programs are necessary to determine whether true changes in prevalence of neoplasia are occurring in free-ranging marine mammals.

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