

## Cystadenocarcinoma simulating hemangiosarcoma of the salivary gland in dog: case report

[Cistoadenocarcinoma simulando hemangiossarcoma de glândula salivar em cão: relato de caso]

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### ABSTRACT

Cystadenocarcinoma is regarded as a rare adenocarcinoma variant in animals. This work reports the case of an 8-year-old female Poodle dog with salivary gland cystadenocarcinoma with morphological characteristics similar to a hemangiosarcoma. Histopathological analysis showed a tumor mass with cystic formations containing a large amount of red blood cells. In order to distinguish these two entities, periodic acid Schiff (PAS) staining and immunohistochemical analysis were carried out with the use of cytokeratin AE1/AE3 (CK) and CD31-specific antibodies. Neoplastic cells were PAS-negative, CK-positive and CD31-negative confirming their epithelial origin. Based on the findings, the diagnosis of high grade cystadenocarcinoma was established.

Keywords: bitch, salivary gland, CD31, neoplastic cells

### RESUMO

*O cistoadenocarcinoma é considerado uma variante do adenocarcinoma de rara ocorrência em animais. Este trabalho relata um caso de cadela Poodle de oito anos, portadora de cistoadenocarcinoma de glândula salivar com características morfológicas semelhantes a de hemangiossarcoma. Na análise histopatológica observou-se uma massa tumoral com formações císticas contendo grande quantidade de hemácias. Para a diferenciação entre as duas entidades realizou-se coloração pelo ácido periódico de Schiff (PAS) e análise imuno-histoquímica com a utilização de anticorpos para citoqueratina AE1/AE3 (CK) e CD31. As células neoplásicas apresentaram-se PAS negativas, CK positivas e CD31 negativas demonstrando a origem epitelial. Com base nos achados firmou-se o diagnóstico de cistoadenocarcinoma de alto grau.*

*Palavras-chave: cadela, glândula salivar, CD31, células neoplásicas*

### INTRODUCTION

Salivary gland tumors are not frequent in dogs and cats, representing about 0.3% of salivary gland diseases (Spangler and Culbertson, 1991). They usually affect old animals, with no differences between breed or sex. The most affected salivary gland is the parotid (Moulton, 1978), and adenocarcinomas are the histological type most frequently found in dogs (Hammer *et*

*al.*, 2001). Cystadenocarcinoma was reported for the first time as a rare adenocarcinoma variant in animals (Head *et al.*, 2002). This tumor has been widely described in human medicine (Shrestha *et al.*, 1994; Johnston *et al.*, 2006; Aloudah *et al.*, 2008; Gallego *et al.*, 2008) and is macroscopically characterized by the formation of masses with varied colors and slow growth which may present cystic areas with mucous content and discrete necrosis (Johnston *et al.*,

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2006; Gallego *et al.*, 2008). Microscopically, it refers to a neoplasm composed of several cysts, which may present papillary projections and sometimes red blood cells inside the cysts (Johnston *et al.*, 2006; Gallego *et al.*, 2008). Most of these tumors produce a mucoid substance, and histochemical techniques such as Periodic Acid Schiff (PAS) or Mayer's Mucicarmine stain (Pollet *et al.*, 1997) evidence this type of material.

When poorly differentiated, malignant tumors characterized by vessel formation, as hemangiosarcomas and lymphangiosarcomas, require the use of an additional tool to help distinguish them from tumors, which mimic the formation of vascular spaces, such as cystadenocarcinomas. The immunohistochemical technique is extremely useful in the differential diagnosis of these histological types (Shrestha *et al.*, 1994; Aloudah *et al.*, 2008). Cytokeratins, intermediate filaments of normal and tumor epithelial cells, are reported in different histogenetic studies as markers used to evidence epithelial neoplasms and to identify micrometastases (Czerniecki *et al.*, 1999; Los Monteros *et al.*, 1999; Yeh and Mies, 2008). Furthermore, antibodies which identify proteins expressed by endothelial cells are used in order to characterize tumors of vascular origin (Lahat *et al.*, 2010).

This work reports a case of salivary gland cystadenocarcinoma, describing its histomorphological and immunophenotypical characteristics and distinguishing it from a high histological grade hemangiosarcoma.

#### CASE DESCRIPTION

An 8-year-old female poodle dog presented an exophytic tumor growth, round and with a friable consistency, in the maxillary region of the left canine tooth. Surgical extirpation was performed as the treatment of choice. Macroscopic analysis showed that the tumor mass had an irregular surface, measuring about 4 x 2 x 2 cm, with whitish areas of friable consistency and black dots. The cut surface presented a homogeneous solid aspect and white in color.

Fragments collected were fixed in neutral buffered 10% formalin solution and included in

paraffin. Afterwards, 4µm-histological sections were obtained from these fragments, stained with Hematoxylin and Eosin and PAS staining protocols. The mitotic index was performed evaluating 10 peripheral tumor areas with microscope (BX-41) magnification (40x) (Dutra *et al.*, 2008).

Microscopical analysis showed cell proliferation in a cystic arrangement, delimited by cuboidal to flattened cells, arranged in one or two layers, filled with red blood cells and/or eosinophilic amorphous material (Fig. 1A). Cells are characterized by high nuclear pleomorphism, fragmented chromatin and an abundant eosinophilic cytoplasm. Furthermore, a cutaneous ulceration was observed, consisting predominantly of a polymorphonuclear inflammatory infiltrate. Cystic neoformations were PAS negative (Fig. 1D). The high mitotic index found (average of 4 mitoses/field), associated with the observation of intravascular tumor emboli and areas of stromal invasion, characterized a neoplasm with high histological grade (Fig. 1B and C).

A biotin-peroxidase system was used for immunohistochemical procedure with identification of the secondary antibody by the polymer (Advance HRP). Antigen retrieval was performed in water bath at 98°C, with a citrate buffer solution pH 6.0 (Target Retrieval Solution) for 20 minutes. In order to block the endogenous peroxidase activity, slides were incubated with a solution of H<sub>2</sub>O<sub>2</sub> 3% in methyl alcohol. Reagents were applied manually, with 1 hour incubation for the primary antibody and 30 minutes for the other reagents, except for diaminobenzidine chromogen, incubated for 5 minutes. The monoclonal antibodies used were pan-cytokeratin (1:50, clone AE1/AE3) and CD-31 (1:100, clone JC70A).

Immunohistochemical analysis showed cytoplasmic immunoreactivity for CD-31 in peritumoral vessels with a high staining intensity, considered an internal reaction control. However, neoplastic cells presented negative staining for the same protein (Fig.1E). High intensity cytoplasmic CK staining was observed in neoplastic cells, delimiting cystic neoformations (Fig. 1F).

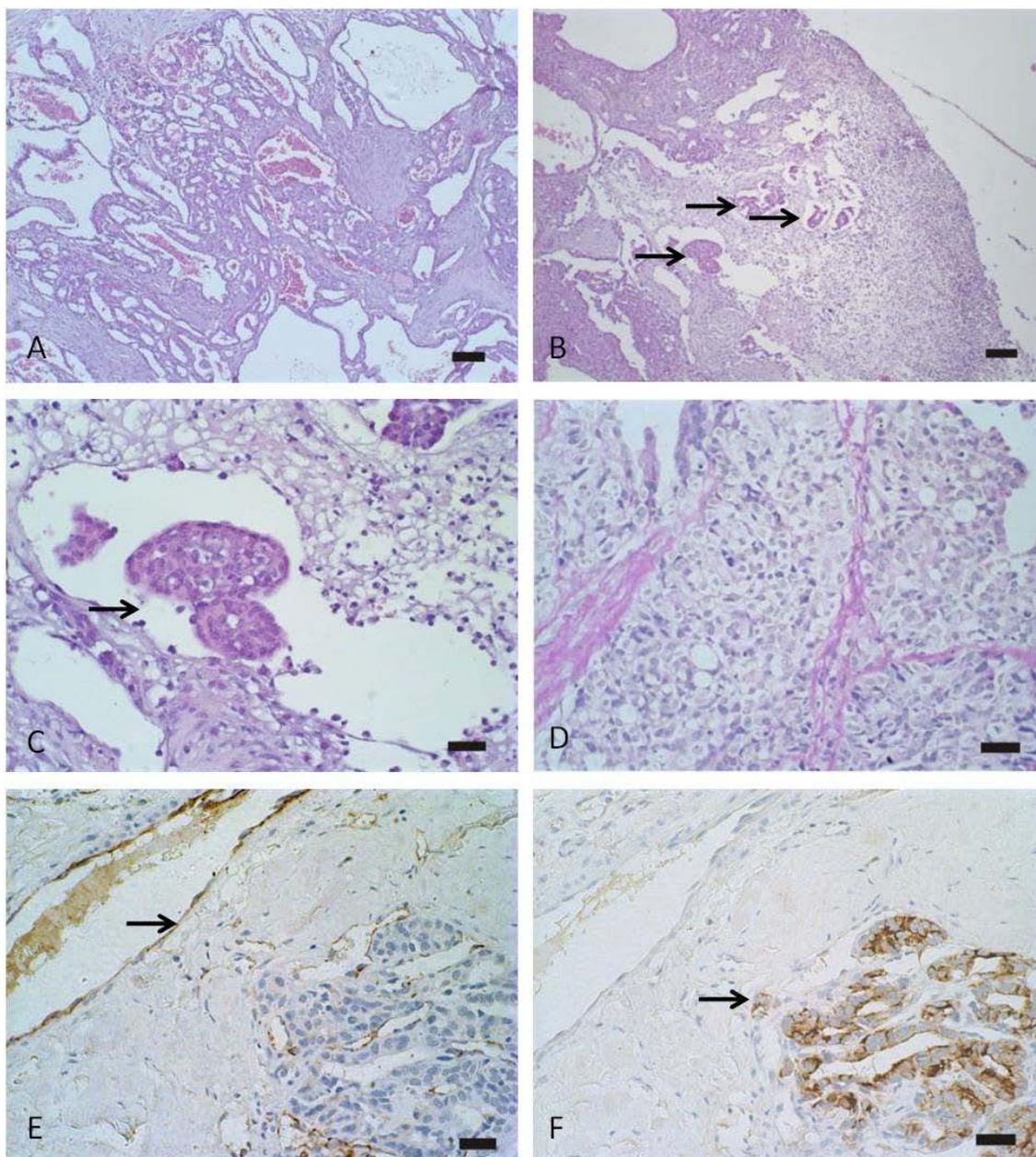


Figure 1. Dog oral mucosa: cystadenocarcinoma. (A). Epithelial cell proliferation in tubulopapillary pattern with dilated duct formation, sometimes containing red blood cells. H&E Bar = 50 $\mu$ m. (B) Neoplastic mass containing areas of vascular invasion (arrow). H&E Bar = 50 $\mu$ m. (C) Vascular tumor emboli (arrow). H&E Bar = 20 $\mu$ m. (D) Neoplastic cells negative for PAS staining (arrow). Bar = 20 $\mu$ m. (E) Positive vascular staining for CD-31 in positive control (normal vascular endothelium) (arrow). IHQ Bar = 20 $\mu$ m. (F) Positive staining of neoplastic epithelial cells for CK AE1/AE3 (arrow). IHQ Bar = 20 $\mu$ m.

## DISCUSSION

Salivary gland cystadenocarcinoma is a salivary adenocarcinoma variant, rarely described in dogs. In 1975 a case of zygomatic

adenocarcinoma (Buyukmihci *et al.*, 1975) was reported, posteriorly considered a possible cystadenocarcinoma (Head *et al.*, 2002) with macroscopical and microscopical characteristics similar to the present study's findings. In

humans, due to its higher frequency, several papers have described cystadenocarcinoma regarding the histochemical and immunophenotypical characteristics (Pollett *et al.*, 1997; Aloudah *et al.*, 2008; Gallego *et al.*, 2008).

In this case, the tumor was microscopically characterized by epithelial cell proliferation in a tubulopapillary pattern with the formation of cystic areas. These findings corroborate with descriptions in humans (Nakagawa *et al.*, 2002; Johnston *et al.*, 2006; Yamada *et al.*, 2007).

In humans, high grade cystadenocarcinomas are described based on tumor behavior, including perineural infiltration, vascular or lymphatic invasion, infiltration of peritumoral connective tissue and metastasis in regional lymph node and high mitotic index (Pollett *et al.*, 1997; Yamada *et al.*, 2007). In the present report, infiltration of neoplastic cells in regional lymphatic vessels and high mitotic index was observed, confirming high histological tumor grade.

In cases with of poorly differentiated salivary gland tumors, the use of PAS staining is usually suggested to determine the histological character of the lesion, showing normal mucin production by tumor cells (Pollett *et al.*, 1997; Militerno *et al.*, 2005). However, this technique is not extremely effective, as cystadenocarcinomas with PAS-negative neoplastic cells have been reported in humans, as well as observed in this report (Kawahara *et al.*, 2009).

In the present report, due to the presence of cystic formations containing a large amount of red blood cells, the histological analysis was followed by an immunohistochemical identification of epithelial and endothelial markers. A molecular study of hemangiosarcomas used CD-31 as a diagnostic tool in order to confirm malignant vascular tumors (Lahat *et al.*, 2010). The same antibody may be used to discard a possible endothelial origin (hemangiosarcoma) (Cushing *et al.*, 2010), as performed in the present study. Currently, several papers have used immunohistochemistry in order to characterize hemangiosarcomas (angiosarcomas), and distinguish them from tumors that mimic the formation of vascular spaces by means of particular antibodies. The positivity for cytokeratins was also reported in

other studies (Pollett *et al.*, 1997; Kawahara *et al.*, 2009), where immunopheno typically characterize human salivary gland cystadenocarcinomas. Immunohistochemical evaluation showed that neoplastic cells were positive for pan-cytokeratin (clone AE1/AE3) and negative for CD-31, showing an epithelial origin. Thus, immunohistochemistry has shown more accuracy, compared to histochemistry, when characterizing the neoplastic histogenesis.

## CONCLUSIONS

Due to the fact that salivary cystadenocarcinomas are rare in domestic animals and present morphological heterogeneity, it is extremely important to report and show the use of tools such as immunohistochemistry to help to establish a differential diagnosis of histomorphologically similar tumor variants.

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