

## **BONE AND PULMONARY METASTASIS FROM A MAMMARY ADENOSQUAMOUS CARCINOMA IN A BITCH**

*(Metástase óssea e pulmonar de adenocarcinoma  
escamoso mamário em cadela)*

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### **ABSTRACT**

*Mammary tumors are the most common in bitches, and bone and pulmonary metastases are not well described. Adenosquamous mammary carcinoma is a rare tumor that can be classified as a type of metaplastic tumor. This report describes the clinical and histopathological findings of this atypical presentation of mammary carcinoma in an 11-year-old, spayed, Poodle. Surgery was performed to remove malignant mammary nodules with a provisional diagnosis of carcinoma on cytologic examination. Thirteen months later, the dog returned showing left pelvic limb lameness that had persisted for 6 months prior to the consultation. The patient showed right pelvic limb lameness and a mass in the same limb. Radiographs of the affected limb revealed a pathological fracture in the femoral diaphysis and proliferation of the periosteum. After clinical staging, a left pelvic limb amputation was performed. A diagnosis of grade II adenosquamous mammary carcinoma was made through histopathology and immunohistochemistry. Although uncommon, bone and pulmonary metastases from mammary tumors, such as adenosquamous carcinoma, can be considered differential diagnoses for bony tumors. This report highlights the aggressive nature and significant metastatic potential of the adenosquamous carcinoma.*

**Keywords:** Dog, Mammary gland, bone metastasis, squamous metaplasia.

### **RESUMO**

Os tumores mamários são os mais comuns em cadelas e as metástases ósseas e pulmonares não são bem descritas. O carcinoma mamário adenoescamoso é um tumor raro que pode ser classificado como um tipo de tumor metaplásico. Este relato descreve os achados clínicos e histopatológicos dessa apresentação atípica de carcinoma mamário em um Poodle de 11 anos de idade, castrado. A cirurgia foi realizada para remover nódulos mamários malignos com diagnóstico provisório de carcinoma no exame citológico. Treze meses depois, a cadela retornou apresentando claudicação do membro pélvico esquerdo que persistia por seis meses antes da consulta. O paciente apresentava claudicação do membro pélvico direito e uma massa no mesmo membro. A radiografia do membro afetado revelou fratura patológica na diáfise femoral, proliferação do periósteo e áreas de osteopenia. Após estadiamento clínico, foi realizada amputação do membro pélvico esquerdo. O diagnóstico de carcinoma adenoescamoso mamário grau II foi feito através da histopatologia e imunohistoquímica. Embora incomuns, as metástases ósseas e pulmonares de tumores mamários, como o carcinoma adenoescamoso, podem ser consideradas diagnósticos diferenciais para tumores ósseos. Este relatório destaca a natureza agressiva e significativo potencial metastático do carcinoma adenoescamoso.

**Palavras-chave: Keywords:** Cão, glândula mamária, metástase óssea, metaplasia escamosa.

### **INTRODUCTION**

Canine mammary tumors are the most common tumors in female dogs. Surgical removal followed by histopathologic analysis is the main treatment however. Adjuvant

chemotherapy can also be indicated for this type of neoplasm (SORENMO *et al.*, 2019). Adenosquamous mammary carcinoma is a rare, aggressive tumor in man (RASOTTO *et al.*, 2017; Ko *et al.*, 2019; Lee *et al.*, 2019). This tumor can be classified as a type of metaplastic mammary tumor (CROUCHER *et al.*, 2016). Adenosquamous mammary carcinoma is a variation of squamous cell carcinoma, which also has glandular structures, and is considered a differential diagnosis (GOLDSCHIMIDT *et al.*, 2016). Studies have reported that the histopathologic findings of adenosquamous mammary carcinoma in dogs are similar to those in human cases (LEE *et al.*, 2019, RASOTTO *et al.*, 2017).

In humans metastases, the skeleton is the third most common site for metastases after the lungs and liver (CROUCHER *et al.*, 2016). In the skeleton, the metastatic process begins with colonization, when circulating cells enter the bone marrow compartment and become involved in specialized microenvironments or niches. The second stage involves survival and dormancy. Disseminated tumor cells adapt to their new microenvironment, avoid the immune system, and reside in a dormant environment for long periods. The third step, the reactivation, and development require the ability to escape from the sleeping state to actively proliferate and form a micrometastasis. The final step, the growth, occurs when cells grow uncontrollably, become independent of the microenvironment and, finally, modify the bone as metastasis grows (GOLDSCHIMIDT *et al.*, 2016).

In human and canine species, adenosquamous mammary carcinoma has a carcinomatous pattern associated with foci of malignant squamous differentiation (KO *et al.*, 2019; GOLDSCHIMIDT *et al.*, 2017). Although uncommon, this type of carcinoma typically shows grades II and III with lymphovascular invasion and metastasis to regional lymph nodes (GOLDSCHIMIDT *et al.*, 2017). Tumors are often deep and infiltrative, however, a low-grade variant with superficial dermal invasion has been reported (KO *et al.*, 2019). The differential diagnosis of adenosquamous mammary carcinoma must include metastatic tumors and tumors with secondary extension into the skin, such as squamous cell carcinoma (KO *et al.*, 2019). Bone metastases are frequently seen in human patients with cancer and result in severe consequences (TROST *et al.*, 2014). However, bone tumors are most often diagnosed as primary tumors and, in most cases are associated with severe bone pain, pathologic fractures and compression of the adjacent muscle and nerves (TROST *et al.*, 2014).

In veterinary medicine, bone metastases are uncommon in bitches with mammary tumors (TROST *et al.*, 2014). Diagnostic imaging methods such as standard radiography are useful techniques for the early detection of metastases, especially in cases where structural bone changes have occurred (TOLEDO *et al.*, 2018). There are few reported veterinary cases describing the imaging, histopathologic findings, and survival time. The purpose of this report was to raise diagnostic suspicion for the diagnosis of this atypical presentation of mammary carcinoma in dogs.

## PATIENT CARE

An 11-year-old, 4kg, spayed female Poodle was admitted to the Veterinary Hospital of the Federal University of Campina Grande (UFCG), Patos, Paraíba, Brazil. Previously, the patient had undergone a bilateral mastectomy due to the presence of nodules in the caudal and inguinal abdominal mammary glands (right and left), with a cytologic diagnosis of carcinoma.

On histopathologic examination the tumor was classified as adenosquamous mammary carcinoma, grade II, the inguinal lymph nodes were free of metastasis. Furthermore, tumor emboli were seen in lymphatic and blood vessels under light microscope. The client declined adjuvant chemotherapy.

Thirteen months later, the patient returned with a six-month history of right pelvic limb lameness and the presence of a mass in the same limb. On physical examination, the mass was uniform throughout the proximal region of the left pelvic limb and had a firm consistency. The femoropatellar joint region was rigid in extension. Neurologic examination showed no abnormalities.

Further examinations were performed to rule out systemic abnormalities and to determine the clinical staging. Blood count revealed normochromic normocytic anemia [hematocrit: 24% (reference 24-46%), red blood cells  $3.75 \times 10^6/\mu\text{L}$  (reference  $5.0-10.0 \times 10^6/\mu\text{L}$ ), hemoglobin 8g/dL (reference 14-18g/dL), mean corpuscular volume: 63.7 fL (reference 60-77 fL) and mean corpuscular hemoglobin concentration: 33.5 g/dL (reference 31-36g/dL). On serum biochemistry there was: mild azotemia (creatinine: 1.6g/dL (reference 0.5-1.5g/dL) and BUN: 146.7mg/dL (reference 21.4-59.92mg/L). The levels of albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma GT (GGT), phosphorus and total protein were within the normal ranges for dogs.

Thoracic radiographs (right and left lateral and ventrodorsal projections) revealed interstitial bronchial opacification due to the aging process. Radiographs of the right pelvic limb in the mediolateral and dorsoventral projections (due to limb stiffness) revealed a pathologic fracture in the femoral diaphysis with osteophytic periosteal proliferation, intense bone lysis at the fracture focus, and osteopenic areas in the femoral diaphysis. In addition, enlargement of soft tissues along the entire length of the femur was noted with mineralization of areas adjacent to the fracture region. Abdominal ultrasound examination was normal.

On this second evaluation, the cytology report suggested a malignant tumor with squamous differentiation. Due to the patient's advanced clinical staging, limb amputation was suggested, and samples were submitted for histopathologic examination.

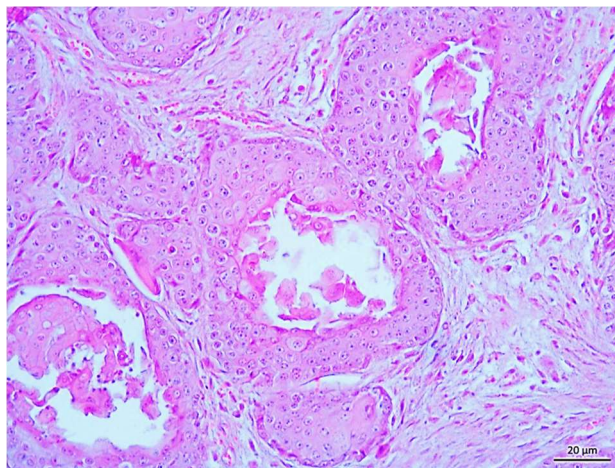
Macroscopically, there was a mass surrounding the femur (Fig. 01). A transverse cut of the femoral mass revealed a complete fracture in the middle third of the femur. The mass was friable with a diffusely irregular and granular surface.



**Figure 01:** Photomicrograph of an adenosquamous mammary carcinoma in a female Poodle.

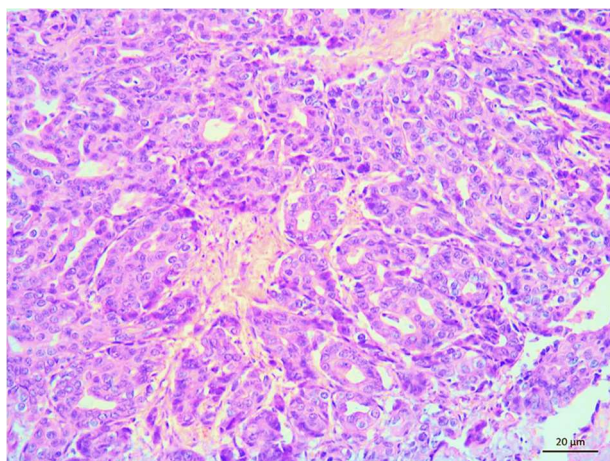
**Obs:** Macroscopy (right femur): note the yellow mass with a firm consistency.

Microscopically, the tumor mass in the adjacent muscles was poorly delimited, unencapsulated, with an infiltrative pattern and carcinomatous proliferation with lobes of several sizes containing acinar arrangements (Figs. 02 and 03). Some lobes showed central necrosis. The acini were covered by up to 10 layers of polygonal cells. The pleomorphism was moderate and no mitosis was observed in the analyzed sections. The nuclei were rounded with sparse chromatin and prominent nucleoli. There was also intense, multifocal, and accentuated squamous differentiation which was organized in trabecular formations. Tumor emboli were present in the lumen of blood vessels.



**Figure 02:** Photomicrograph of an adenosquamous mammary carcinoma in a female Poodle.

**Obs:** Histopathology (adjacent muscles): neoplastic squamous epithelial cells forming islands and trabeculae. Bar 20µm, higher magnification 40x, hematoxylin-eosin (HE).



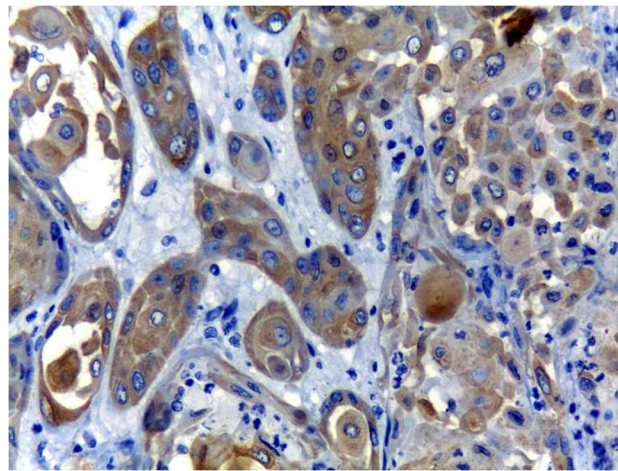
**Figure 03:** Photomicrograph of an adenosquamous mammary carcinoma in a female Poodle.

**Obs:** Histopathology (right femur). Expansive neof ormation featuring islands, trabeculae, and central necrosis. carcinomatous cells show a broad, eosinophilic, and polyhedral cytoplasm. The acini are covered by layers of polygonal cells. Bar 20µm, higher magnification 40x, HE.

On histopathologic examination of the right femur, the tumor found to have infiltrated the femur with predominantly squamous differentiation. The left popliteal lymph node showed

a loss of architecture due to intense tumor infiltration, similar to that described in the right pelvic limb. Based on these findings, together with the patient's history, a diagnosis of metastasis of adenosquamous carcinoma to the femur, with the involvement of the adjacent musculature, was made (Figs. 01, 02 and 03).

An immunohistochemical panel was performed to increase the accuracy of histopathological diagnosis. The antibodies used were: CK Pan - intermediate filaments of epithelial cells, clone AE1/AE3 (Fig. 04); High molecular weight CK (squamous and ductal epithelium marker, clone 34betaE12); CK5 (High molecular weight cytokeratin, clone XM26). Additionally, the marker CK7 (Ductal, glandular superficial and transition squamous marker, clone OV-TL12) showed positive immunostaining in the pelvic limb and the lung parenchyma. All the samples showed positive immunostaining for tumor cells located in the left pelvic limb.



**Figure 04:** Photomicrograph of an adenosquamous mammary carcinoma in a female Poodle.

**Obs:** Immunohistochemistry photomicrograph. Histological section of metastasis in the left pelvic limb showing strong immunostaining for antibody AE1/AE3. Bar 50µm, higher magnification 40x. Polymer complex linked to peroxidase, Harris Hematoxylin.

Four weeks after surgery, the patient returned showing signs of dyspnea, anorexia, and pale mucous membranes. Several abnormalities were seen in the blood count: normochromic normocytic anemia [hematocrit of 15.5% (reference: 24-26%), red blood cells  $2.28 \times 10^6/\mu\text{L}$  (reference 5.0-10.0  $\times 10^6/\mu\text{L}$ ), hemoglobin 5.2g/dL (reference 14-18g/dL), mean corpuscular volume: 68fL (reference: 60-67fL), mean corpuscular hemoglobin concentration 33g/dL (reference: 31-36g/dL), neutrophilic leukocytosis with values of  $13.700 \times 10^3/\mu\text{L}$  (reference 4-12  $\times 10^3/\mu\text{L}$ ), thrombocytopenia 30.000µ/L (reference 200.000 – 500.000µ/L)], azotemia [creatinine values 3.5g/dL (reference 0.5-1.5g/dL), BUN 295.7mg/dL (reference 21.4-59.92 mg/dL) and hyperphosphatemia 13.5mg/dL (reference 2.6-6.2mg/dL)]. Thoracic radiographs suggested pulmonary metastasis with a diffuse nodular pattern. The client elected to euthanize the patient. At autopsy, there were multiple nodules in the lung and the microscopic analysis of the lung parenchyma showed the same pattern described in the tumor mass and the femur (Fig. 03). Between the diagnosis and the euthanasia, 13 months passed after the diagnosis of adenosquamous mammary carcinoma

## RESULTS AND DISCUSSION

We report a case of bone and lung metastasis from mammary adenocarcinoma in a 11-year-old female Poodle. This dog presented the profile for mammary tumors and the most affected breeds are Poodle, Cocker Spaniel, and German Shepherd, aged between 9 and 12 years (SALAS *et al.*, 2015; DE NARDI *et al.*, 2016).

The mammary gland, prostate and urinary bladder are the most common primary sites of tumors with bone metastatic potential (COOLEY e WATERS,1998). Bone metastases are rare in dogs and less common than primary bone tumors (TOLEDO *et al.*, 2018, Simmons *et al.*, 2015). The rib, spine, and femur are the sites most affected in dogs with metastatic mammary carcinomas. The most common location is the metaphyseal region of the long bones and vertebral body (COOLEY e WATERS, 1998, SIMMONS *et al.*, 2015). Bitches with mammary carcinomas were the most prevalent cases (39 cases) and those that most often presented bone metastases (8/21, 38%). In most cases (7/8), bone metastases originated from simple variants of mammary carcinoma (SIMMONS *et al.*, 2015). The common metastasis sites due to mammary carcinoma were humerus, femur and vertebra (thoracic and lumbar), with four cases of each (trost) (SIMMONS *et al.*, 2015). In this case, the site of the primary tumor and the bone metastasis were similar. However, this report describes a bone and lung metastasis from adenosquamous carcinoma, a histological type rarely reported in bitches. (SIMMONS *et al.*, 2015).

In the present case, the femur was diffusely affected, resulting in a pathologic fracture in the diaphysis. In the bone, the microenvironment has a rich blood supply that facilitate the occurrence of bone metastases (RASOTTO *et al.*, 2017). Single metastases located in the proximal regions of long bones must be carefully differentiated from primary bone tumors also found frequently in these locations (MORELLO *et al.*, 2010). Furthermore, it is believed that long bones are more often affected due to their abundance of bone marrow suggesting that the metastatic route is hematogenous (RASOTTO *et al.*, 2017). Probably, the same occurred in this case report because neoplastic emboli were observed inside the blood vessels.

Cytology and histopathology were essential for the final diagnosis of a metastatic process (CASSALI *et al.*, 2014). In present study, cytologic examination of the left pelvic limb was useful in detecting the carcinomatous origin of the tumor, as well as excluding the possibility of other tumors with similar features (osteosarcoma, soft tissue sarcomas, histiocytic sarcoma and tumors of neuromuscular origin) (SANTANA *et al.*, 2016). A diagnosis of squamous cell carcinoma was considered due to the cytologic presentation of a malignant tumor with carcinomatous differentiation. However, the macroscopic appearance of the tumor with preserved cutaneous integrity and bone and musculature involvement, suggested a metastatic malignant tumor with squamous differentiation was more likely (FERREIRA *et al.*, 2003; CASSALI *et al.*, 2014). Histopathologic analysis of the left pelvic limb (femur and muscles) confirmed a diagnosis of malignant tumor with squamous differentiation similar to the adenocarcinoma likely (FERREIRA *et al.*, 2003; CASSALI *et al.*, 2014). This finding, linked to the previous history of adenosquamous mammary carcinoma, led to the diagnosis of a metastasis of this tumor to the left pelvic limb.

Survival times for adenosquamous mammary carcinoma in bitches are usually longer than for other subtypes such as anaplastic carcinoma and carcinosarcoma. This type of

carcinoma has the highest recurrence when compared with other histopathologic subtypes (RASOTTO *et al.*, 2017). Thus, the chances of cure are reduced due to the biological behavior of the squamous metaplastic component which has an infiltrative behavior pattern, similar to other tumors with squamous cell differentiation, such as the squamous cell carcinoma (RASOTTO *et al.*, 2017). In the present report, the bone metastasis occurred 13 months after the first surgery, similar to the time for metastatic development described by other Rasotto *et al.* (2017), who confirmed that the rate of distant metastases from adenosquamous mammary carcinoma was 60% (6/10) (mean survival time 18 months). Pulmonary metastasis was diagnosed 15 months after diagnosis of the mammary tumor, at the time of autopsy, corroborating findings the authors (RASOTTO *et al.*, 2017). However, the mammary tumor in the present study was grade II. This grade was one of the least common in the study, in which, of 10 bitches with adenosquamous carcinoma, 10% (1/10) were grade I, 20% (2/10) were grade II and 70% (7/10) were grade III (RASOTTO *et al.*, 2017).

## CONCLUSIONS

In conclusion, even though uncommon, bone metastases from mammary tumors can be included as differential diagnoses along with the most common bony tumors, especially in dogs with a previous history of mammary tumors with squamous differentiation. Finally, pathological fracture may be the first clinical sign resulting from metastasis in these patients.

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