NOTE *Wildlife Science*

Gastric carcinoma in a South American sea lion (*Otaria flavescens*)

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ABSTRACT. A 22-year-old captive male South American sea lion (*Otaria flavescens*) developed an undifferentiated carcinoma originating in the cardiac region of the stomach. Clinical symptoms included vomiting, anorexia and weight loss. Ultrasonography and endoscopy showed gastric wall thickness. At necropsy, the gastric wall had significant thickening around the cardiac region, and metastases were found in some organs. Histologically, samples from the stomach wall and metastases showed the same tumor tissue. Immunohistochemistry was positive for epithelium markers. Ductal growth, keratinocytes or signet ring cells were absent. The tumor was classified as an undifferentiated carcinoma using the World Health Organization's (WHO) guide to international classification of tumors in domestic animals. This is the first report of a primary gastric carcinoma in a pinniped.

KEY WORDS: carcinoma, endoscopy, sea lion, stomach, ultrasonography

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Several tumors in pinnipeds have been reported, and almost all of the reports to date have been on Californian sea lions [11]. An ovarian interstitial cell tumor and a hemangiosarcoma are the two sole reports of tumor in the South American sea lion [2, 19]. In domestic animals, gastric carcinomas are very rare compared with their occurrence in humans. However, in the veterinary setting, gastric carcinoma commonly occurs in dogs [7]. The only reports to date about tumor in the alimentary system of pinnipeds include one of a squamous cell carcinoma of the tongue [15] and a metastatic tumor of the stomach [10, 17]. Therefore, it is extremely rare, and this is the first report of a primary gastric undifferentiated carcinoma in a pinniped.

A 22-year-old captive male south American sea lion showed an increase in γ-GTP over four years and also showed signs of physical deterioration, which was treated with ursodeoxycholic acid. The animal had clinical symptoms of vomiting, anorexia and weight loss for three months before its death. In the final month, the animal presented with hematemesis and melena. Ultrasound revealed thickening of the gastric wall and mucosal irregularity around the cardiac region (Fig. 1). Endoscopy was further performed and confirmed thickening and ulcers of the gastric mucosa around the same region. Treatment of the ulcer and administration of steroid medication did not improve symptoms.

At necropsy, the gastric wall from the cardiac region to the corpus showed significant thickening and was found to be extremely hard. The gastric mucosal folds were markedly raised with a swelling like appearance. The swelling extended from the cardiac region for a total length of 17 cm. There were also many ulcers of varying sizes (Fig. 2). The thickness of the gastric wall was 4.3 cm in the cardiac region and 3 cm at the fundus. There are no data of normal value of thickness in each part of the stomach in this species, but the thickness of the wall in the pyloric region that looks as usual was 1.5 cm. The serosa of the gastric wall thickening had multiple small white nodes, especially around the cardiac region. Part of the diaphragm serosa had adhered to the serosa of the gastric wall, and there were also multiple small white nodes. At the lesser curvature of the stomach, a tumor (7.5 × 2.3 × 2 cm) was found, which was likely to be a gastric lymph node and it was stuck to the thoracic aorta. The inside of the tumor showed white, solid tissue, and the center contained necrotic tissue. Additionally, there were multiple white nodes, 0.1 cm to 1 cm in diameter, in the parenchyma of the diaphragm, liver, spleen and lung, and some conjunct white nodes were observed along the hilum of the spleen. The distal portion of the esophageal wall was also thickened.

All organs were fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut into 4 μm thick sections and stained with hematoxylin and eosin (HE). Some tumor sections were also stained with periodic acid-Schiff (PAS), Alcian Blue and Watanabe's method for the reticulum. Immunohistochemistry (IHC) was applied to the paraffin wax sections. The samples were incubated with primary antibodies to cytokeratin AE1/AE3 (Dako, Carpintera, CA, U.S.A.), cytokeratin CAM5.2 (Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.) and carcinoembryonic antigen (Dako). Peroxidase-conjugated secondary antibody (Histofine, NICHIREI, Tokyo, Japan) was then used and visualized using diaminobenzidine.

Histologically, the gastric wall was also markedly thickened by tumor cell growth, and infiltration was present from immediately beneath the gastric mucosal epithelium to the submucosal layer of the tissue (Fig.3). Tumor tissue replaced

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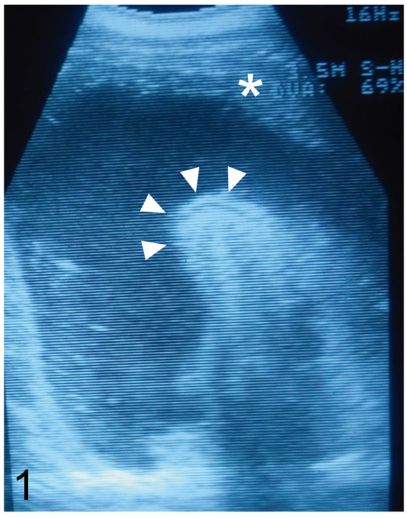
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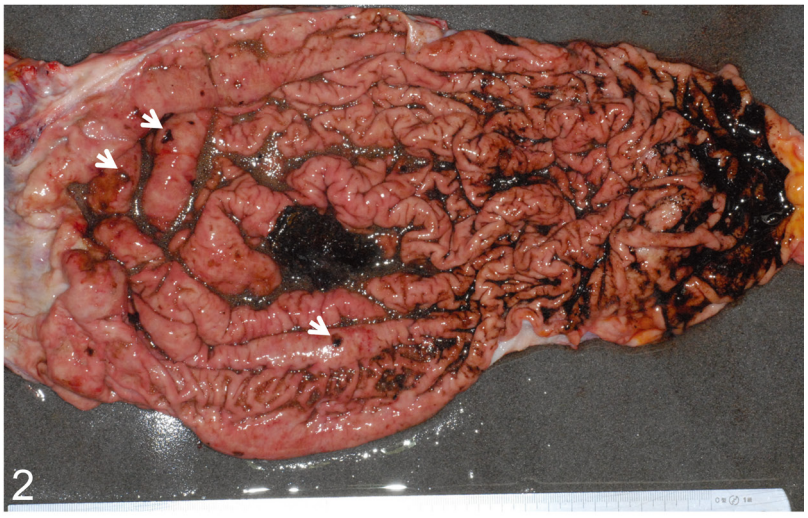
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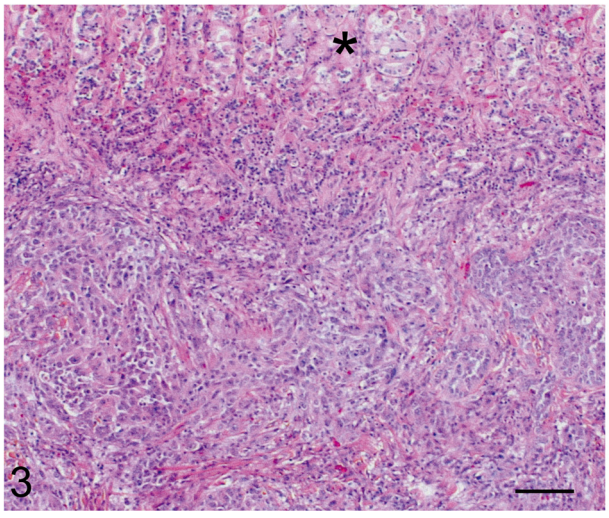
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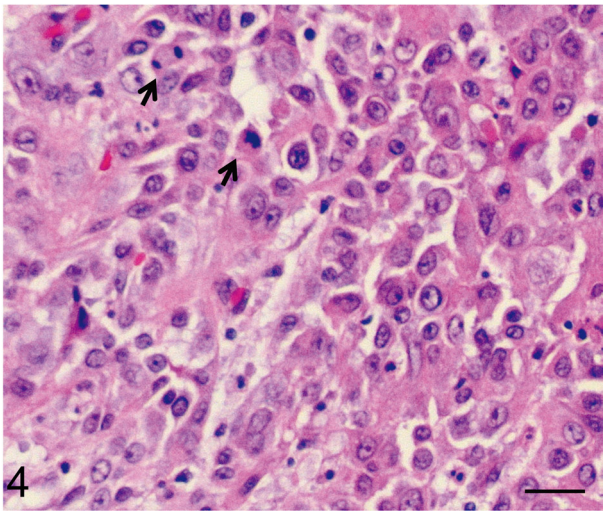
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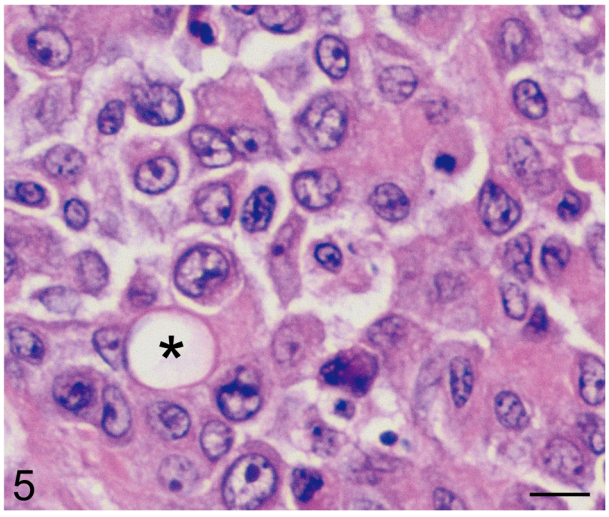
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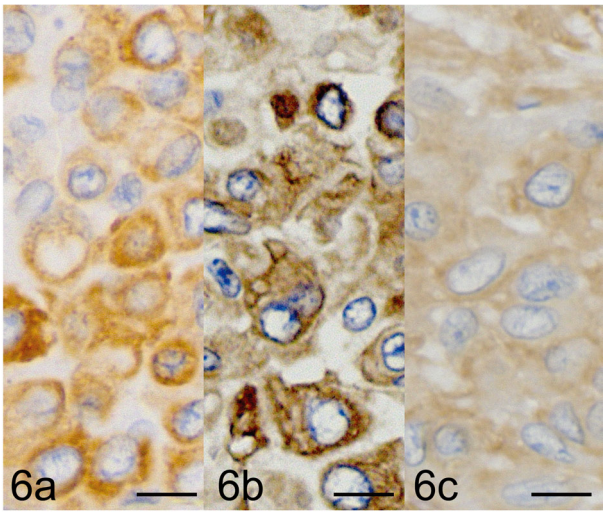
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the normal gastric tissue in the lesion, and it spread to the muscular layer and serosa. Some tumor cells were present within the vasculature as well as lymphatic vessels. The tumor formed polycystic lesions, and there was solid or cribriform growth with hyperplasia of the fibroblast cells. The core of the polycystic lesions was necrotic. The tumor cells were ovoid or round in shape and had an eosinophilic cytoplasm. The cells were highly cellular and had an atypical nucleus and nucleus-cytoplasmic ratio. Many mitotic figures were detected in the tumor cells (Fig. 4). Some tumor cells formed a single layer that imitated tube-like structures, however, the basement membrane was not visible and the mucosa in these was often positive for Alcian Blue (Fig. 5). The tumor tissue was positive for cytokeratin AE1/3, CAM5.2 and CEA (Fig. 6), and tissue of the gastric mucosal epithelium was also positive for cytokeratin AE1/3 and CAM5.2. Similar histological results were observed in the metastatic foci in the thicker part of the esophagus, the gastric and splenic lymph nodes, diaphragm, liver, spleen and lung. In this case, the tumor tissue occupied almost a half of the stomach and had spread to all layers except the mucosal epithelium of the gastric tissue. Apart from the gastric and surrounding regional lymph nodes, the size of all nodes were small and less than 1 cm. The results of IHC in the tumor tissue removed from the sea lion were similar to that seen in a canine or human gastric carcinoma [5, 8, 18]. Because of these reasons, we diagnosed this case as a primary gastric carcinoma. Additionally, in the tumor that was removed, solid growth of immature cells was significant, and the tissue did not show complete ductal growth, keratinocytes or the presence of signet ring cells. Therefore, the tumor was classified as an undifferentiated carcinoma using the WHO international histological classification of tumor in domestic animals [7]. Although gastric carcinomas often occur in the pyloric region in canines and humans [3, 12], in the current case, the cardiac region was the most affected and this suggests the possibility that this tumor originated there. Gastric carcinoma is well documented in domestic animals, particularly that of canines [6, 7]. It is characterized by hyperplasia of fibroblast cells and wide lateral invasive growth [4, 12, 16] as was observed in this case. Canine gastric carcinoma often consists primarily of poorly differentiated signet ring cells when examined histologically, however, in this case, there were no signet ring cells observed, indicating a different tissue type to that seen in the canine cases [7]. In this case, we observed metastasis in the esophagus, gastric and splenic lymph nodes, diaphragm, liver, spleen and lung. This pattern of metastasis is similar to that seen in cases of canine gastric carcinoma [4, 8, 14]. According to the histological growth pattern, it is likely that the tumor metastasized to the esophagus invasively and then spread to the liver, spleen, lung and the diaphragm through the circulation. The sea lion in this case exhibited typical clinical symptoms of gastric dysfunction including vomiting, anorexia and weight loss [1, 8, 9, 13], however, these symptoms are not specific for canine gastric tumors [13]. A biopsy of the stomach tissue was not performed, and therefore, we were unable to reach a diagnosis of gastric carcinoma before the animal died. The gastric carcinoma in this case was severely advanced at the necropsy, and the advanced tumor might have been associated with the observed clinical symptoms of cachexia, vomiting and anorexia for a period of more than three months.

In the final stages, the animal developed serious pulmonary edema and myocardial degeneration, which lead to respiratory and cardiac insufficiency and subsequently death. Tumors of the alimentary system have rarely been reported in the pinniped. This report of carcinoma in a South American sea lion has demonstrated similarities to that observed in canine and human cases of gastric carcinoma. While we were unable to definitively diagnose this case as gastric carcinoma prior to the death of the animal, ultrasonography and endoscopy have proven to be useful tools for assessing the alimentary tract and monitoring and improving the health of wildlife.

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Fig. 1. Cross-sectional ultrasound image of the gastric cardia. It shows thickening of the gastric wall (arrowheads) and mucosal irregularity (asterisk) of the gastric cardia.

Fig. 2. Gross appearance of the gastric mucosa. The gastric mucosa folds remarkably and is raised showing stromas from the gastric cardia to the corpus; many ulcers in varying sizes are visible (arrows). The thickness of the gastric wall was 4.3 cm in the cardiac region and 3 cm at the fundus.

Fig. 3. Appearance of the tumor tissue from the gastric mucosa at low magnification. The lower tissues layer of the tumor shows the presence of polycystic lesions with hyperplasia of fibroblasts and solid or cribriform growth. The top layer is the normal gastric mucosa (asterisk). Hematoxylin and eosin staining; bar=100 μm

Fig. 4. Appearance of the tumor tissue from the gastric mucosa at high magnification. The tumor shows the presence of polycystic lesions with hyperplasia of fibroblasts. Mitosis was frequently observed in 18 cells/10 high-power fields (arrows). Hematoxylin and eosin staining; bar=20 μm

Fig. 5. Appearance of the tumor cells at high magnification. The tumor cells are ovoid or round, with rich eosinophilic cytoplasm. The cells show high cellular and nuclear atypia with a high nuclear-cytoplasmic ratio. Some tumor cells formed a single layer similar to tube-like structures (asterisk). Hematoxylin and eosin staining; bar=10 μm

Fig. 6. Immunohistochemistry results of the tumor tissue. All tumor tissues showed positivity for the epithelium markers, CK AE1/AE3 (a), CAM5.2 (b) and CEA (c); bars=10 μm

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