

Tubulopapillary carcinoma of the mammary gland in a maned wolf (*Chrysocyon brachyurus*): histopathological and immunophenotypical analysis

[*Carcinoma túbulo-papilar da glândula mamária em lobo guará fêmea (Chrysocyon brachyurus): análise histopatológica e imunofenotípica*]

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ABSTRACT

A maned female wolf (*Chrysocyon brachyurus*) showed nodules in the inguinal and left abdominal cranial mammary glands. The mammary gland was surgically excised, and microscopic analysis revealed epithelial cell proliferation in a tubular and papillary pattern; delicate fibrovascular stalks presenting numerous layers of moderately pleomorphic epithelial cells were observed. This histologic appearance was compatible with a diagnosis of mammary tubulopapillary carcinoma. The immunohistochemical profile revealed nuclear positivity for estrogen (70%) and progesterone (at least 90% of the neoplastic cells). The myoepithelium-associated with neoplastic cells lacked integrity, as evidenced by failed smooth muscle alpha actin reactivity in microinvasive areas. A low proliferation index was observed (3.4%). To the authors' knowledge, the present case represents the first finding of female tubulopapillary carcinoma in a mammary gland in this species.

Keywords: *Chrysocyon brachyurus*, female maned wolf, immunohistochemistry, mammary gland, tubulopapillary carcinoma

RESUMO

Um lobo guará fêmea (*Chrysocyon brachyurus*) apresentou nódulos nas mamas inguinal e abdominal cranial esquerda. As glândulas mamárias foram cirurgicamente excisadas, e na análise microscópica revelaram proliferação de células epiteliais em arranjo tubular e papilar com tecido conjuntivo de sustentação delicado e formação múltiplas camadas de células epiteliais moderadamente pleomórficas. Os achados histopatológicos foram compatíveis com carcinoma túbulo-papilar da glândula mamária. O estudo do perfil imuno-histoquímico mostrou positividade nuclear para estrógeno (70%) e progesterona (em pelo menos 90% das células). Observou-se perda da integridade da camada mioepitelial, adjacente a proliferação epitelial, evidenciada pela perda de reatividade para alfa actina de músculo liso em áreas de microinvasão. Índice de proliferação celular baixo foi observado (3,4%). O presente relato representa a primeira descrição de um carcinoma túbulo-papilar da glândula mamária em uma fêmea desta espécie.

Palavras-chave: *Chrysocyon brachyurus*, lobo guará fêmea, imuno-histoquímica, glândula mamária, carcinoma túbulo-papilar

INTRODUCTION

The maned wolf (*Chrysocyon brachyurus*) is an extinction-threatened species widely distributed in South America (Argentina, Bolivia, Brazil,

Colombia, Paraguay, Peru and Uruguay) (Brasil..., 2003). The Brazilian savannah, or Cerrado, constitutes the largest portion of the species range and is thought to harbor 20.000 maned wolves (Sollmann *et al.*, 2010).

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Tumors in the mammary gland are well-known and common in canines, but they are rare in wildlife and exotic species (Carpenter *et al.*, 1980). Mammary tumors have been reported previously in a European pine marten (*Martes martes*) (Williams *et al.*, 1989), Blackfooted ferrets (*Mustela nigripes*) (Carpenter *et al.*, 1980), Mexican gray wolf (*Canis lupus baileyi*) (Federico *et al.*, 2010), red fox from Austria (*Vulpes vulpes*) (Janovsky and Steineck, 1999), Guinea pig (*Cavia porcellus*) (Suárez-Bonnet *et al.*, 2009) and male Maned wolf (*Chrysocyon brachyurus*) (Cassali *et al.*, 2009). This is the first report that describes pathological and immunophenotyping findings of a female maned wolf tubulopapillary carcinoma of the mammary gland.

CASE DESCRIPTION

A consistent nodule was identified in the left inguinal mammary gland in a maned wolf born at the Zoo-Botanic Foundation of Belo Horizonte. The left abdominal cranial mammary gland revealed additional nodules, and enlargement of the inguinal lymph node was observed. Surgical excision of the left inguinal lymph node and radical unilateral mastectomy was performed. Pulmonary radiography did not show a metastatic focus.

Macroscopic analysis showed two distinct nodulations: the first was identified between cranial and caudal abdominal mammary glands and the second in an inguinal mammary gland. The cerebriform masses presented whitish and friable consistency measuring about 3.0cm and 3.5cm, respectively. White parenchyma with cystic areas measuring about 1.5cm were observed in the cut surface of the first nodule. The second nodule showed similar findings in addition to friable consistency dots and large hemorrhagic lesions. There was no macroscopic alteration in the lymph nodes. Tumor specimens were collected, fixed in 10% neutral buffered formalin solution and embedded in paraffin. Afterwards, 4µm histological sections were obtained from these fragments and stained by Hematoxylin and Eosin.

Microscopic analysis showed epithelial cell proliferation in tubular and papillary patterns with delicate fibrovascular stalks, presenting numerous layers of moderately pleomorphic epithelial cells (Fig. 1A). In situ carcinoma associated with intravascular tumor emboli and stromal invasive areas was observed (Fig. 1B). A mitotic index was calculated based on 10 peripheral tumor areas measured on a microscope (BX-41^a) magnification of 400x; the mitotic index was low (average of 1 mitosis/field) (Dutra *et al.*, 2008). Lymph node metastasis was not seen.

A biotin-peroxidase system was used for the immunohistochemical procedure with the identification of the secondary antibody by polymer (ADVANCE HRP). The monoclonal antibodies used were pan-cytokeratin (CK AE1/AE3) (Clone AE1/AE3, Dako, 1:10); estrogen receptor (ER) (Clone 1D5, Dako, 1:20); progesterone receptor (PR) (Clone hRPa2, Neomarkers, 1:20); smooth muscle α -actin (ASMA) (Clone 1A4, Dako, 1:100) and Ki-67 (Clone MIB-1, Dako, 1:25).

A semi-quantitative method was used for scoring ER and PR into five categories: < 1% negative cells (-); 1 - 25% positive cells (+); 26 - 50% positive cells; 51 - 75% positive cells; >75% diffusely positive cells (Hammond *et al.*, 2010). Qualitative analysis was used for ASMA and CK AE1/AE3. A normal mammary gland and skin sample were used as a positive control. Negative controls consisted of replacing the primary antibody with phosphate buffered saline. MIB-1 nuclear staining was assessed through the determination of the percentage of positive cells among 1,000 tumor cells (400x) (Dutra *et al.*, 2008).

Immunohistochemical analysis of epithelial cells presented strong cytoplasmic staining for CK AE1/AE3, ER immunoreactivity +++ (70% of the cells with nuclear staining) and PR immunoreactivity (over 90% of the cells with nuclear staining) (Fig. 1C, D, E). Immunostaining for MIB-1 showed a low proliferation index (3.4%). Additionally, loss of myoepithelium integrity was evidenced by failed ASMA reactivity in microinvasive areas (Fig. 1F).

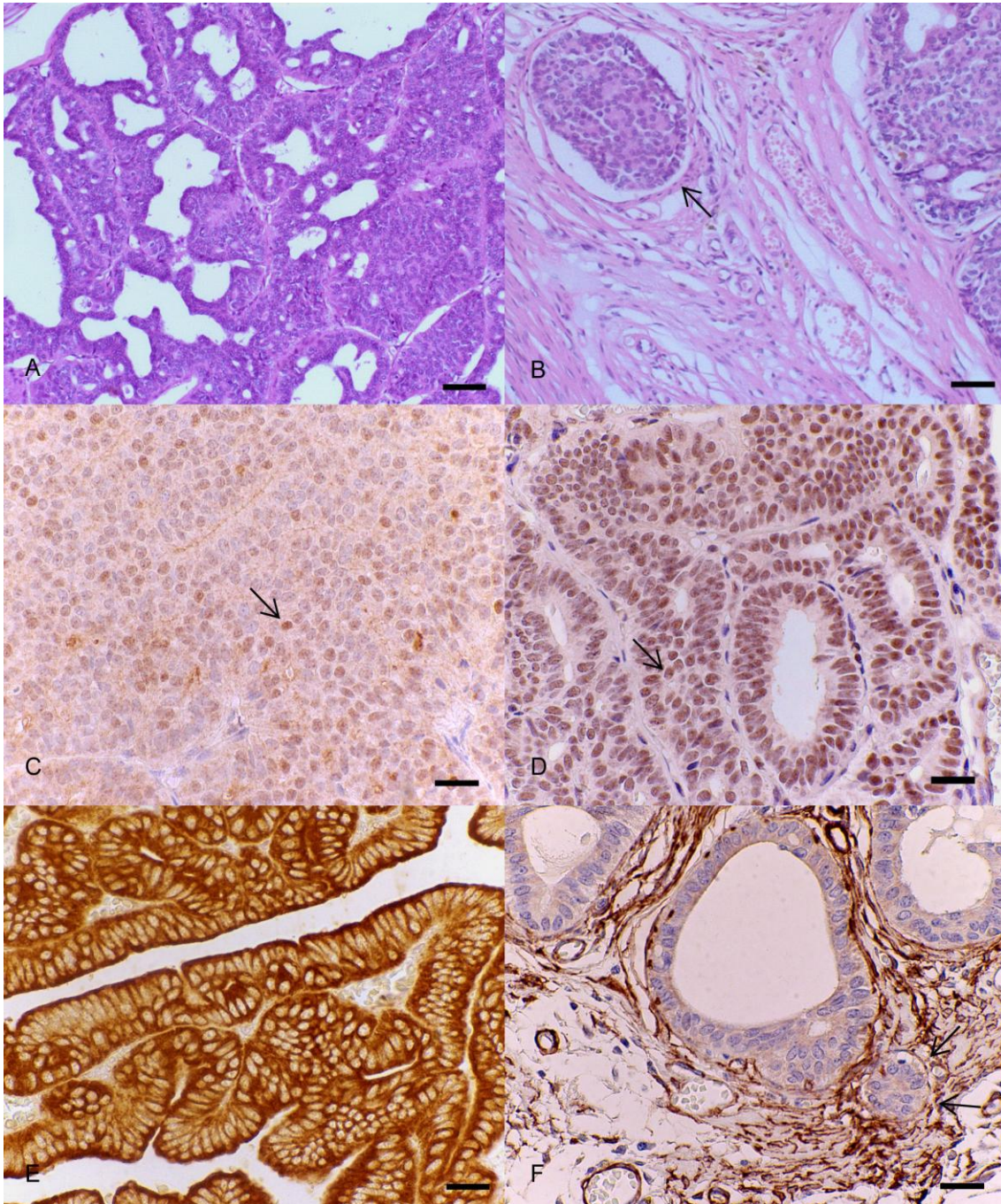


Figure 1. Female maned wolf mammary gland: Tubulopapillary carcinoma. (A) Malignant epithelial cells of the tubulopapillary carcinoma with tubular and papillar features. Hematoxilin & eosin. Bar: 50µm. (B) Lymphatic vessel invasion (arrow). Hematoxilin & eosin. Bar: 20µm. (C) Nuclear imunostaining for estrogen receptor (arrow). Counterstained with Harris' hematoxylin. Bar: 20µm. (D) Nuclear imunostaining for progesterone receptor (arrow). Counterstained with Harris' hematoxylin. Bar: 20µm. (E) Cytoplasmic epithelial cell imunostaining for cytokeratin AE1/AE3. Counterstained with Harris' hematoxylin. Bar: 20µm. (F) Cytoplasmic imunostaining for smooth muscle alfa actin. Microinvasive areas was observed (arrow). Counterstained with Harris' hematoxylin. Bar: 20µm.

DISCUSSION

Although tumors have been reported in maned wolves and are a significant cause of death in nondomestic canids, the prevalence of neoplasia in this species has not been assessed (Munson and Montali, 1991). Neoplasms have been described in captive maned wolves (Munson and Montali, 1991; McNulty *et al.*, 2000; Cracknell *et al.*, 2009). Until now, only one case of a male maned wolf mammary neoplasm has been described (Cassali *et al.*, 2009). This is the first report of a malignant mammary tumor in a female maned wolf.

Canine simple mammary carcinomas can be classified as tubulopapillary, solid or anaplastic based on their differentiation and biologic behavior. The canine tubulopapillary carcinoma has a strong tendency to infiltrate surrounding tissues and lymphatic vessels (Misdorp *et al.*, 1999). In this report, a maned wolf tubulopapillary carcinoma with lymphatic vessel and stromal invasions was described.

Epithelial histogenesis was confirmed by positive cytoplasmic staining for cytokeratin AE1/AE3. Smooth muscle alpha actin, CK5 and p63 antibodies were used to highlight canine mammary gland myoepithelial cells (Ramalho *et al.*, 2006). Nuclear myoepithelial staining for p63 has been utilized in a male maned wolf benign mammary tumor, and the non-infiltrative nature of the tumor was observed (Cassali *et al.*, 2009). In this paper, myoepithelial cell smooth muscle alpha actin reactivity at the epithelial-stromal junction demonstrated myoepithelium integrity loss and microinvasive areas, confirming an infiltrative tubulopapillary carcinoma.

Hormonal receptors have been considered as prognostic factors of canine mammary neoplasms (Millanta *et al.*, 2005). Canine benign mammary tumors with higher estrogen receptor staining than malignant mammary tumors have been reported, and negative staining for estrogen receptor was observed in the simplest carcinomas (Las mulas *et al.*, 2005). A separate study demonstrated that most canine-infiltrating carcinoma had PR negativity and ER positivity (Millanta *et al.*, 2005). In the male maned wolf, a benign mammary neoplasm was detected in low numbers of epithelial cells positive for ER. In

this report, hormonal receptors positive for tubulopapillary carcinoma were observed and low mitotic and cell proliferation indices confirming their prognostic values.

CONCLUSIONS

To the authors' knowledge, the present case represents the first finding of female tubulopapillary carcinoma in a mammary gland in this species. Although not very common, mammary tubulopapillary carcinoma should be included in the differential for mammary gland lesions in maned wolves.

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