

## ESOPHAGEAL SQUAMOUS CELL CARCINOMA IN SIX HARBOR SEALS (*PHOCA VITULINA* SPP.)

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**Abstract:** Six cases of esophageal squamous cell carcinoma were identified in six captive adult Pacific (*Phoca vitulina richardsii*;  $n = 2$ ) and Atlantic (*Phoca vitulina concolor*;  $n = 4$ ) harbor seals. These seals presented with intermittent dysphagia, regurgitation, inappetence, and abnormal posturing. Common clinical pathology findings in these seals included azotemia, hyperproteinemia, hyperglobulinemia, and leukocytosis. Gastrointestinal endoscopy commonly revealed an ulcerated mass near the gastroesophageal junction. Each seal was euthanized ( $n = 3$ ) due to poor prognosis, subsequently died while undergoing an anesthetic procedure ( $n = 2$ ), or found dead ( $n = 1$ ). The diagnosis of squamous cell carcinoma was confirmed via biopsy of esophageal mucosa during endoscopy or histopathologic examination of affected tissues after necropsy. On the basis of clinical and postmortem findings, esophageal squamous cell carcinoma should be considered as a differential diagnosis in aged harbor seals exhibiting clinical signs of regurgitation, decreased appetite or anorexia, vomiting, and/or abnormal posturing.

**Key words:** Esophagus, harbor seal, neoplasia, *Phoca vitulina*, regurgitation, squamous cell carcinoma.

### INTRODUCTION

The number of reports of neoplasia in marine mammals continues to increase and may be a result of the increased number of animals annually examined by pathologists.<sup>7</sup> As many deaths in wild populations go unnoticed, or autolysis limits necropsy findings, the most detailed pathology information is often from single cases involving marine mammals under human care, where historical and treatment information may be available.<sup>18</sup> Physical, chemical, and infectious agents have all been associated with neoplasms in other species, and the likelihood of developing neoplasms may be further modulated by age, hormones, and genetics.<sup>7</sup>

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This report describes six cases of squamous cell carcinoma of the esophagus in harbor seals (*Phoca vitulina* spp.) maintained in aquarium or zoologic institutions (Table 1). Contrast radiography, gastrointestinal endoscopy, and computed tomography (CT) were helpful diagnostic modalities in these cases. The diagnosis of squamous cell carcinoma was confirmed via biopsy of esophageal mucosa during endoscopy or histopathologic examination of affected tissues after necropsy in all cases.

Conservative medical therapy was used in most cases for supportive care and alleviation of clinical signs; however, medical therapy, although palliative in some instances, ultimately was not successful. Surgical intervention for tumor removal was not pursued in any of the described cases in which the diagnosis was known antemortem due to high risk of anesthetic complications and resection limitations. These cases emphasize the need for advanced diagnostics in harbor seals displaying clinical signs of regurgitation, vomiting, long-term decreased appetite or anorexia, and abnormal posturing or apparent postprandial gastrointestinal discomfort, and that esophageal squamous cell carcinoma should be considered as a differential diagnosis in aged seals with this clinical presentation.

### CASE REPORTS

#### Case 1

A 31-yr-old, 120-kg intact male Pacific harbor seal (*Phoca vitulina richardsii*) was evaluated for a 4-

**Table 1.** Summary of cases of esophageal squamous cell carcinoma in Pacific harbor seals (*Phoca vitulina richardsii*) and Atlantic harbor seals (*Phoca vitulina concolor*), including case number, species, age, sex, and primary clinical signs.

Case	Species	Age (year)	Sex	Primary clinical signs
1	Pacific harbor seal ( <i>Phoca vitulina richardsii</i> )	31	Male	Inappetance, regurgitation, vomiting, arched dorsal posture after eating, dysphagia
2	Atlantic harbor seals ( <i>Phoca vitulina concolor</i> )	28	Female	Intermittent discomfort, lethargy, regurgitation, vomiting, decreased training compliance, increased social anxiety, stiffness, reaction to manipulation of pedal flippers
3	Atlantic harbor seals ( <i>Phoca vitulina concolor</i> )	35	Female	Intermittent discomfort, regurgitation, decreased training compliance, increased social anxiety, stiffness, reaction to manipulation of pedal flippers
4	Atlantic harbor seals ( <i>Phoca vitulina concolor</i> )	29	Female	Inappetance, vomiting and regurgitation, dysphagia, lethargy, decreased training compliance
5	Pacific harbor seal ( <i>Phoca vitulina richardsii</i> )	27	Male	Intermittent regurgitation
6	Atlantic harbor seals ( <i>Phoca vitulina concolor</i> )	32	Male	Diarrhea, regurgitation, vomiting, decreased training compliance, floating with a hunched posture with eyes closed

wk history of intermittent inappetance, regurgitation, vomiting, and arched dorsal posturing after eating. The seal was housed in a 120,000-gallon outdoor synthetic seawater facility with two adult female stellar sea lions (*Eumetopias jubatus*). The previous medical history of this animal included bilateral mature cataracts, gingival hyperplasia, and mild gingivitis. The initial clinical signs resolved after 3 wk of gastrointestinal protectants, including sucralfate (Teva Pharmaceuticals USA, Sellersville, Pennsylvania 18960, USA; 1,200 mg p.o. b.i.d.), ranitidine (Amneal Pharmaceuticals of New York, Hauppauge, New York 11788, USA; 300 mg p.o. s.i.d.), and simethicone (Advance Pharmaceutical Inc., Ronkonkoma, New York 11779, USA; 180 mg p.o. b.i.d.). However, 4 wk later, the seal again began refusing fish, posturing with its back arched or neck stretched after eating, and regurgitating. Ingested fish were uniquely regurgitated with the heads digested but the bodies intact.

Gastroprotectants, as described above, were no longer effective as the seal began to exhibit dysphagia and continued discomfort after eating, as evidenced by rolling on its side, licking its lips, and scratching its side with its pectoral flipper. Complete blood count and serum biochemistry panel performed at this time revealed a mild neutrophilic leukocytosis (13,500 cells/ $\mu$ l; in house reference range, 5,100–10,800 cells/ $\mu$ l; neutrophil count 10,260 cells/ $\mu$ l; in-house reference range, 3,900–7,200 cells/ $\mu$ l) and monocytosis (1,485 cells/4,800 cells/ $\mu$ l; in-house reference

range, 50–550 cells/ $\mu$ l) with mild hyperfibrinogenemia (261 mg/dl; in-house reference range, 150–250 mg/dl). The seal was administered penicillin G benzathine/penicillin G procaine (Combi-Pen-48<sup>®</sup>, Bimeda Inc., Le Sueur, Minnesota 56058, USA), 990,000 IU i.m. once; and ceftiofur crystalline-free acid (Excede<sup>®</sup>, Pfizer Inc., New York, New York 10017, USA), 1,200 mg s.c. once for antibiotic coverage; and dexamethasone sodium phosphate (Dexium-SP<sup>™</sup> Bimeda Inc.), 36 mg i.m. s.i.d. to control suspected inflammation. Diazepam (Hospira Inc., Lake Forest, Illinois 60045, USA; 8.4 mg i.m. s.i.d.) was administered for appetite stimulation. Famotidine (Baxter Healthcare Corporation, Deerfield, Illinois 60015, USA; 60 mg i.m. s.i.d.) and metoclopramide (Hospira Inc.; 40 mg i.m. s.i.d.) were administered to decrease gastric acid production and reduce regurgitation in hopes of improving the seal's appetite. Although incidences of regurgitation and vomiting decreased substantially after starting these medications, the seal continued to intermittently refuse food and show signs of postprandial discomfort.

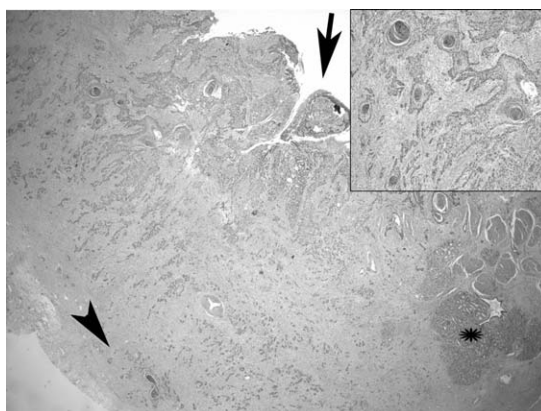
Differential diagnoses included esophageal foreign body, gastric or esophageal ulcerations, esophageal or gastrointestinal obstruction, and esophageal or gastric neoplasia. Repeated blood work 1 wk later revealed a persistent neutrophilic leukocytosis (16,300 cells/ $\mu$ l; neutrophil count 14,018 cells/ $\mu$ l). Due to this finding and to improve the antibiotic spectrum, marbofloxacin (Zeniquin<sup>®</sup>, Pfizer Inc.; 500 mg, p.o. s.i.d.) was

initiated. Full-body radiographs and abdominal ultrasound performed under behavioral control at this time did not reveal any substantial abnormalities.

Three days following the change in antibiotics, the seal appeared increasingly lethargic and exhibited intermittent episodes of coughing, sneezing, and white or foamy nasal discharge. Complete blood count and serum biochemistry panel indicated a marked mature neutrophilic leukocytosis (white blood cell count 24,400 cells/ $\mu$ l; neutrophil count 23,400 cells/ $\mu$ l) and lymphopenia (732 cells/ $\mu$ l; reference range, 1,230–2,500 cells/ $\mu$ l) with mild anemia (45%; reference range 50–57%); progressive hyperfibrinogenemia (365 mg/dl) with mild hyperproteinemia (8.3 g/dl; in-house reference range, 7.0–7.8 g/dl) and mild hyperglobulinemia (4.3 g/dl; reference range, 3.1–3.9 g/dl). Lung auscultation was unremarkable, although development of an upper respiratory infection was suspected.

Due to the lack of improvement during marbofloxacin treatment and while awaiting culture results of the nasal discharge, the seal's antibiotics again were changed: (levofloxacin, Levoquin<sup>®</sup>, Janssen Pharmaceuticals Inc., Titusville, New Jersey 08560, USA; 4.8 mg/kg p.o. s.i.d.); and iron supplementation (Ferocon<sup>®</sup> Breckenridge Pharmaceutical, Inc., Boca Raton, Florida 33487, USA; 120 mg p.o. s.i.d.) was initiated due to the newly presented anemia. One week following this change, the seal's white blood cell count (14,000 cells/ $\mu$ l) and neutrophil count (13,160 cells/ $\mu$ l) had decreased, but severe lymphopenia had developed (280 cells/ $\mu$ l). Culture results of the nasal discharge revealed normal respiratory flora.

Due to lack of improvement at this time in the clinical presentation, the seal was anesthetized for upper gastrointestinal endoscopy and advanced imaging with CT. The seal was premedicated with atropine sulfate (Med-Pharmex Inc., Pomona, California 91767, USA; 2.4 mg i.m.) prior to anesthetic induction with diazepam (Hospira Inc.; 18 mg i.v.). The animal was intubated for anesthetic maintenance with isoflurane (Isoflo<sup>®</sup>, Abbott Laboratories, Abbott Park, Illinois 60064, USA) in oxygen. Esophagoscopy revealed a pale yellow ulcerated mass in the caudal esophagus approximately 4 cm cranial to the lower esophageal sphincter. The mass was irregular, proliferative, erythematous, and contained a small but invasive crater containing caseous material. A separate 5-cm linear ulcer was present 4 cm cranial to the mass lesion. Multiple endoscopic



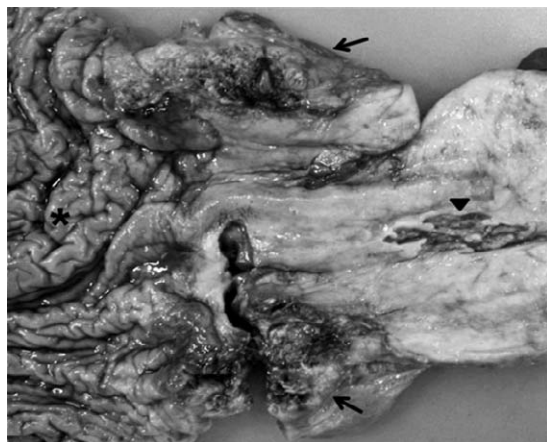
**Figure 1.** Esophageal squamous cell carcinoma of a Pacific harbor seal (*Phoca vitulina richardsii*). Hematoxylin and eosin (H&E;  $\times 20$ ) staining of paraffin-embedded sections demonstrates the cords of epithelial cells invading the underlying tissue through the tunica muscularis (arrowhead). Note the ulcerated surface of the mass (arrow) and esophageal glands present within the muscularis externa (asterisk). Inset: H&E ( $\times 100$ ) demonstrates irregular cords and nests of neoplastic epithelial cells and concentric lamellated pattern of keratinization (keratin pearls, arrows).

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pinch biopsies were obtained from within and around the crater of the mass. Biopsies from the esophageal mass were submitted on saline-dampened gauze for flash freezing and histopathology. On the frozen section, islands and trabeculae of squamous epithelial cells were identified, invading through the basement membrane to the underlying submucosa (Fig. 1), which was consistent with squamous cell carcinoma.

CT for further characterization and determination of potential surgical resection of the mass was performed during the same anesthetic procedure from the level of the midthorax to the gastric pylorus. Images showed that the caudal esophageal wall was thickened (2.5 cm) and irregular, resulting in narrowing of the esophageal lumen and changes extended for 1 cm into the gastric cardia. Several pinpoint gas lucencies were noted along the mucosal surface of the esophagus. These results were suggestive of an intramural mass with superficial ulceration.

Biopsy results became available at the completion of CT imaging. Because it was determined that complete resection was unlikely due to the location, involvement of the lower esophageal sphincter, and locally invasive nature of the tumor, euthanasia was performed (Euthasol<sup>®</sup>, Virbac Corporation, Fort Worth, Texas 76161,



**Figure 2.** Esophageal squamous cell carcinoma of a Pacific harbor seal (*Phoca vitulina richardsii*). Cut section of the mass (arrows) proximal to gastroesophageal junction. Mass has a grey-black center and tan-pink periphery; ulcer (arrowhead) on the esophageal mucosa proximal to the mass; gastric rugae (asterisk).

USA; 10,140 mg i.v.) without recovering the seal from anesthesia.

Gross necropsy findings revealed a slightly thin body condition along with previously diagnosed ocular abnormalities, including bilateral lenticular opacities and lens luxations. Cranial to the gastroesophageal junction at the level of lower esophageal sphincter, an  $8 \times 5 \times 4$  cm, cauliflower-like, grey-tan, irregularly spherical, firm, transmural, ulcerated mass was identified (Fig. 2). On the cut section of this area, minimal turbid yellow fluid discharge was identified, and the mass had a grey-black center with a tan-pink periphery. An esophageal mucosal ulcer ( $5 \times 1.5$  cm) was cranial to the mass on the ventral surface and may have been associated with pooling of refluxed gastric juices contributing to ulcer formation.

Microscopically, the lesion extended from the ulcerated mucosa and invaded the submucosa and muscular layers. The mass was poorly circumscribed, unencapsulated, and composed of polygonal cells arranged in cords, trabeculae, nests, and occasional individualized cells, supported by moderate amounts of dense collagenous stroma (desmoplasia). The cells had variably distinct cell borders and contained abundant amphophilic to eosinophilic cytoplasm. Nuclei were round to oval and had finely stippled chromatin and 1 to 2 distinct magenta nucleoli. Although few epithelial cells had undergone keratinization individually, cords and nests of cells often had central, eosinophilic accumulations of compact lamellated keratin (keratin pearls; Fig. 1). Occasionally,

intercellular bridges (desmosomes) were identified between epithelial cells. Mitotic figures averaged 3 per 10 high-power ( $400\times$ ) fields and a moderate degree of anisokaryosis and anisocytosis was noted. Within the ulcerated area and between the neoplastic cells were a moderate number of neutrophils, few lymphocytes, and necrotic cell debris. These findings remained consistent with a squamous cell carcinoma. Of note, esophageal glands were present within the tunica muscularis, which is an unusual finding and may be particular to the seal esophagus (Fig. 1). No evidence of lymphatic or vascular invasion by the neoplastic cells, no metastatic foci in other organs, and no other significant pathologic findings were noted.

Culture results from the esophageal tumor obtained at necropsy revealed moderate growth of *Morganella morganii*. Although not confirmed histologically, it was suspected that infection of the mass itself, along with microperforations of the esophagus and the subsequent mild leakage of esophageal contents into the body cavity had led to the leukocytosis noted in this case. The grossly observed ulceration cranial to the tumor was likely associated with regurgitation of fish and repeated mechanical trauma from fish spines. The observed regurgitation of fish with bodies intact but the heads digested suggests that the fish bodies may have been lodged between the mass and the caudal esophageal wall, only allowing digestion of the head portion.

## Case 2

A 28-yr-old, 70-kg intact female wild-born Atlantic harbor seal (*Phoca vitulina concolor*) was evaluated for an 11-wk history of intermittent discomfort, regurgitation, decreased training compliance, increased social anxiety, and stiffness and reaction to manipulation of pedal flippers. The seal was housed in an 185,000-gallon outdoor synthetic seawater seal pool facility with one other Atlantic harbor seal and two grey seals (*Halichoerus grypus*). The previous medical history of this animal included intermittent episodes of presumptive pox viral lesions on the rostrum and flanks, intermittent blepharospasm associated with changes in water quality, and a fractured maxillary incisor tooth.

On initial veterinary inspection, the seal was bright, alert, responsive, and interactive. Abdominal palpation performed at the time revealed no negative or painful reactions. Due to the reported nonspecific gastrointestinal signs, the seal was started on famotidine (Wockhardt USA, LLC.,

Parsippany, New Jersey 07054, USA; 50 mg p.o. s.i.d.) but displayed only mild improvement in the frequency of regurgitation.

The seal continued regurgitating intermittently for the next 10–14 days, but the abdomen appeared of appropriate contour, and no pain was noted on palpation. Most likely differentials included a gastrointestinal foreign body or ulceration, but a partial gastrointestinal obstruction or more diffuse ulceration were also considered. As diagnostics were limited without sedation and the seal had become noncompliant with venipuncture during operant conditioning, symptomatic treatment efforts were continued for the following week without improvement.

When the seal exhibited continued stiffness and reaction to manipulation of the pedal flippers, it was hypothesized to be consistent with a musculoskeletal presentation (e.g., osteoarthritis) rather than abdominal pathology because it was thought that an infectious etiology or severe gastrointestinal ulceration would have progressed differently by this third week of observation. Further, the seal's geriatric signalment and moderate obesity were risk factors for development of musculoskeletal disease. For these reasons, a nonsteroidal anti-inflammatory medication, carprofen (Pfizer Animal Health; 400 mg p.o. s.i.d. for 7 days), was incorporated into the treatment regimen. Gastroprotectants were continued, as previously prescribed, to minimize adverse effects of the nonsteroidal anti-inflammatory medication.

The following day, the seal displayed marked improvement in appetite, attitude, energy level, and training compliance. This rapid improvement in approximation to the analgesic measures was considered supportive of a causal link, so carprofen and gastroprotectants were continued for a total of 3 wk. During this period, the seal appeared bright, alert, responsive, and with good energy. Toward the end of this 3-wk period, the seal began intermittently vomiting after entering the pool and had periods of increasing lethargy and discomfort. At this point carprofen was discontinued, although gastroprotectants were maintained.

It was suspected that the type and size of fish offered was influencing this seal's regurgitation patterns and clinical signs. For this reason, only small capelin was offered for the next 4 days. Despite the change in the size of fish offered, the seal again became depressed, unresponsive to training, and displayed a decreased appetite and continued intermittent regurgitation. Because the seal was refusing medicated fish, flunixin was

administered intramuscularly (flunixin meglumine, Bimeda MTC Animal Health, Inc., Cambridge, Ontario, Canada; 90 mg i.m. once) pending additional diagnostics 2 days later. The seal was administered diazepam (Hospira, Inc.; 25 mg p.o.) to facilitate manual restraint for blood collection. The hemogram was consistent with published species normal ranges.<sup>10</sup> Biochemistry results showed mild elevations in blood urea nitrogen (47 mg/dl; reference range, 31–46 mg/dl) and creatinine (2.4 mg/dl; reference range, 1.0–1.8 mg/dl), which was consistent with anorexia and dehydration, as well as hyperproteinemia (8.7 g/dl; reference range, 7.0–7.8 g/dl) with hyperglobulinemia (5.9 g/dl; reference range, 3.1–3.9 g/dl).<sup>10</sup> All other ancillary testing, including *Dirofilaria immitis* antigen and leptospirosis microscopic agglutination test (MAT) were negative, and serum zinc concentrations were within normal limits. Radiographs performed at this time revealed no evidence of gastric or duodenal metallic foreign body and no obvious skeletal lesions that would be consistent with osteoarthritis. Ultrasonographic findings showed normal hepatic and renal size, and echogenicity and the gastric lumen and gastrointestinal tract appeared appropriately oriented within the abdominal cavity. No radiographic or ultrasonographic explanation could be found for the seal's clinical signs.

Flunixin meglumine (90 mg i.m. once) was repeated, and vitamin E (Neogen Vet Corporation, Lexington, Kentucky 40511, USA; 400 IU i.m. once) was administered. During the procedure, 0.9% sodium chloride (Abbott Labs; 1 L s.c.) was administered to enhance the seal's hydration. Due to the seal's advanced age, the slow progression of disease and presented hyperglobulinemia, neoplasia was suspected. Intramuscular injections of flunixin meglumine (75 mg i.m. s.i.d.) were continued for an additional 7 days, with little response from the seal. It was concluded that deterioration of condition warranted full immobilization to exhaust pre-exploratory diagnostic armament.

For this procedure, the seal was premedicated with midazolam (Hospira, Inc.; 14 mg i.m.) and underwent anesthetic induction using isoflurane in oxygen via facemask and followed by endotracheal intubation. The physical examination performed at this time remained within normal limits. Radiographs showed bilaterally increased pulmonary opacity, marked shifting of the gastrointestinal tract gas pattern to the left cranial abdominal quadrant, and uniform soft tissue opacity of the right cranial abdomen. These

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findings were suggestive of a possible mass effect in the right cranial abdominal quadrant.

Gastroscopy performed at this time demonstrated marked esophagitis just cranial to the cardia and lower esophageal sphincter. In this area, the esophagus was irregular with red plaques, inflammation, and excessive mucus. Moderate resistance was encountered upon entry into the stomach. Examination of the stomach did not reveal any foreign object or ulcerations, and the mucosa appeared healthy.

A contrast gastrointestinal series was performed over the next hour with barium sulfate (Humco, Texarkana, Texas 75501, USA; 90 mg p.o. once) mixed with sucralfate (Axcan Pharma US, Inc., Birmingham, Alabama 35242, USA; 1 g p.o. once) gavigated into the stomach. A series of radiographs were taken, and no filling defects were detected in the stomach or cranial small intestines. The esophagus could not be evaluated during this study, as the barium had been gavigated directly into the stomach.

Upon completion of the barium study, the seal was administered 0.9% saline (6 L s.c. once), vitamin E (400 IU i.m. once), famotidine (60 mg i.m.), enrofloxacin (Bayer Healthcare LLC., Shawnee Mission, Kansas 66201, USA; 350 mg i.m.), and vitamin B complex (Sparhawk Laboratories, Inc., Lenexa, Kansas 66215, USA; 200 mg s.c. once). The abnormal radiographic findings suggested that exploratory surgery would be warranted as neoplasia was considered the most likely diagnosis in this geriatric seal.

Although it was planned to begin empiric therapy to treat the esophagitis the following day, the seal remained anorexic, and all active prescriptions were cancelled. It was determined prudent to move forward promptly with exploratory surgery due to the progressive nature of the seal's presentation. The seal was anesthetized, as previously described. Presurgical radiographs were consistent with previous films, and the midline ventral abdominal exploratory was largely as would be expected for a normal seal. Incisional biopsies were performed along the edges of liver and spleen and submitted for culture and histopathology, as no other findings were noted to account for the radiographic presentation. As abdominal wall closure was initiated, the seal suddenly went into cardiac arrest. Resuscitation was attempted, and although these efforts returned the seal's heart to normal sinus rhythm, the pupils were fixed and dilated, and the seal maintained agonal respiratory patterns. The seal was supported in this state for 1 hr

before recovery was concluded unsuccessful, and euthanasia was performed (Euthasol, Virbac Corporation; 2,340 mg i.v.).

On gross necropsy, gastroesophageal masses and fibrosis were noted with severe esophagitis in the unexplored area cranial to the diaphragm. In addition, the seal had pancreatic and splenic adhesions, marked pancreatic lymphadenopathy and multifocal abscesses, right nephrolithiasis, lumbar spondylosis, prescapular lymphadenomegaly, and aortic arteriosclerosis. The primary microscopic diagnosis and primary contributor to mortality on necropsy was determined to be esophageal squamous cell carcinoma with multiple ulcerations, regional invasion and adhesions, and multifocal vascular invasion. Histopathologic findings of the esophageal squamous cell carcinoma were consistent with those previously described in Case 1.

### Case 3

An estimated 35-yr-old, 57-kg intact female wild-born Atlantic harbor seal was evaluated for an 18-day history of intermittent discomfort, regurgitation, decreased training compliance, increased social anxiety, stiffness, and reaction to manipulation of pedal flippers. The seal was housed in the same collection as Case 2 and presented 18 mo after euthanasia of the first animal. The previous medical history of this animal included a cataract in the right eye and associated intermittent blepharospasm.

On initial veterinary inspection, the seal was bright, alert, responsive, and active but with poor appetite. Two days later, the seal was displaying an abnormal curled posture and was intermittently listless. When the seal was on the deck, an obvious dorsal arching of the thorax was noted, but in the water, this posture was not as apparent. During training sessions, the seal was moving slowly and spitting out fish. Although in the past, these behaviors had been observed seasonally, the prior case had increased an index of suspicion, and progressed diagnostic sedation more quickly than the first case.

The following day the seal was anesthetized following premedication with midazolam (12 mg i.m.) and anesthetic induction with isoflurane in oxygen via facemask followed by endotracheal tube. The hemogram and chemistry panel were largely within normal limits, except minor GGT (19 IU/L; reference range, 2–12 IU/L) and total bilirubin (0.7 mg/dl; reference range, 0.2–0.6 mg/dl) elevations and a mild elevation in creatinine (1.9 mg/dl; in-house reference range, 1.0–1.8 mg/

- 24 dl) likely associated with anorexia.<sup>10</sup> All other ancillary testing, including *Dirofilaria immitis* antigen and leptospirosis MAT were negative, and serum zinc concentrations were within normal species limits.

Radiographs performed at this time were unremarkable besides an old displaced dorsal spinous process fracture of T2. No abnormal findings were obvious on abdominal ultrasound examination. Gastroscopy revealed normal esophageal mucosa until the cardiac level and entry to gastric lumen, where slight ulceration was present, as well as nodules and fibrotic stippling of the mucosa. The gastric mucosa and structure was within normal limits and without foreign body or ulceration. Multiple pinch biopsies of abnormal esophageal mucosa were taken. Cytologic preparations of the esophageal biopsies presented multiple sheets of cells with criteria of malignancy, suggestive of an adenocarcinoma, which supported the clinical impression at gastroscopy. However, histopathology of biopsy samples revealed chronic neutrophilic esophagitis, and definitive confirmation of malignant neoplasia was not possible.

Prior to recovering the seal from anesthesia, it was treated with 0.9% saline (2 L s.c.), vitamin E (400 IU i.m.), meloxicam (Norbrook Laboratories, Ltd., Newry, County Down, Northern Ireland; 11.4 mg s.c.), famotidine (28.5 mg i.m.), enrofloxacin (282 mg s.c.), and sucralfate (2 g p.o. via gavage tube). With the location of the neoplastic changes, surgical resection was considered not possible, and gradual wasting from anorexia was expected with marked poor quality of life.

Pending histopathology, the seal was initiated on a course of once daily meloxicam (5.5 mg i.m.), enrofloxacin (285 mg i.m.), and famotidine (28.5 mg i.m.). It was decided that if no return to appetite or progressive clinical signs occurred during the treatment period, then euthanasia was indicated, as marked weight loss would have been reached during this time. Although the seal initially showed improvement on this treatment regime, after 10 days, the seal began exhibiting a snorting behavior not associated with feeding and a very wide mouth gape behavior during feedings. This latter behavior was suspected to be related to swallowing, as the seal's head was extended caudally with full neck extension concurrent with the open mouth.

As the abnormal behavior and anorexia continued for an additional 5 days, the seal was anesthetized, as previously described for this

animal. Plain radiographic and ultrasonographic results were consistent with this seal's previous exam. Barium was introduced (54 mg p.o.) into the esophagus for better evaluation of the mucosal surface. Radiographs of the thorax confirmed the presence of a circular mass in the left cranial mediastinum (2.5 × 1.5 cm) that was extramural to the esophagus. The differential diagnoses at that time included intramural esophageal mass, mediastinal or lung neoplasia, or lymphadenopathy. Contrast radiographs also highlighted esophageal mural changes at the cardia with stricture identified. The contrast media was very slow to move from the esophagus to the stomach. Gastroscopy revealed abnormal esophageal mucosa approximately 5–10 cm cranial to the previously diagnosed lesions with grey and stippled appearance. Continued and progressive ulceration and general nodularity and fibrotic webbing of the mucosa were observed at the cardia with apparent adhesions.

Given that surgical resection of the affected areas were not possible, even if a definitive diagnosis was concluded, and progression of the lesions at the cardia had occurred despite daily treatment over the past several days, a grave to guarded prognosis was given with reduction in quality of life. Due to the progressive nature of disease and its nonresectable location for diagnosis or resolution, euthanasia was performed with pentobarbital (Euthasol, Virbac Corporation; 8,800 mg i.v.).

Gross examination during necropsy revealed regional distortion at the gastroesophageal junction, and the esophageal mucosa was multifocally effaced by an infiltrative, poorly demarcated neoplastic mass. Gross necropsy also revealed severe multifocal lymphadenomegaly, vaginal and endometrial cysts, and mitral valvular endocardiosis. The diagnosis of the esophageal mass was confirmed via histopathology as a squamous cell carcinoma.

#### Case 4

A 29-yr-old, 59-kg intact female wild-born Atlantic harbor seal was found dead in its pool after a brief period of inappetance and regurgitation of undigested fish. The seal had initially been housed for 25 yr at an aquarium on the East coast and had produced eight offspring prior to initiating treatment with medroxyprogesterone acetate (Depo Provera®, Pharmacia & Upjohn Company, Kalamazoo, Michigan 49001, USA; 200 mg i.m. monthly for three summer months each year for 2 yr). It subsequently was transferred to another

facility and housed in two natural seawater pools (75,000 and 90,000 gallons, respectively) with three Atlantic harbor seals and two Pacific harbor seals for the next 4.5 yr until death.

In addition to reproductive season DepoProvera treatment, the previous medical history of this animal included recurrent uveitis and chronic glaucoma due to bilateral cataracts and anteriorly luxated lenses. Despite intermittent treatment during flare-ups with buffered aspirin (CVS Pharmacy Inc., Woonsocket, Rhode Island 02895, USA; 10 mg p.o. b.i.d.), this problem progressed to retinal detachment and blindness in the right eye.

This seal participated in multiple research protocols involving periodic blood sampling and intermittent butorphanol tartrate sedation (Dolorex®, Merck Animal Health, Summit, New Jersey 07901, USA; 23.6 mg i.m.) to facilitate small blubber biopsies and intravenous catheterization procedures. Periodic routine health assessments recorded complete blood cell counts (CBC), with mild to moderate neutrophilic leukocytosis (12,000 to 14,600 cells/ $\mu$ l; published reference range, 4,800–14,250 cells/ $\mu$ l), which was attributed to the chronic uveitis and serum chemistry values that were similar to published values, with the exception of serum alkaline phosphatase, which was persistently mildly elevated (154–233 U/L; reference range, 11–176 U/L) and frequent mild increases in fibrinogen (400–500 mg/dl; reference range 120–324 mg/dl).<sup>4</sup> Intermittent episodes of lethargy and decreased participation in training sessions were attributed to pain caused by episodes of severe blepharospasm with anterior uveitis and were treated with oral aspirin for several weeks. When the seal exhibited decreased appetite and possible salt water ingestion, observed as open mouth gaping in the pool, the animal was sedated with butorphanol (22 mg i.m.) for endoscopic examination that revealed scattered shallow pinpoint reddened areas of gastric ulceration presumed to be associated with chronic aspirin use. Oral anti-inflammatory therapy was changed to gastric coated aspirin (Ecotrin® Prestige Brands, Tarrytown, New York 10591, USA; 325 mg p.o. b.i.d.) plus ranitidine (75 mg p.o. b.i.d.), resulting in improved activity and training compliance. The gastric-coated aspirin was then continued twice daily to control chronic ocular discomfort.

Approximately 1 yr later, regurgitation of food, vomiting, and decreased appetite recurred. The seal was sedated again with butorphanol (22 mg i.m.), and endoscopic examination of the esoph-

agus and stomach at that time revealed several discrete small foci of pale gastric mucosa and an irregular, linear shallow erosion near the duodenal sphincter, and pinch biopsies were collected. Histopathologic examination of gastric mucosal biopsies was unremarkable. Aspirin was discontinued; sucralfate (1 g p.o. b.i.d.) was initiated, ranitidine was continued twice daily, and the seal was moved to a back holding pool for observation. Topical anti-inflammatory ophthalmic solution (flurbiprofen 0.05%, Pacific Pharmaceuticals, Irvine, California 92612, USA) was started for ocular analgesia in lieu of aspirin.

The seal's appetite remained fair to good but activity was decreased, training behaviors were marginal, and regurgitation and difficulty swallowing again were noted approximately 4 wk later. Metoclopramide (Watson Pharma Private Ltd., Verna, Salcette Goa 403722 India; 10 mg p.o. b.i.d.) was administered in addition to the ranitidine and sucralfate, and food was cut into smaller pieces prior to feeding. No further difficulties were noted until a free feed was offered 2 wk later. The seal regurgitated several fish after rapidly eating most of the food, then proceeded to reingest all the regurgitated fish from the pool. The animal voluntarily positioned for examination by a consulting veterinary ophthalmologist a few days later, and no new ocular abnormalities were noted. Despite normal eating patterns that evening, the seal was found dead the following morning with multiple large blood clots in the water. Postmortem radiographs of entire body revealed increased density of both lungs and moderate gas throughout the intestinal tract.

Gross necropsy findings included a large firm mass in the submucosal layer of the mid-thoracic esophagus that had ruptured into the esophageal lumen with massive acute intraluminal hemorrhage. Death was due to hypotensive shock and cardiovascular collapse complicated by septicemia and pulmonary congestion, edema, and emphysema. Additional necropsy findings included an abscessed pancreatic lymph node, liver capsular fibrosis, adrenocortical and ovarian atrophy, right atrioventricular valve fibrosis, right-sided epicardial fibrosis, chronic buphthalmos, superficial keratitis, and retinal detachment in the right eye as well as bilateral hypermature cataracts with anterior lens luxation.

Histopathologic findings included squamous cell carcinoma of the esophagus, suppurative mediastinitis, mild neutrophilic and lymphocytic cholangiohepatitis, reactive lymphoid hyperplasia, a focal chronic ulcer on the tongue, and

marked atrophy of fat. Esophageal samples were evaluated by polymerase chain reaction for porcine herpes virus, and immunohistochemistry for bovine papilloma virus was negative. The peripancratic mass represented a lymph node with metastatic foci of squamous cell carcinoma, and the thyroid gland had marked variability in follicular luminal diameter, hypertrophy of follicular cells, reduced colloid content, and in some areas, complete absence of follicular lumen with no dysplastic changes. Thyroid findings were reported to be most consistent with diffuse hyperplastic goiter. Secondary bacterial infection associated with the primary neoplasm had resulted in suppurative mediastinitis and low-grade septicemia. The suboptimal nutritional status at time of death was attributed to the neoplastic process, and the ulcer in the tongue most likely due to stress or trauma from chewing or regurgitation. No infectious agents were noted in the tongue ulcer, and no viral inclusions were noted in the examined sections.

#### Case 5

A 27-yr-old, 84-kg intact male Pacific harbor seal died under anesthesia due to cardiopulmonary arrest during endoscopic examination to remove gastric foreign objects. This animal presented with a 6-wk history of intermittent regurgitation and reconsumption of regurgitated apparently digested fish. The seal was housed in a 139,000-gallon outdoor freshwater seal pool facility with two adult female California sea lions (*Zalophus californianus*) and one male and one female Pacific harbor seal. The previous medical history for this seal included intermittent treatment over 3 yr for bilateral hypermature cataracts and glaucoma OS with topical administration of topical dorzolamide (dorzolamide HCl/timolol maleate ophthalmic solution, Bausch & Lomb Inc., Tampa, Florida 33637, USA; b.i.d.) to the left eye, and treatment of apparent pinniped keratitis with doxycycline (West-Ward Pharmaceutical Corp., Eatontown, New Jersey 07724, USA; 400 mg p.o. b.i.d.) and topical triple antibiotic ophthalmic drops (Falcon Pharmaceuticals, Fort Worth, Texas 76134, USA; t.i.d.) as needed. Four years prior to death, this seal had gastric endoscopy and gastrotomy performed to remove 17 large landscaping rocks from the gastric lumen following a brief episode of inappetence and apparent abdominal pain.

Radiographs performed under behavioral control 5 wk prior to endoscopy identified the presence of five radiopaque gastric foreign objects

that appeared to be rocks. Although intermittent vomiting continued, the animal continued to appear behaviorally and clinically normal between episodes, and abdominal pain was not identified or elicited with palpation. Radiographs were repeated 2 wk prior to endoscopy, confirming that the rocks remained within the gastric lumen. As decreased appetite and weight loss were noted, anesthesia for gastric endoscopy was performed. The seal was sedated with midazolam HCL (ZooPharm, Inc., Windsor, Colorado 80550, USA; 21 mg i.m.). When the seal did not become adequately sedated after this initial dose, a second dose of midazolam (10 mg i.m.) was administered 25 min later. Although the seal did not become sedate enough to allow for mask induction, he was trained to allow for chamber induction with inhalant anesthetics. Isoflurane (Phoenix Pharmaceuticals, St. Joseph, Missouri 64507, USA) in oxygen was administered initially at 5%, then reduced to 2% until the animal was sedate enough to allow for intubation.

Endoscopically, five small rocks and three short sticks were identified within the gastric lumen. An irregularity was noted at the gastroesophageal junction during endoscopy, but a biopsy was not performed at that time. An ulcerated mass was also noted on the ventral surface of the tongue, and a punch biopsy of this site was obtained. Three rocks and two sticks were removed via endoscopy prior to the animal developing cardiopulmonary arrest and eventual death under anesthesia.

Necropsy revealed a mass located at the distal esophagus that extended approximately 2 cm from the mucosa into the lumen. The mass demonstrated a friable center surrounded by proliferative tissue. An enlarged lymph node was located on the serosal surface of the stomach slightly aboral to the gastroesophageal junction. An ulcerated glossal lesion was noted adjacent to the recent biopsy site.

Histopathology showed a poorly demarcated and unencapsulated esophageal mass that infiltrated the mucosa and submucosa. This mass was confirmed as a squamous cell carcinoma with metastasis to a regional lymph node. The lesion observed on the ventral tongue was an epithelial ulceration of unknown cause with chronic inflammation. The esophageal tumor was likely the cause of the regurgitation described in this case; however, it was not suspected to be immediately involved in the death of the animal. It is likely that this animal died due cardiac arrest as a complication of anesthesia.

### Case 6

A 32-yr-old, 90.3-kg intact male Atlantic harbor seal (*Phoca vitulina concolor*) initially presented with a 2-day history of diarrhea, vomiting, and changed behavior of floating in a corner in a hunched posture with eyes closed. The seal was housed in a 77,000-gallon outdoor, freshwater exhibit with two other Atlantic harbor seals. The previous medical history of this seal included bilateral mature cataracts, anterior lens luxation OD, and gingival hyperplasia caudal to maxillary incisors.

At initial presentation, the seal had a reduced appetite and was not participating well in training sessions, so a physical examination under behavioral control was not possible at that time. Due to this seal's primary clinical signs of gastrointestinal origin, empirical therapy was started with sucralfate (Teva Pharmaceuticals USA; 1 g p.o. s.i.d.) and metronidazole (Teva Pharmaceuticals USA; 500 mg p.o. b.i.d.) for 5 days. Differential diagnoses in consideration were gastroenteritis, neurologic disease, renal disease, or hepatic neoplasia. On day 5, appetite had improved to ~75% of diet, but partially digested fish parts were noted at the bottom of the pool, so treatment was extended for an additional 5 days. By that time, appetite appeared to have returned to normal, with no further reports of regurgitation or vomiting.

As the seal's behavioral compliance was improved at that time, physical examination and blood sampling were performed under behavioral control. Physical examination was unremarkable; hematology and serum chemistry panel were within normal limits. Serologically, *Dirofilaria* antigen and Eastern equine encephalitis antibody detection were negative, and postvaccine West Nile virus titer via serum neutralization was 80.

Four weeks later, partially digested fish fragments were again noted at the bottom of the pool, and the seal's appetite was decreased. Metronidazole was reinitiated, but no response was noted. Feeds were broadcast in an attempt to administer sucralfate and amoxicillin (Dava Pharmaceuticals, Inc., Fort Lee, New Jersey 07024, USA; 1,000 mg p.o. b.i.d.) in addition to metronidazole for 14 days. Within 10 days, the seal's appetite was improving, and behavioral compliance during training sessions had improved; however, the seal began tearing herring in half before consumption. At approximately 8 wk from initial presentation, partially digested fish were again noted intermittently in the pool. Because of the chronic nature of the vomiting, sucralfate was continued, and

omeprazole (Apotex Corp., Weston, Florida 33326, USA; 10 mg p.o. s.i.d.) was initiated.

Within 3 days of omeprazole initiation, the seal's appetite began to decline until it refused all herring and only consumed halved capelin. Due to this marked progression of clinical signs, the seal was anesthetized for advanced diagnostics. The seal was premedicated with midazolam (Hospira, Inc.; 10 mg, i.m.), then induced with propofol (Abbott Laboratories; 300 mg i.v.), followed by intubation and maintenance with isoflurane (Abbott Laboratories) in oxygen. Thoracic and abdominal radiographs were unremarkable. Abdominal ultrasound was nondiagnostic. Full-body CT scan revealed increased soft tissue density in right nasopharynx and a subjectively small and somewhat nodular liver. Gastroscopy revealed a large esophageal ulcer in the cranial one-third of the esophagus, but the stomach and duodenum did not contain any gross evidence of ulceration, mass effect, or other inflammatory processes. No parasites were seen. Three gastric and three duodenal pinch endoscopic biopsies were obtained for histopathology. The endoscope was retroflexed to evaluate the nasopharyngeal soft tissue density noted on advanced imaging. A nasopharyngeal swelling was noted that appeared inflammatory in nature and was presumed to be secondary to previous episodes of regurgitation. Perioperative enrofloxacin (Bayer HealthCare LLC; 690 mg in 0.9% saline s.c.) and meloxicam (Norbrook Laboratories Ltd.; 6.9 mg s.c.) were administered prior to obtaining surgical laparoscopic liver biopsies. During laparoscopic examination, the liver appeared fibrotic, and it was difficult to obtain representative biopsy samples. Once the hepatic biopsy procedure was completed, the seal was recovered from anesthesia. Postsurgical recovery was uneventful. Postsurgical treatments included enrofloxacin, sucralfate, omeprazole, tramadol (Amneal Pharmaceuticals, Glasgow, Kentucky 42141; 50 mg p.o. b.i.d.), and metoclopramide (Teva Pharmaceuticals USA; 15 mg p.o. s.i.d.).

Hematologic and blood chemistry values were within normal limits. Histopathology revealed hepatic cholestasis with centrilobular necrosis and moderate portal fibrosis as well as biliary hyperplasia and moderate lymphoplasmacytic duodenal enteritis. Copper staining was negative for copper in the liver. Aerobic, anaerobic, and fungal cultures of liver tissue were negative.

Based on histopathology results, S-adenosylmethionine (Nutramax Laboratories, Inc., 946 Quality Drive, Lancaster, South Carolina 29720,

USA; 425 mg p.o. b.i.d.), ursadiol (Epic Pharma, LLC, Laurelton, New York 11413, USA; 600 mg, p.o., s.i.d.), prednisolone (Wedgewood Pharmacy, 405 Heron Drive, Swedesboro, New Jersey 08085, USA; 35 mg p.o. b.i.d.), and tramadol 50–75 mg p.o. b.i.d. were initiated. Although appetite and demeanor initially improved for 4 wk, appetite and comfort waned after that time despite continued treatment. Approximately 12 wk into this regimen, it was determined that quality of life had deteriorated significantly, and euthanasia was performed (Euthansia-III, Med-Pharmex, Inc.; 5,460 mg i.v.).

Gross necropsy revealed thin body condition, as well as the previously described ocular pathology. The previously noted proximal esophageal ulcer seen on gastroscopy was resolved. At the level of the esophageal sphincter, the tissue appeared grossly thickened. Within the esophagus, fragments of partially digested fish and mucus were noted just cranial to the lower esophageal sphincter. The mucosal wall at the level of the sphincter contained a raised pink nodule measuring 1 × 1.5 cm. The mucosal tissue cranial to the raised lesion was friable and contained yellow-tan exudate when penetrated. On cross section, the sphincter wall was grossly thickened and irregular around approximately 80% of the esophagus circumferentially and was 5 cm thick at its widest point. Histologically, the wall of the esophagus was effaced by a squamous cell carcinoma. The tumor was comprised of anastomosing cords and nests of neoplastic epithelial cells that differentiated synchronously and asynchronously from peripheral basal-type cells to central squamous cells, sometimes oriented around accumulations of necrotic cell debris or squamous pearls. The neoplastic cells had moderate anisokaryosis, prominent nucleoli, and frequent mitoses. The tumor was invoking a scirrhous response, was ulcerated, and variably inflamed.

## DISCUSSION

Seals, as in any mammal that have clinically important esophageal disorders, generally manifest with dysphagia and regurgitation, especially evident after feeding.<sup>2</sup> Phocids tend to consume their fish head first, and once ingested, the gastric acid will digest the body quicker than the head. Therefore, under normal circumstances a seal's vomitus of ingested fish would have the head intact, while the body is partially digested. The seal in case one exhibited the opposite, likely because of the partial esophageal obstruction in

the aboral esophagus, allowing only partial passage of the fish, head first, into the stomach. The seals described in Cases 2–5 also exhibited dysphagia and regurgitation, usually associated with the immediate postprandial period, whereas the seal in Case 6 exhibited only intermittent vomiting and decreased appetite.

Identification of an esophageal disorder is based on characteristic clinical signs of regurgitation, and dysphagia and may be confirmed with plain and contrast radiographs, endoscopic examination, transesophageal ultrasound, and advanced imaging; however, CBC and clinical chemistry are useful to assess systemic complications associated with esophageal disease.<sup>21</sup> Symptomatic treatment includes maintenance of energy and fluid requirements, motility modifiers, antibiotics to address secondary infections, and reduction of gastric acid secretion, but ultimately, provides no specific resolution.<sup>21</sup>

Some reported neoplasms in pinnipeds include carcinomas, leiomyomas, granulosa cell tumors, and lymphosarcomas.<sup>1,3,5,6,8,11–13,14,18,19,22</sup> One case of esophageal squamous cell carcinoma leading to thoracic metastasis was diagnosed postmortem in a California sea lion, and this animal displayed similar clinical signs to the harbor seals presented here (Fravel, pers. comm., July 2013). Although gastrointestinal neoplasms appear rare in phocids, one report of a gastrointestinal adenocarcinoma in a ringed seal (*Phoca hispida*) has been made.<sup>17</sup> Although squamous cell carcinomas have been described and documented throughout *Pinnipedia* in tissues, such as skin, uterus, gingiva, lung, pharynx, and vagina,<sup>18</sup> these six cases expand the literature with squamous cell carcinomas originating from the esophagus.

Reports in the literature of marine mammal neoplasia as secondary to infectious or toxic agents have been made.<sup>7,9,15,16,18,20,22</sup> However, as in these six cases, the etiology of most marine mammal tumors remains unknown.<sup>7</sup> Further investigation into potential etiologies for this condition in harbor seals is warranted. Clinical evaluation of ill phocids displaying gastrointestinal signs, including hematology, radiographs with or without contrast, and gastrointestinal endoscopy, should be pursued to screen for the primary cause of disease. On the basis of compatible clinical findings, which can be confirmed at postmortem exam, esophageal squamous cell carcinoma should be considered as a differential diagnosis in adult seals, exhibiting clinical signs of regurgitation or chronic vomiting, decreased appetite or anorexia, and abnormal posturing or

apparent gastrointestinal discomfort. As represented by this case series, the prevalence of squamous cell carcinoma of the esophagus in phocids may be more common than previously described.

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#### LITERATURE CITED

1. Acevedo-Whitehouse KA, Constantino-Casas F, Auriolles-Gamboa D, Rodriguez-Martinez HA, Godinez-Reyes CR. Hepatic carcinoma with spleen metastasis in a California sea lion from the Gulf of California. *J Wildl Dis.* 1999;35(3):565–568.
2. Aiello SE, Mays A. Esophagus. In: Merck veterinary manual, 8th ed. Whitehouse Station (NJ): Merck Publishing; 1998. p. 128.
3. Backlin BM, Eriksson L, Olovsson M. Histology of uterine leiomyoma and occurrence in relation to reproductive activity in the Baltic gray seal (*Halichoerus grypus*). *Vet Pathol.* 2003;40:175–180.
4. Bossart GD, Reidarson TH, Dierauf LA, Duffield DA. Clinical pathology. In: Dierauf LA, Gulland FMD (eds.). CRC handbook of marine mammal medicine. 2nd ed. Boca Raton (FL): CRC Press; 2001. p. 394–395.
5. Brown RJ, Smith AW, Morejohn GV, DeLong RL. Metastatic adenocarcinoma in two California sea lions, *Zalophus californianus*. *J Wildl Dis.* 1980;16(2):261–266.
6. Fauquier D, Gulland FMD, Haulena M, Spraker T. Biliary adenocarcinoma in a stranded northern elephant seal (*Mirounga angustirostris*). *J Wildl Dis.* 2003;39(3):723–726.
7. Gulland FMD, Lowenstine LJ, Spraker TR. Neoplasia. In: Dierauf LA, Gulland FMD (eds.). CRC handbook of marine mammal medicine, 2nd ed. Boca Raton (FL): CRC Press; 2001. p. 522–528.
8. Gulland FMD, Trupkiewicz JG, Spraker TR, Lowenstine LJ. Metastatic carcinoma of probable transitional cell origin in 66 free-living California sea lions (*Zalophus californianus*), 1970 to 1994. *J Wildl Dis.* 1996;32(2):250–258.
9. Howard EG, Britt FO, Jr., Simpson JG. Neoplasms in marine mammals. In: Howard EG (ed.). Pathobiology of marine mammal disease, Volume 2. Boca Raton (FL): CRC Press; 1983. p. 95–162.
10. International Species Inventory System. Physiological data reference. Values for *Phoca vitulina*. [CD-ROM] Apple Valley (MN): International Species Information System.
11. Joseph BE, Cornell LH. Metastatic squamous cell carcinoma in a beached California sea lion (*Zalophus californianus*). *J Wildl Dis.* 1986;22(2):281–283.
12. Lipscomb TP, Scott DP, Garber RL, Krafft AE, Tsai MM, Lichy JH, Taubenberger JK, F.Y. Schulman, Gulland FMD. Common metastatic carcinoma of California sea lions (*Zalophus californianus*): evidence of genital origin and association with novel gamma herpesvirus. *Vet Pathol.* 2000;37:609–617.
13. Matsuda M, Hashinura S, Une Y, Sirouzu H, Nomura Y. Two distinct carcinomas of mammary gland origin in a California sea lion. *J Wildl Dis.* 2003;39(1):241–243.
14. Mawdesley-Thomas LE. An ovarian tumor in a Southern elephant seal. *Vet Pathol.* 1971;8:9–15.
15. Mawdesley-Thomas LE. 1974. Some aspects of neoplasia in marine mammals. In: Russell FS, Yonge B (eds.). Advances in marine biology, Volume 12. New York (NY): Academic Press; 1974. p. 151–231.
16. Mawdesley-Thomas LE, Bonner WN. Uterine tumors in a gray seal (*Halichoerus grypus*). *J Pathol.* 103; 205–208.
17. Mikaelian I, Leclair D, Inukpuk J. Adenocarcinoma of the small intestine in a ringed seal from Hudson Bay. *J Wildl Dis.* 2001;37:379–382.
18. Newman SJ, Smith SA. Marine mammal neoplasia: a review. *Vet Pathol.* 2006;43:865–880.
19. Sato S, Kitamura H, Masaaki M, Fukazawa M, Takeda M, Kadota K. Adenocarcinoma of the lung in a Steller sea lion (*Eumetopias jubatus*). *J Vet Med Sci.* 1998;60(12):1349–1351.
20. Sato T, Higuchi T, Shibuya H. Lingual squamous cell carcinoma in a California sea lion (*Zalophus californianus*). *J Zoo Wildl Med.* 2002;33:367–370.
21. Spillmann T. Esophageal diseases diagnostic and therapeutic approaches. In: Proc. World Small Anim Vet Assoc Ann Congress; 2007. p. 19–23.
22. St. Leger J. Pathology of marine mammals. In: Proc. C. L. Davis Found Diagn Pathol Dis Aer Terr Aquat Wildl; 2008. p. 14.

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