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Case Report

Colonic papillary adenocarcinoma in a crab-eating fox (*Cerdocyon thous*): Anatomopathological and immunohistochemical features

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Abstract

A captive 17-year-old male crab-eating fox (*Cerdocyon thous*) presenting clinical signs of hyporexia and constipation for two weeks was found dead. On autopsy, a large and firm mass expanding the large intestine wall and totally obstructing the colon lumen was identified. Histopathology revealed a non-encapsulated poorly demarcated adenocarcinoma composed of cuboidal to pseudostratified columnar epithelium forming papillary projections of variable sizes. The cytoplasm of the neoplastic cells was immunopositive for CK7 and PAN-CK, but negative for VIM and CK20. Immunohistochemistry revealed an immunophenotype different from the enteric adenocarcinomas reported in humans and domestic dogs. This is the first case report of colonic papillary adenocarcinoma in a crab-eating fox.

Key words: Wildlife, wild canids, fox, digestive system, tumor, immunohistochemistry, histopathology.

Introduction

The crab-eating fox (*Cerdocyon thous*) is a medium-sized canid found in all biomes of Brazil and other South American countries (38). Three out of the five existing subspecies of *C. thous* are found in Brazil: *C. t. entrerianus* (South and Southeast), *C. t. azarae* (Southeast, Midwest, North and Northeast) and *C. t. thous* (North and Northeast) (4). In these species, malignant insulinoma in a *C. thous* (28), is the only neoplasm reported so far.

Intestinal adenocarcinomas are malignant epithelial tumors that originate from the mucosa of the gastrointestinal tract and are considered uncommon in most animal species (25). In dogs, most of these tumors are found in the large intestine (1, 13, 16, 25), mainly in the colon and the rectum (1), and although infrequent, enteric adenocarcinomas have already been diagnosed in other species of domestic animals such as cats, horses, cattle, sheep, goats and swine (10).

In wild animals, cases of colonic adenocarcinomas are often described in non-human primates (9, 12, 20, 27, 33), being occasionally documented in other species, such as snakes (8, 26) and African hedgehog (*Atelerix albiventris*) (21). In wild canids, intestinal adenocarcinomas are extremely rare and only one case affecting the small intestine of a swift fox (*Vulpes velox*) has been reported (10). This report describes the anatomopathological and immunohistochemical features of a colonic papillary adenocarcinoma in a captive crab-eating fox.

Case description

A 17-year-old male crab-eating fox from the Dois Irmãos State Zoo Park, Recife, Northeastern Brazil, was presented with a 15 days history of anorexia, weight

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loss, emaciation, tenesmus and abdominal distention. The fox had no previous history of enteric disease. Ultrasound examination revealed a hyperechoic abdominal mass but no evidence of gastrointestinal obstruction. The fox underwent intensive therapy that included parental administration of fluid therapy with 0.9% crystalloid solution, B vitamins and parenteral nutrition consisting of 30 kcal of carbohydrates, 6 kcal of amino acids and 9 kcal of lipids. In addition, for gastric protection, 1mg/kg ranitidine was administered subcutaneously every 12 hours. After these procedures, the animal started to spontaneously ingest small portions of food, but due to low energy intake, it was submitted to an enteral diet by esophageal tube, however, no significant evolution of its clinical condition was observed, being found dead in the enclosure five days after placement of the esophageal tube.

Grossly, a 3.0 cm x 2.7 cm x 1.8 cm, brownish-gray, non-ulcerated nodular firm mass was observed on the descending colon. On the cut surface, it had a brown-gray polypoid appearance and was markedly infiltrating the mucosa and obstructing the colon lumen (Fig. 1A). The intestinal segments, particularly on the stenotic and obstructed area, with the oral segment of the colon adjacent to the tumor were severely distended. In these areas the mucosa was mildly congested, edematous and the serosal vessels were congested. In addition, moderate peritonitis characterized by yellow fibrillary material (fibrin) covering the spleen and segments of the small and large intestine loops was observed. The lungs were edematous and had multifocal areas of consolidation and interlobar emphysema. No other tumor growth was identified in any of the remaining organs.

Samples of the mass, small and large intestine, liver, spleen, kidneys, heart and lungs were fixed in 10% neutral buffered formalin (PBS 0.01M and pH 7.2), routinely processed in paraffin wax and stained with haematoxylin and eosin (HE). Immunohistochemical labeling was carried out using monoclonal antibodies against pan-cytokeratin (PAN-CK), cytokeratin 7 (CK7), cytokeratin 20 (CK20) and vimentin (VIM). Briefly, the sections were subjected to endogenous peroxidase blockage with 0.03% hydrogen peroxide. The polymer EnVision® System Labeled Polymer (Dako) was used as a secondary antibody. The reaction was developed with 0.3 mg/mL of 3,3'-diaminobenzidine (DAB) and counterstained with Harris' hematoxylin. Additional information is summarized in table 1.

Microscopic examination of the colon demonstrated a non-encapsulated and poorly demarcated neoplasm in the mucosa layer, infiltrating and extending to the submucosa, muscularis and serosa (transmural infiltration). The neoplasm was composed of well differentiated cuboidal to columnar epithelial cells originating from the intestinal glands. Occasionally, a pattern of epithelial pseudostratification was noted. Neoplastic cells were organized in a papillary pattern, containing projections of varying sizes, supported by a well-vascularized connective tissue. Areas of glandular fusion, which also had elongated crypt glands with secondary branching, was noted (Fig. 1B and 1C). Neoplastic cells exhibited moderate to abundant eosinophilic cytoplasm with nuclei varying in size and sometimes in shapes (anisokaryosis), with finely stippled to condensed chromatin. Nucleoli were evident, ranging from one to three. Anisocytosis was mild. Mitosis figures were low, ranging from zero to two mitoses in ten 40x fields (mean 0.5). Papillary projections were ectasic, sometimes presenting mucinous eosinophilic material, cellular debris, and epithelial desquamation in its lumen. In areas close to the serosa, the neoplasm formed lobes that occasionally contained mucous secretion positive for Periodic acid–Schiff (PAS) (Fig. 1D).

The cytoplasm of neoplastic cells labelled strongly for intermediate epithelial cell filaments (PAN-CK, clone AE1/AE3) (Fig. 1E) and for ductal and glandular epithelium markers (CK7, clone OV-TL 12/30) (Fig. 1F) but was immunonegative for CK 20 and VIM.

Approximately 15% of pulmonary alveoli and bronchioles were filled with macrophages, edema and fibrin, and there was moderate edema of the interlobular septa and multifocal areas of emphysema.

Table 1. Antibodies used for diagnosis of colonic papillary adenocarcinoma in a crab-eating fox:

Monoclonal antibodies	Target tissue	Manufacturer	Dilution	Clone	Antigen retrieval protocol
Pan-CK	Epithelial cells	Dako	1:100	AE1/AE3	Sodium citrate solution (pH 6)
CK7	Ductal and glandular epithelium	Dako	1:25	OV-TL 12/30	Trypsin 2%
CK20	Intestinal and gastric epithelium	Dako	1:25	Ks20.8	Trypsin 2%
Vimentin	Mesenchymal cells	Dako	1:200	V9	Sodium citrate solution (pH 6)

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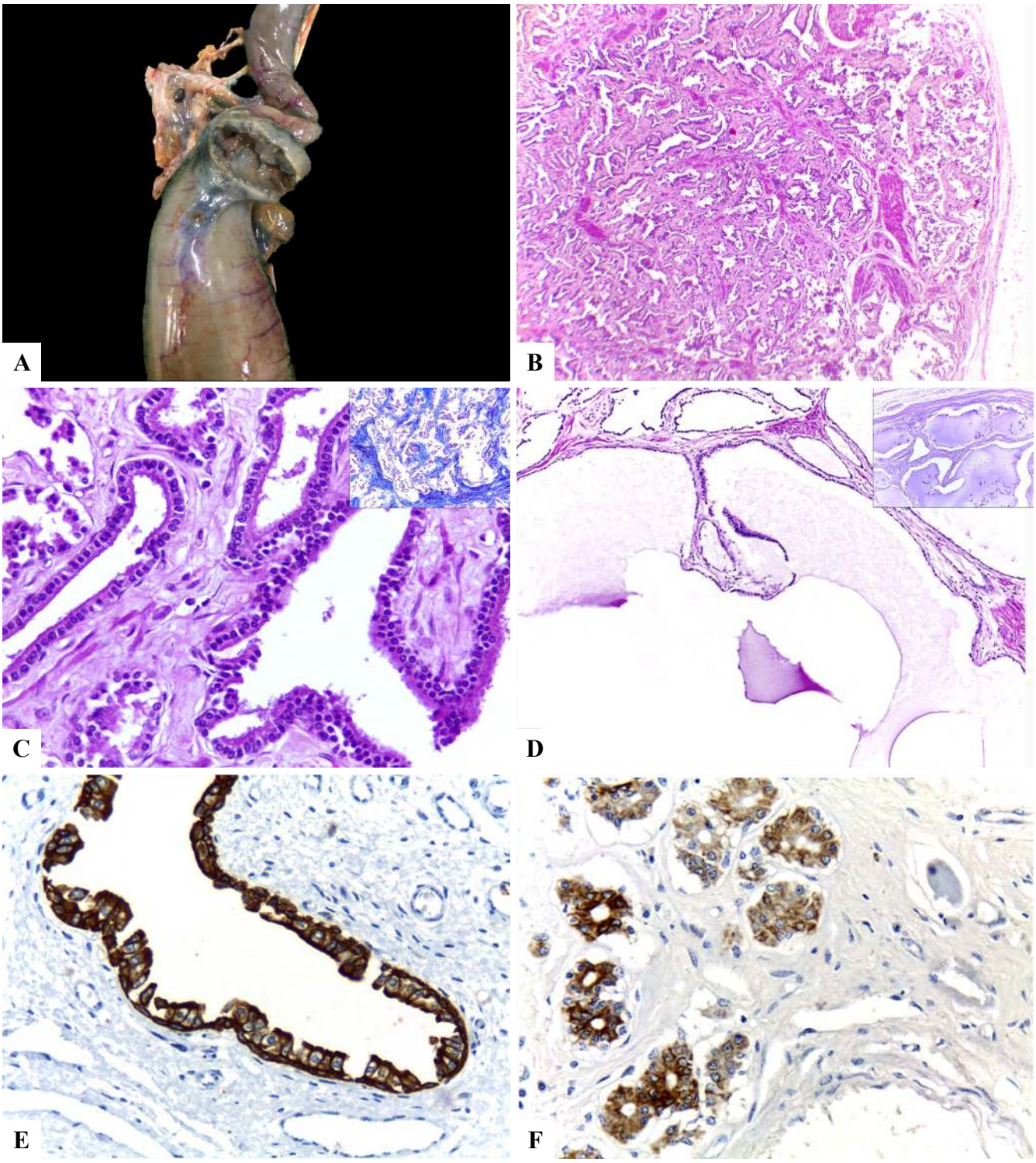

Figure 1. Papillary adenocarcinoma in the colon of a crab-eating fox (*Cerdocyon thous*). A. Neoplasm characterized by a firm mass with an irregular surface, with brownish to grayish areas and well adhered to the colon mucosa. B. Malignant, infiltrative epithelial neoplasia, forming papillary projections and supported by fibrous connective tissue (HE, 4x). C. Papillary projections are formed by epithelium ranging from simple to pseudostratified. Neoplastic cells exhibit eosinophilic cytoplasm with basophilic nuclei varying in size, with condensed to stippled chromatin and evidence of nucleoli. Anisocytosis and anisokaryosis are discrete (HE, 40x). Note the fibrovascular stroma evidenced in Masson's trichrome (MT, 10x) (inset). D. Occasionally, inside the papillary projections there was eosinophilic and amorphous material suggestive of mucin (HE, 20x), positive for Periodic acid–Schiff (PAS, 10x) (inset). E. Intense immunolabeling for pan-cytokeratin in neoplastic cells of the epithelium of the papillary projections (IHC). F. Intense immunolabeling for CK 7 in neoplastic cells of the intestinal gland epithelium (IHC).

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Discussion

The neoplasm described in this fox is similar to intestinal papillary adenocarcinomas of dogs and was characterized by wide projections with multiple enfolding papillary structures lined by tall columnar cells with elongated vesicular nuclei and prominent nucleoli and a well-developed fibrovascular stroma which was evidenced by Masson's trichome staining. However, in dogs mitotic figures are frequent, and many goblet cells lining the crypt glands, and villi can be observed (10). This pattern of mitosis was not observed, and goblet cells were rare in the present tumor.

Immunohistochemical results revealed positive cytoplasmic immunostaining for CK 7+ and negative for CK20- (CK7+/CK20-), which is the first immunohistochemical profile of a papillary adenocarcinoma in *C. thous*. These results differ from cases of colon and colorectal adenocarcinoma in humans, where 82% exhibit a CK 7-/CK20+ immunohistochemical profile, 10% are CK 7-/CK20, 8% are CK 7+/CK20+ (17), and only 1% to 4% are CK 7+/CK20- (2, 3, 22, 32). Regarding PAN-CK, papillary adenocarcinomas of the large intestine are immunopositive for this antibody and negative for VIM, as in the present study, which reinforces their epithelial origin (30, 37).

Neoplasms have been reported in several genera of foxes worldwide. *Vulpes* is the genus with more reports of neoplasms, including an adenosquamous carcinoma in the nasal and oral cavity (18), a carcinoma of C cells of the thyroid (23), an insulin-producing islet cell tumor in an ectopic pancreas (15), a mammary gland adenocarcinoma (24), a pinealoma (35) and a hepatocellular carcinoma (29). Reports of neoplasms in other genera include a nephroblastoma in an adult fennec fox (*Fennecus zerda*) (14) and ceruminous gland tumors in Santa Catalina Island foxes (*Urocyon littoralis catalinae*), which has been associated with ear mite infestations with intense inflammatory response (39).

Most reports of neoplasms in Brazilian wild canids have been described in the maned wolf (*Chrysocyon brachyurus*), including ovarian tumors (dysgerminomas, granulosa cell tumors, and ovarian adenopapillomas) (31), a mammary ductal papilloma in a male (7), a mammary tubulopapillary carcinoma (19), a pancreatic gastrinoma (5) and an urinary bladder teratoma (17). Other neoplasms affecting wild canids in Brazil include an extranodal lymphoma, a cutaneous mast cell tumor in bush dog (*Speothos venaticus*) (6, 34), and a pancreatic adenoma in a hoary fox (*Lycalopex vetulus*) (36).

So far, there is only one peer-reviewed case report of neoplasms in *C. thous*, being an malignant insulinoma in a ten-year-old female that was maintained in a Zoo in Minas Gerais State (28). The only reported case of an enteric adenocarcinoma in a non-domestic canid was identified in the jejunum of a female swift fox from the Sunset Zoo in Manhattan in the state of Kansas, USA (10), however, immunohistochemical data is not available.

To the authors knowledge, this is the first case of a papillary adenocarcinoma in the colon of a *C. thous*. This neoplasm should be considered a differential diagnosis in geriatric crab-eating foxes with clinical signs of weight loss, tenesmus, and abdominal distension. Immunohistochemistry was an important tool for the outcome of the case, which revealed an immunophenotype different from colon and colorectal adenocarcinomas described in humans. In addition, the immunohistochemical profile of enteric adenocarcinomas in domestic dogs, so far, did not show CK7+ and CK20- immunostaining as presented in the current study.

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