



Metastatic Grade II simple tubulopapillary mammary carcinoma in a free-living female maned wolf (*Chrysocyon brachyurus*)

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ABSTRACT: This study described the pathological aspects of a metastatic grade II simple tubulopapillary mammary carcinoma in a free-living senile female maned wolf (*Chrysocyon brachyurus*). The diagnosis was based on histopathological findings of malignant epithelial cells arranged in tubular and papillary patterns, with marked anisocytosis and anisokaryosis and metastases to regional lymph nodes, lungs, parietal pleura, and pericardium. Also associated to positive immunolabeling of neoplastic cells for Cytokeratin AE1/AE3 and negative for P63, Calponin and Cox-2, added to the cell proliferation index of 26.8%. The findings demonstrated that free-ranging wild canids develop mammary neoplasms similar to those observed in domestic canids.

Key words: *Chrysocyon brachyurus*, tubulopapillary mammary carcinoma, wild animals.

Carcinoma mamário simples túbulo-papilar metastático Grau II em lobo-guará fêmea (*Chrysocyon brachyurus*) de vida livre

RESUMO: Este estudo descreve os aspectos patológicos de um carcinoma mamário simples túbulo-papilar metastático grau II em um lobo-guará, fêmea, senil de vida livre. O diagnóstico foi baseado nos achados histopatológicos de células malignas epiteliais arranjadas em um padrão tubular e papilar, com acentuada anisocitose e anisocariose e metástases para os linfonodos regionais, pulmão, pleura parietal e pericárdio. Também associado a marcação imuno-histoquímica positiva das células neoplásicas para Citoqueratina AE1/AE3 e negativas para P63, Calponina e Cox-2, somado ao índice de proliferação celular de 26,8%. Os achados demonstram que canídeos silvestres de vida livre podem desenvolver neoplasias mamárias similares às observadas em canídeos domésticos.

Palavras-chave: *Chrysocyon brachyurus*, carcinoma mamário túbulo-papilar, animais silvestres.

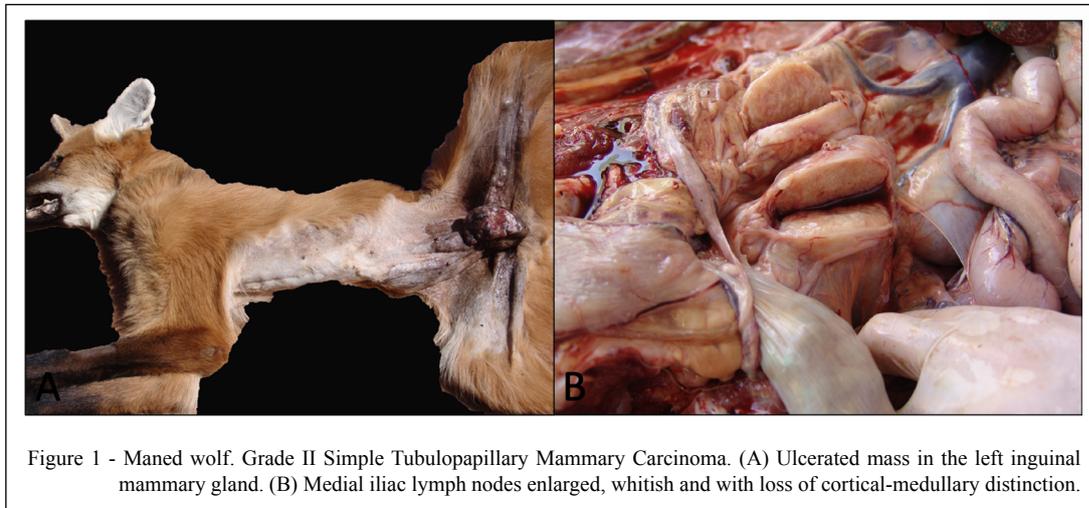
The maned wolf, considered the largest canid in South America, is listed among the endangered species by the International Union for Conservation of Nature (PAULA & DE MATTEO, 2016).

Mammary neoplasms, commonly observed in domestic dogs (CASSALI et al., 2020), are rare in wild animals (JANOVSKY & STEINECK, 1999), being described only in captive canids, such as the Mexican gray wolf (*Canis lupus baileyi*) (FEDERICO et al., 2010), Austrian red fox (*Vulpes vulpes*) (JANOVSKY & STEINECK, 1999) and in a male (CASSALI et al., 2009) and female (GAMBA et al., 2011) maned wolf (*Chrysocyon brachyurus*). The present study described the pathological aspects of metastatic grade II simple tubulopapillary mammary carcinoma in a free-living female maned wolf.

A female maned wolf, found in a residence in the city of São Tomé das Letras, Minas Gerais

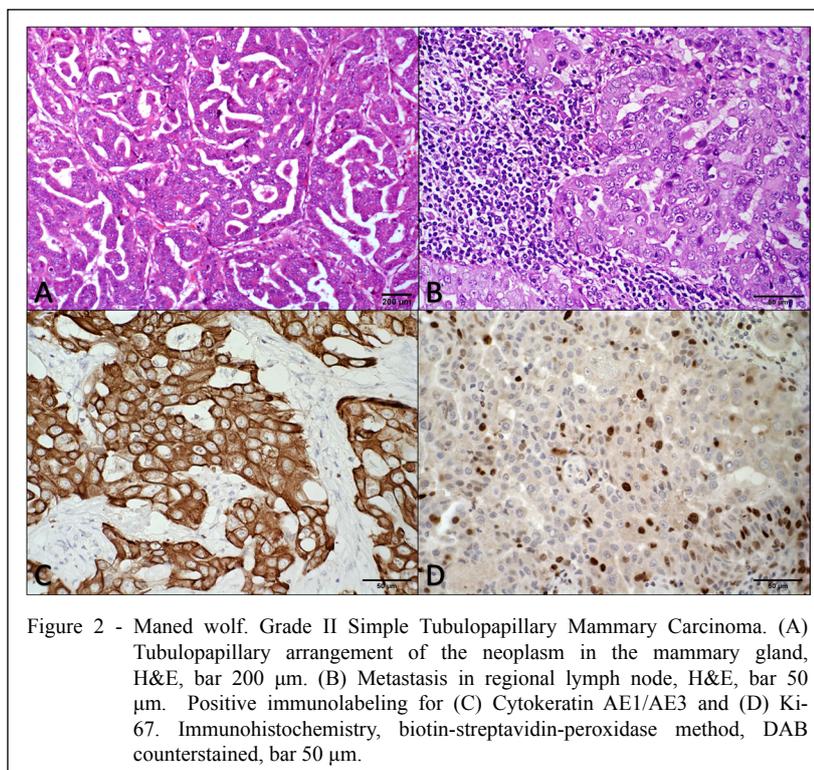
State, Brazil, was forwarded by the Environmental Police to the Wild Animals Ambulatory of the Federal University of Lavras (UFLA). After physical examination, blood count, radiography and ultrasonography, the maned wolf was euthanized due to the unfavorable prognosis and referred for necropsy at the Veterinary Pathology Sector of UFLA.

The canid, considered senile due to the accentuated wear of the incisor teeth and bilateral cataracts, had a low body score, pale mucous membranes and a mass in the left inguinal mammary gland measuring 21 x 8 x 6 cm, firm, ulcerated (Figure 1A) with a yellowish and lobulated cut surface. Smaller nodules were seen in the left caudal abdominal mammary gland. The superficial inguinal, axillary and medial iliac lymph nodes were enlarged, whitish and with loss of cortical-medullary distinction (Figure 1B). In addition, there were multifocal whitish nodules,



measuring up to 1 cm in diameter in the parietal pleura, pericardial sac, and lungs. Other necropsy findings included discrete endocardiosis in the left and right atrioventricular valves; enlarged left kidney and markedly reduced volume in the right kidney, with a 12.5 cm specimen of *Dioctophyma renale* in the renal pelvis surrounded by a thin layer of renal cortex. Tissue samples were collected in 10% neutral-buffered formalin and routinely processed for histopathology.

The histological exam revealed proliferation of malignant luminal epithelial cells arranged in tubular and papillary patterns (Figure 2A) with eosinophilic cytoplasm, oval nucleus, granular chromatin, one to four nucleoli, supported by thin fibrovascular stroma revealed by the Masson Trichrome staining. There were marked anisocytosis and anisokaryosis (Figure 2 A-B) and nine mitotic figures in 10 highest magnification fields (area of 2.37mm²). Multifocal



necrosis, lymphatic and blood vessel invasion were also observed. Metastatic epithelial cells were found in the lymph nodes (Figure 2B), lungs, parietal pleura, and pericardium.

Neoplasm samples were sent to the Laboratory of Comparative Pathology (LPC-UFMG) for immunohistochemical analysis. Monoclonal antibodies for Ki-67 (1:50 dilution Dako), Cox-2 (1:50 dilution; Invitrogen); Calponin (1:300 dilution; Cell Marque), P63 (1:100 dilution; Dako) and cytokeratin AE1/AE3 (1:500 dilution; Dako) were used. The binding sites of the primary monoclonal antibodies were identified by the Novolink Polymer Detection System method (Leica Biosystems, Newcastle Upon Tyne, UK) and diaminobenzidine (DAB, Dako) as chromogen. The Ki-67 evaluation was carried out by manual counting of a total of 500 neoplastic cells in 10 fields with the highest magnification in the areas of greatest positivity (hot spot/hot zones) using the Image J software (version 1.42q), with a point of cutoff $\geq 20\%$, as described by NAKAGAKI et al. (2021). Tumor cells in the mammary gland and regional metastases were positive for cytokeratin AE1/AE3 (Figure 2C) and negative for P63 and Calponin (myoepithelial markers) and Cox-2. The cell proliferation index by Ki-67 immunolabeling was 26.8% (Figure 2D).

The diagnosis of metastatic grade II simple tubulopapillary mammary carcinoma was based on necropsy, histopathology (GOLDSCHMIDT et al., 2011) and immunohistochemical findings, with strong cytoplasmic staining of epithelial cells for AE1/AE3 cytokeratin (GAMBA et al., 2011). Tumor grade was determined according to the scoring system based on three morphological characteristics: 1) degree of differentiation assessed by tubular formation; 2) nuclear pleomorphism and 3) mitotic count in the high-power field diameter (PEÑA et al., 2013)

The findings were moderate tubular formation between 10-75% (score 2), marked nuclear pleomorphism (score 3) and mitotic count with nine mitotic figures in 10 highest magnification fields (score 2), with a total score of 7, being classified as grade II (PEÑA et al., 2013).

There was no immunolabeling for Cox-2 in the case studied; however, the possibility that the antibody is not suitable for this animal species cannot be totally excluded, since in domestic canines Cox-2 expression is more frequent in mammary neoplasms with a lower degree of differentiation (CARVALHO et al., 2016). Antibody Ki-67 is a cell cycle marker, frequently used in canine neoplasms. The cell marking above the cutoff point ($>20\%$) seen in the present case is observed in malignant mammary neoplasms and

indicated a greater chance of metastasis, worse prognosis and lower survival rate (KASZAK et al., 2018).

The findings demonstrated that free-ranging wild canids can develop mammary neoplasms similar to those observed in domestic canids. The histological features, the immunolabeling and the proliferation cell rate corroborated the diagnosis of grade II simple tubulopapillary mammary carcinoma.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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