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Anaplastic mammary carcinoma in a captive jaguar (*Panthera onca*)

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ABSTRACT

A captive female of jaguar (*Panthera onca*), 12 years old, presenting an 8-day history of anorexia and lethargy, died in its enclosure in Manaus (Amazonas, Brazil). The necropsy revealed an abdominal multinodular ventral plaque into the mammary region. Additionally, in several locations of the subcutaneous tissue, there were multiple metastatic nodules of firm texture. Metastases were also observed in the lymph nodes, heart and lungs. The immunohistochemistry results revealed the cytoplasmic staining for cytokeratin on neoplastic cells, mild staining (+) for E-cadherin, moderate staining (++) for estrogen and progesterone receptors, and about 50 to 70% of staining for Ki-67. The neoplastic cells were negative for vimentin staining. Therefore, the histopathological examination led to a diagnosis of metastatic anaplastic mammary carcinoma, which is an unusual condition in wild felines.

KEYWORDS: Amazon, Felidae, neoplastic disease, wild feline

Carcinoma mamário anaplásico em uma onça-pintada (*Panthera onca*) criada em cativeiro

RESUMO

Uma onça pintada (*Pantera onca*) de cativeiro, fêmea, de 12 anos, com história clínica de 8 dias de anorexia e letargia, foi encontrada morta em seu recinto em Manaus (Amazonas, Brasil). Na necropsia foi observada uma placa abdominal ventral multinodular na região mamária. Em várias regiões do tecido subcutâneo, foram observados diversos nódulos metastáticos de consistência firme. Metástases também foram observadas nos gânglios linfáticos, coração e pulmões. Os resultados da imuno-histoquímica (IHQ) revelaram coloração citoplasmática para citoqueratina nas células tumorais, coloração leve (+) para E-caderina, moderada (++) para receptores de estrogênio e progesterona e cerca de 50 a 70% de coloração para Ki-67. As células neoplásicas foram negativas na IHQ para vimentina. Assim, o exame histopatológico conduziu a um diagnóstico de carcinoma mamário anaplásico metastático, que é uma condição incomum em felinos selvagens.

PALAVRAS-CHAVE: Amazônia, Felidae, doença neoplásica, felino selvagem

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In nondomestic felids, mammary neoplasms, usually of malignant development, have been reported with higher incidence in tigers, *Panthera tigris* L., than in other captive felids like jaguar, *Panthera onca* L., lion, *Panthera leo* L., leopard, *Panthera pardus* L., puma, *Puma concolor* (Linnaeus, 1771), and jungle cat, *Felis chaus* Schreber, 1777 (Harrenstien et al. 1996; Munson and Moresco 2007; Sadler et al. 2016). Most of the mammary tumors observed in felids kept in zoos had an aggressive pattern, often invasive and metastatic, similarly to when diagnosed in domestic cats, *Felis catus* L. (Finotello et al. 2011; Munson and Moresco 2007; Sadler et al. 2016). Here we describe the gross histological and immunohistochemical features of an anaplastic mammary carcinoma in a captive jaguar, a condition which so far had not been reported in *P. onca*.

A captive female jaguar, about 12 years old, from Manaus, Amazonas state, Brazil), died in its enclosure (in a private hotel mini zoo) after an 8-day history of anorexia and lethargy. The necropsy revealed a multinodular plaque into the mammary region (inguinal to abdominal direction), measuring 12.0 x 8.0 x 1.0 cm. The skin over this specific region had areas of ulceration. Through a surface cut, it was observed that the mass was extending from the superficial dermis to the subcutaneous tissue (Figure 1a). Another mass was observed adjacent to the plaque measuring 6.0 x 2.5 x 1.5 cm, being solid/firm on surface cut and diffusely white. Into the subcutaneous tissue, in several regions of the animal body, there were multiple nodules, white to reddish (Figure 1c), with firm texture, and varying from 0.3 to 2.0 cm in diameter (Figure 1b). The parenchyma of the superficial axillary and inguinal lymph nodes was replaced by a firm and white tissue. In the myocardium, there were multiple nodules, small, firm and whitish with not more than 0.3 cm in diameter. Likewise, there were some well delimited nodules in the lungs, firm, whitish and measuring no more than 1.5 cm in diameter. From the lungs, after incision, flowed a large amount of liquid and there was a white foam throughout the length of the trachea, indicating a possible pulmonary edema.

Figure 1. A – Anaplastic mammary carcinoma (AMC) in a captive jaguar (*Panthera onca*) from Manaus (Amazonas, Brazil), with neoplastic tissue extending from the superficial dermis to the subcutaneous tissue; B – Metastasis of the AMC with firm nudules in the subcutaneous tissue (thoracic and abdominal lateral region); C – Surface cut of the nodules in high magnification presenting white with reddish areas. This figure is in color in the electronic version.

The samples were fixed by immersion in buffered 10% formalin for later microscopic evaluation. The histopathological examination revealed that the tumor tissue was composed of exceedingly invasive and scattered individualized or small grouped neoplastic cells, with a round, oval, or polygonal shape, showing mild to moderate anisocytosis (Figure 2a). Some of the neoplastic cells had lost the junctional properties, as typically observed in cells of epithelial origin. The nuclei of neoplastic cells are generally huge and round or oval. However, in this case, they are highly pleomorphic and exhibit moderate anisocariosis, multiple nucleoli and several multinucleated cells. In addition, many mitotic figures were seen (mitotic index: 10 mitotic figures/10 high-power fields/2.37 mm^2). Moderate desmoplasia was observed around the neoplastic cells. Peritumoral neoplastic emboli were observed within lymph vascular vessels, with mild to moderate inflammatory infiltrations. Histological analysis revealed the presence of some large pleomorphic epithelial cells, grouped in small clusters with eventual presence of bizarre nuclei. Our case was classified as grade II based on tubule formation, nuclear pleomorphism and mitotic count. Because neoplastic cells are very anaplastic,

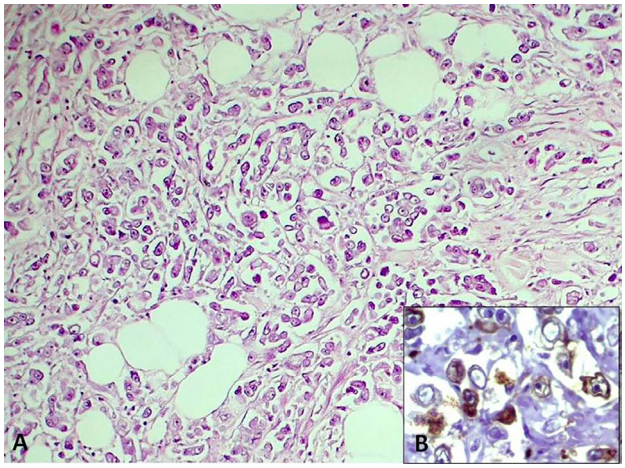

Figure 2. A – Histopathology of the mammary gland tumor of a captive jaguar (*Panthera onca*) from Manaus (Amazonas, Brazil) showing tumor cells with infiltrative growth into the surrounding connective tissue (H.E); B – Immunohistochemical result showing neoplastic cells positive for cytokeratin. Magnification 40x, scale bar = 50 µm. This figure is in color in the electronic version.

immunohistochemistry was performed with cytokeratin and vimentin (Sawa et al. 2017), E-cadherin (Furusawa et al. 2021), estrogen and progesterone receptors and Ki-67 antibodies (Dagher et al. 2019a). The immunohistochemistry results revealed the cytoplasmic staining of cytokeratin in neoplastic cells (Figure 2b), mild (+) staining for E-cadherin, moderate (++) staining for estrogen and progesterone receptors and about 50 to 70% of staining for Ki-67. The neoplastic cells were negative for vimentin staining.

Studies on tumors in wild felines are uncommon. A review of medical records and necropsy reports in Knoxville Zoological Gardens (USA) from 1979 to 2003 reported 40 neoplasic tumors in 26 felids (comprising the integumentary-mammary, endocrine, reproductive, hematopoietic-lymphoreticular, digestive, and hepatobiliary systems), amounting to 28% of all death cases or reason for euthanasia of those felids (Owston et al. 2008).

Failure of early detection of tumors in zoo felids is likely due to late diagnostic procedures, such as physical examination, since animals are examined only opportunistically, and depend on scheduled anesthesia. In this context, early clinical detection is difficult, as the absence of prominent nodules prevents early clinical suspicion. However, there are indications that mammary cancer of high grade and metastatic potential develops early in the course of carcinogenesis in both domestic and zoo felids (McAloose et al. 2007; Munson and Moresco 2007; Zappulli et al. 2015; Sadler et al. 2016).

Mammary gland tumors have been associated with the use of the synthetic progestins melengestrol acetate (MGA) (Harrenstien et al. 1996) and medroxyprogesterone acetate (MPA) as a method of contraception (Black et al. 1979; Misdorp 1991) in both, domestic and zoo felids (McAloose at al. 2007). A correlation has been found between clinically aggressive mammary carcinomas and MGA treatment in wild captive felids (Black et al. 1979). However, in the present case, there is no record of use of any contraceptive method.

Anaplastic mammary carcinoma is difficult to treat though surgery procedure, due to its early and extensive infiltration into the surrounding tissues and the lymphatic system (Kim et al. 2015), as observed in the present case. The regional lymph nodes and lungs are the organs that are most frequently affected by metastases (Misdorp 2002). The most frequently observed area of metastases in felines was the lungs (76.9%), followed by the skin (23.1%) (Togni et al. 2013). Pleura, liver, spleen, kidneys, bones, and brain may also be affected (Hayes et al. 1981). In our case, metastases were observed in skin, inguinal and axillary lymph nodes, lung and heart.

The historical lack of consensus on morphological classification of domestic animal mammary tumors reflects controversies over histogenesis (Misdorp 2002). In domestic and zoo cats, morphologic patterns of mammary cancer are like breast cancer in women (Munson and Moresco 2007). Most cancers have some areas of tubulopapillary growth with formation of solid, cribriform patterns and both intraductal and infiltrating components (McAloose et al. 2007). Our case supports the literature, as we observed peritumoral neoplastic emboli within lymphatic vascular vessels, with mild to moderate inflammatory infiltrate.

Histological grading is a good parameter to stratify tumors according to their biological aggressiveness. The Elston and Ellis (EE) histologic grading system, originally developed for human breast cancer, is commonly used to grade feline mammary carcinomas (Dagher et al. 2019b) and has a strong correlation with prognosis, therefore was used in our case report. Most diagnosed zoo felids had high-grade mammary cancer, mostly with metastatic disease at the time of diagnosis, regardless of their history of progestin exposure (Castagnaro et al. 1998; McAloose et al. 2007). Mammary carcinomas in domestic cats have comparable aggressive features, high tumor grades (II or III), and evidence of metastasis at the time of diagnosis (Dagher et al. 2019b). These features are associated with decreased survival in domestic cats, as in human mammary cancer patients, and also are likely important prognostic indicators in zoo felids (Castagnaro et al. 1998; McAloose et al. 2007).

Although feline mammary malignant tumors are frequent in non spayed female cats, anaplastic carcinomas have not yet been described in jaguars. The lack of information of mammary tumors in *P. onca* may be due to a low prevalence of the disease in this species, associated with the difficulty in its clinical recognition and/or accurate diagnosis in wild or captive animals (McAloose et al. 2007). In contrast to other types of mammary carcinomas, the anaplastic type in felids is highly invasive and exhibits extensive amounts of collagen fibres (scirrhous carcinoma), which is, in comparison, not the typical presentation of anaplastic carcinomas in dogs and humans (Misdorp 2002).

We confirmed that the cellular origin of the tumor in our case was epithelial based on the immunohistochemical positivity for cytokeratin, and negative staining for vimentin (Kim et al. 2015). The low proportion of mammary cancer positive for either progesterone (PR) or estrogen receptors (ER) in zoo felids is similar to that reported for mammary carcinomas in domestic cats, which have fewer ER and PR receptors than in normal mammary gland or benign mammary tumors (Dagher et al. 2019a). PR status does not appear to correspond to prognosis in zoo felids, as both PR-positive and -negative cancers were of high grade with metastasis (De Las Mulas et al. 2000; McAloose et al. 2007).

The strong expression of Ki-67 in our case, especially in the tumor cells, demonstrating mitotic figure, indicates that the tumor is highly malignant and aggressive. Similarly, most reports on zoo felids stated a high-grade cancer with aggressive metastasis (McAloose et al. 2007; Yun et al. 2014).

Anaplastic mammary carcinoma is uncommon in feline species and in this case, it was not associated with the use of contraceptives. The cytokeratin-positive immunohistochemistry contributed for the diagnostic of an anaplastic mammary carcinoma. No published reports on this type/classification of neoplasm existed so far for wild felines.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author Alexandre Alberto Tonin.