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CASE REPORT

Metastatic exocrine pancreatic adenocarcinoma in a giant otter (*Pteronura brasiliensis*)

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ABSTRACT

Neoplasms of the exocrine pancreas are uncommon in domestic animals and rarely occur in wildlife. This article describes the clinical and pathological findings of one case of metastatic exocrine pancreatic adenocarcinoma in an 18-year-old giant otter (*Pteronura brasiliensis*) in captivity with a history of inappetence and apathy. Abdominal ultrasonography was inconclusive, and tomography revealed a neoplasm affecting the urinary bladder and hydroureter. During the anaesthesia recovery, the animal presented a cardiorespiratory arrest and died. Grossly, there were neoplastic nodules in the pancreas, urinary bladder, spleen, adrenal glands, and mediastinal lymph node. Microscopically, all nodules were composed of a malignant hypercellular proliferation of epithelial cells with acinar or solid disposition, supported by a sparse fibrovascular stroma. Neoplastic cells were immunolabeled with antibodies to Pan-CK, CK7, CK20, PPP and chromogranin A. Approximately 25% of the cells were positive for the presence of Ki-67 too. Pathological and immunohistochemical findings confirmed the diagnosis of metastatic exocrine pancreatic adenocarcinoma.

KEYWORDS

giant otter, immunohistochemistry, pancreatic adenocarcinoma, oncology, wildlife disease

The giant otter (*Pteronura brasiliensis*) is a carnivorous wild mammal widely distributed in South America. It belongs to the family Mustelidae within the order Carnivora. It lives in groups of three to nine individuals, and the adult females reach up to 1.8 m and 22–26 kg (Carter and Rosa, 1997; Cabral et al., 2010). In the past, reports on neoplasms in animals of the Mustelidae family were rare: a study of 125 necropsies in mustelids over 30 years yielded no case of neoplasm (Ratcliffe, 1933). However, in the last decades, more neoplastic cases have been documented in these animals, including hepatocellular adenoma (Bee et al., 2007), lymphoma (Bartlett et al., 2010), melanoma (Weber and Mecklenburg, 2000), trichoblastoma and mammary adenoma (Fuke et al., 2021) in Eurasian otter (*Lutra lutra*). Pancreatic islet cell (Andrews et al., 1997) and testicular tumors (Batista-Arteaga et al., 2011) were diagnosed in ferrets (*Mustela putorius furo*). Additionally, the occurrence of lymphoma (Kim et al., 2002), sarcoma (Burek-Huntington et al., 2012), seminoma (Reiner and Lipscomb, 1998) and

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cholangiocellular adenocarcinoma, leiomyoma and phaeochromocytoma (Stetzer et al., 1981) have been reported in sea otters (*Enhydra lutris*). However, spontaneous neoplasms have not been reported in *P. brasiliensis*. This article describes the anatomopathological and immunohistochemical findings of a rare case of metastatic exocrine pancreatic adenocarcinoma in a giant otter. To the authors' best knowledge, this is the first case of exocrine pancreatic neoplasm in this species.

An 18-year-old female giant otter at the Zoological Garden Foundation of Brasília, Brazil, developed a 2-day history of inappetence, prostration, compromised locomotion, and apathy. Anaesthesia was performed with midazolam (1 mg kg^{-1}) and ketamine (10 mg kg^{-1}), and the maintenance with propofol (0.5 mg kg^{-1} min) and isoflurane 0.5%. Abdominal ultrasound examination revealed nonspecific findings, such as mild ascites. Computer tomography (CT) revealed hydroureter and numerous round nodules affecting the urinary bladder and gall bladder. During the recovery from anaesthesia, the animal had a cardiorespiratory arrest and died despite attempts at emergency cardiopulmonary resuscitation maneuvers.

On gross examination, the conjunctival and oral mucosa were moderately pale. There was a 7.0 × 6.5 × 1.2 cm sized, whitish, lobulated, firm mass infiltrating and replacing the pancreas, adjacent mesentery and omentum (Fig. 1A). The gallbladder was distended and almost obstructed by a lobulated, irregular, smooth, brown mass, 3.5 cm in diameter, which occupied approximately 90% of the lumen of the gallbladder. There were also numerous millimetric, firm, white nodules in the edges of the spleen. Moderate swelling of the adrenal glands and some mediastinal lymph nodes was also noted. In the mucosa of the urinary bladder, numerous coalescent, white, firm, non-ulcerated nodules ranging from 0.5 to 2.0 cm in diameter were seen (Fig. 1B). In addition, bilateral hydroureter, hydronephrosis, and renal cysts were found. There were no changes in the skin, cardiovascular, respiratory, gastrointestinal, genital, neurological and skeletal organs. Samples of affected tissues and other routine samples were collected, fixed in 10% neutral buffered formalin, routinely processed for histopathology, and stained with haematoxylin and eosin (HE).

Histologically, all the affected organs (pancreas, spleen, urinary bladder, mediastinal lymph node, and adrenal glands) were composed of non-encapsulated, highly cellular neoplastic proliferations constituted by epithelial cells arranged in acini, tubules, or solid sheets that were sustained by a scarce fibrovascular stroma (Fig. 1C and 1D). The neoplastic cells were cubic to polygonal with few distinct cytoplasmic borders and had mild basophilic cytoplasm and medium to large, hypochromatic, round nuclei with one or two prominent nucleoli. Anisocytosis and anisokaryosis were moderate, and there were seven mitoses seen in ten high power fields (2.4 mm^2). There was a papillary adenoma in the gallbladder.

Selected tissue sections from the tumor were tested by immunohistochemistry (IHC) for the detection of various tissue and tumor markers including chromogranin A (CgA), pancreatic polypeptide (PPP), pan-cytokeratin (pan-CK AE1/AE3), CK7, CK20, Ki-67, gastrin, glucagon, insulin, somatostatin and synaptophysin (Munday et al., 2017). The antibody panel, used in the immunohistochemical studies, is presented in Table 1.

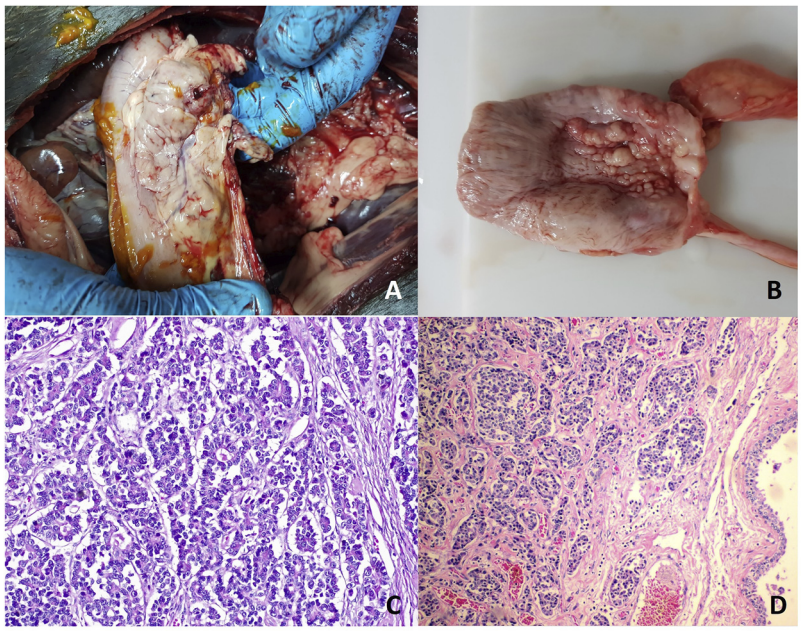

Fig. 1. Macroscopic and microscopic findings in the case of metastatic exocrine pancreatic adenocarcinoma in giant otter (*Pteronura brasiliensis*). A. Whitish, lobulated, firm mass infiltrating and replacing the pancreas. B. Numerous solitary or coalescent, firm, white nodules of various sizes in the mucosa of the urinary bladder. C. Neoplastic proliferation replacing the pancreas. The mass is constituted of epithelial cells arranged in acini, tubules or solid sheets that are sustained by a scarce fibrovascular stroma. HE, ×200. D. The lamina propria and muscular layer of the urinary bladder, infiltrated by numerous metastatic epithelial neoplastic proliferations. HE, ×100

Table 1. Panel of the antibodies used in the current study

Antibody affinity	Clone	Manufacturer	Dilution
Ki-67	MIB-1	Dako Omnis, Santa Clara, USA	1:50
Insulin	K36aC10	DBS, Pleasanton, USA	1:1000
Glucagon	Polyclonal	Cell Marque, Rocklin, USA	1:50
Somatostatin	Polyclonal	Dako Omnis, Santa Clara, USA	1:5000
Gastrin	Polyclonal	DBS, Pleasanton, USA	1:50
Synaptophysin	DAK-SYNAP	Dako Omnis, Santa Clara, USA	RTU
Chromogranin A (CgA)	Polyclonal	Abcam. Cambridge, UK	1:300
Pancreatic polypeptide (PPP)	Polyclonal	Monosan, Uden, The Netherlands	1:1000
Pan-CK	AE1/AE3	Dako Omnis, Santa Clara, USA	RTU
CK7	OV-TL12/30	Cell Marque, Rocklin, USA	1:25
CK20	Ks20.8	Zeta Corporation, Arcadia, USA	1:50

*RTU = ready to use.

Four-micron-thick sample sections from the primary tumorous mass were prepared on gelatin-coated slides for IHC. The Ki-67 antibody was submitted to the streptavidin biotin-peroxidase complex method with commercial detection anti-mouse/anti-rabbit system (Novolink Polymer Detection System TM; Leica Biosystems, Newcastle, United Kingdom). For the other antibodies the Envision FLEX HRP Magenta Substrate Chromogen System (High pH, Dako Omnis - Agilent Technologies, Santa Clara, United States) was used according to the manufacturer's instructions. The slides were incubated with the primary antibodies, as listed in Table 1, at 4 °C overnight. The reagents were applied manually and the immuno-reactivity was visualized by incubating the slides with diaminobenzidine chromogen (DAB Substrate System; Dako/Envision FLEX) (Nakagaki et al., 2022). An insulinoma and normal pancreas from a dog served as positive controls; whereas the negative control consisted of substituting primary antibodies with normal serum (Lab Vision Ultra V Block; Fisher Scientific, Loughborough, UK).

The neoplastic cells had strong IHC labelling with antibodies to Pan-CK (Fig. 2A), CgA (Fig. 2B), CK7 (Fig. 2C), CK20 (Fig. 2D) and PPP (not shown). Approximately 25% of the cells were positive for Ki-67. There was no reaction with the remaining antibodies such as gastrin, glucagon, insulin, somatostatin and synaptophysin. Based on the morphological and immunohistochemical findings, we diagnosed the lesion as a metastatic exocrine pancreatic adenocarcinoma.

The giant otter is the largest semiaquatic carnivore in South America. In addition, it is considered one of the most endangered mustelids in the world (IUCN, 2022). These animals live approximately twenty years in captivity, as in the current case. In dogs, pancreatic adenocarcinoma shows no sex or breed predisposition. The neoplasm usually occurs in older animals, as detected in the giant otter of this study. Neoplasms have rarely been described in *P. brasiliensis*, and this case is the first exocrine pancreatic adenocarcinoma with multiple metastases reported in this species. There are some descriptions of metastatic adenocarcinomas affecting the mammary gland, prepuce, sebaceous gland, adrenal gland, intestine, stomach, and paranasal sinus in other animals of this genus (Miwa et al., 2009).

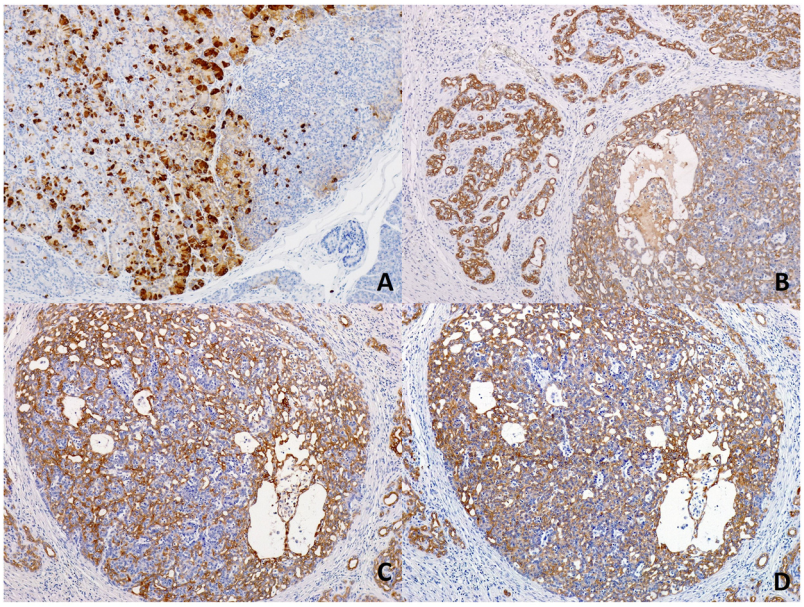

Fig. 2. Immunohistochemical findings in tissue slides prepared from the primary tumor originating from the pancreas. Intensive positive reaction was seen with antibodies to Pan-CK (A); chromogranin A (B); CK7 (C) and CK20 (D). Magnification: ×100

The clinical signs of the case reported here were associated with neoplastic dissemination and infiltration, including multiple metastases to many organs. Ultrasonography and mainly CT are essential and valuable tools to evaluate and localize intra-abdominal masses during the physical examination, as performed in the present case. Metastatic pancreatic adenocarcinoma was considered a relevant diagnosis in this giant otter because it was associated with the clinical signs and contributed with the death of the animal. This neoplasm is uncommonly diagnosed in dogs and cats and rarely in other domestic or wild animals, such as cattle, horses, sheep, and pigs (Church et al., 1987; Kelley et al., 1996; Munday et al., 2017).

The pathological findings observed in the current case are similar to those described for pancreatic adenocarcinomas in other domestic animals, mainly dogs (Munday et al., 2017). Immunohistochemical evaluation using a wide panel of primary antibodies was essential to define the primary site of neoplastic proliferation. Based on positive immunolabelling to CgA, PPP, Pan-CK, CK7 and CK20, we established a diagnosis of adenocarcinoma of the exocrine pancreas with metastases to the spleen, lymph node, urinary bladder and adrenal glands. Neoplastic epithelial cells were confirmed by positive immunoreaction to Pan-CK, CK7 and CK20, whereas the exocrine pancreatic origin was established by the positive immunolabelling with the CgA- and PPP-specific antibodies. Identical IHC pattern was detected in the metastatic tumors. A diffuse reaction to CgA observed in the neoplastic cells was associated with neuroendocrine differentiation of the neoplasm; this feature has been noted in some exocrine pancreatic adenocarcinomas in humans (Gullo et al., 1992; Pour et al., 1993). In addition, the negative reactions with antibodies to insulin, glucagon, somatostatin and gastrin excluded the diagnosis of endocrine or neuroendocrine carcinoma or gastrinoma.

Neoplasms have not been described in giant otters, thus our case is an important contribution to the knowledge of diseases in this species. Future epizootiological and pathological studies are needed to determine the prevalence and the aetiology of this type of neoplasms in giant otters.

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