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PATHOLOGY

Metastatic mammary carcinoma in a cow

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ABSTRACT: *Mammary gland neoplasms in cattle are rarely observed in the field veterinary diagnostics routine. Therefore, the objective of this study is to report a metastatic mammary carcinoma in a fourteen-year-old Holstein cow in the state of Santa Catarina, Brazil. The animal was diagnosed by the field veterinarian with clinical mastitis that was unresponsive to treatment, and was euthanized due to the poor prognosis. At the necropsy, multiple yellow, firm, and sometimes friable nodules, ranging from 0.1 to 20cm were observed in all mammary glands, lymph nodes, kidneys, spleen, liver, pancreas, mediastinal lymph nodes, heart, and lungs. The final diagnosis of mammary carcinoma was established through the association of clinical, necropsy, histopathological, and immunohistochemical findings. Differential diagnoses included diseases such as bovine tuberculosis and chronic fungal or bacterial mastitis.*
Key words: *cattle diseases, mammary neoplasia, metastasis, chronic mastitis, cytokeratin.*

Carcinoma metastático mamário em uma vaca

RESUMO: *Carcinomas de glândula mamária em bovinos raramente são vistos na rotina de diagnóstico no campo. Portanto, o objetivo do trabalho é relatar a ocorrência de um carcinoma de mama metastático em uma fêmea bovina de 14 anos, holandesa, no estado de Santa Catarina, Brasil. O animal foi diagnosticado pelo veterinário com mastite irresponsiva a tratamento clínico, e foi eutanasiada devido ao prognóstico desfavorável. Na necropsia foram observados múltiplos nódulos amarelados, firmes, e por vezes friáveis, variando entre 0.1 e 20cm, disseminados em todas as glândulas mamárias, linfonodos, rins, baço, fígado, pâncreas, coração e pulmão. O diagnóstico de carcinoma de glândula mamária foi baseado em achados clínicos, macroscópicos, histopatológicos e de imuno-histoquímica. Os diagnósticos diferenciais incluíram doenças como tuberculose bovina, mastites crônicas de origem bacteriana ou fúngica.*
Palavras-chave: *enfermidades em bovinos, neoplasia mamária, metástases, mastite crônica, citoqueratina.*

Mammary gland morphogenesis involves the regulatory function of several signaling pathways, growth factors and physiologic hormones. During the tumorigenic process the signaling is deregulated, thus allowing the mammary epithelium to expand, proliferate, and invade neighboring tissue (hyperplasia) (MIHEVC & DOVČ, 2013). However, unlike in domestic carnivores, mammary carcinomas are rare in cattle.

It is believed that the low incidence of mammary carcinomas in cattle is related to factors such as successive pregnancies, low exposure to estrogen during calving process, early culling, and high lactation rate (MIHEVC & DOVČ, 2013). In a literature review, FORD et al. (1989) described only 41 cases of mammary gland tumors in cattle since 1902.

According to POVEY & OSBORNE (1969), cows older than 8 years are more likely to develop cellular proliferative changes in the mammary gland. ELDER et al. (1954) reported a case of a three-year-old Aberdeen Angus heifer, which in late pregnancy developed an undifferentiated mammary gland carcinoma, associated with mastitis and abortion. The purpose of this article is to report a metastatic tubulopapillary carcinoma of mammary gland in a cow from Alto Bela Vista, Santa Catarina State, diagnosed by the Laboratory of Veterinary Pathology of the Instituto Federal Catarinense - Campus Concórdia.

A fourteen-year-old Holstein cow was diagnosed with clinical mastitis for the first time during its productive life, after its tenth calving.

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According to the field veterinarian, treatment based on penicillin, 20000Ukg^{-1} and flunixin meglumine, 2mg kg^{-1} was conducted.

The animal did not show clinical improvement, and a fistula draining moderate amount of purulent exudate appeared in the caudal aspect of the right rear quarter of the udder. Due to the unfavorable clinical progression, anorexia and weight loss, and the poor prognosis, the animal was submitted to euthanasia and necropsy.

Samples of mammary gland, liver, kidneys, lymph nodes, spleen, pancreas, intestines, lungs, heart, and central nervous system were collected, fixed in 10% buffered formalin, paraffin embedded, cut in sections ranging from 3 to 5µm, and stained with Hematoxylin and Eosin (H&E). Ziehl-Neelsen stain was also performed on the mammary gland tissue. Paraffin-embedded mammary gland samples were sent to the Departament of Compared Anatomy and Pathology of the University of Córdoba (UCO), for immunohistochemistry analysis, using anti-cytokeratin (CK) and anti-calponin primary antibodies. The method of immunohistochemistry analysis was performed according to PEÑA et al. (2014).

At necropsy, the mammary gland was severely enlarged, and presented a fistula draining a moderate amount of suppurative exudate on the right rear quarter (Figure 1A). The cut surface of all mammary gland quarters revealed multiple firm and yellowish nodules, ranging from 5 to 20cm, disseminated in the udder parenchyma. The larger nodules presented softened areas in the center (Figure 1B). Metastases were observed in the mammary lymph nodes, kidneys, spleen, liver, pancreas, mediastinal lymph nodes, heart, and lungs. These organs were enlarged, and presented multifocal, firm, white to yellowish nodules, which ranged greatly in size. Kidneys were the most severely affected organs, showing multifocal nodules ranging from 0.1 to 12cm, on the organ surface and parenchyma (Figure 1C). Mediastinal lymph nodes were severely enlarged, firm, and presented caseous pattern on cut surface. Nodules varying from 1 to 5cm were also observed in the lungs and heart.

Histological evaluation revealed a malignant neoplastic proliferation of epithelial cells, which was presented as an infiltrative, non-delimited, and poorly encapsulated mass, arranged in tubules and papillae with moderate amount of connective stroma. Cells presented abundant eosinophilic cytoplasm, sometimes vacuolated, and rounded to oval-shaped nuclei, ranging from 3 to 5µm (Figure 1D). The nucleoli were large, prominent, and usually single and chromatin was granular. Intense cellular atypia and rare mitotic figures were observed. Additionally, there were multiple foci of intraneoplasic necrosis. The same pattern was seen in the kidneys, lymph nodes, liver, spleen, pancreas, lungs, and heart, which characterized metastasis.

The immunohistochemical examination with anti-CK antibody (AE1/AE3 clones) (Figure 1E) showed intense immunoreactivity in the cytoplasm of many neoplastic cells, which confirmed the diagnosis of carcinoma. Neoplastic cells were negative for calponin, ruling out myoepithelial cells. The diagnosis of metastatic simple tubulopapillary carcinoma of mammary gland was established through the association of the clinical history, clinical signs, gross, histological, and immunohistochemical findings (DE LAS MULAS et al., 2005). Tumor classification was based on mammary carcinomas in female dogs, since there is no standardized classification described for cattle (GOLDSCHMIDT et al., 2011). In the present case we observed a tubulopapillary arrangement as described in the classification of mammary neoplasias in domestic canines.

In 2014, neoplastic diseases accounted for 5.2% of the diagnoses in cattle (5/95) established by the Laboratory of Veterinary Pathology of the Instituto Federal Catarinense - Campus Concórdia, and one case (20%) corresponded to the mammary gland carcinoma (1/5). In a retrospective study, LUCENA et al. (2011) reported one case (0.17%) of mammary gland primary neoplasia amongst 586 diagnoses of neoplastic diseases in cattle (8.7% of the total diagnoses in this species). There are few reports of mammary gland carcinomas in cattle; therefore, establishing the metastatic pattern may not be possible. PETRITES-MURPHY (1992) compiled 10 cases of mammary gland carcinomas reported in the literature, whereas six presented metastasis in the lymph nodes. Out of these six cases, three animals also showed metastasis in the lungs, of which two showed metastasis in other organs, including liver, diaphragm, pleura, pericardium, and kidneys. BEAMER & SIMON (1983) reported a case of mammary gland carcinoma presenting metastasis in the mammary lymph nodes. According to TESSELE & BARROS (2016), among 65 tumors found in cattle from slaughterhouses, only one corresponded to a mammary neoplasia, classified as mammary adenocarcinoma.

In this report, metastases were observed in several organs, including lungs and lymph nodes. Multiple nodules scattered in the affected organs were reported; however, they all had the same morphologic and histopathological patterns. The cut surface revealed friable, granular, and yellowish

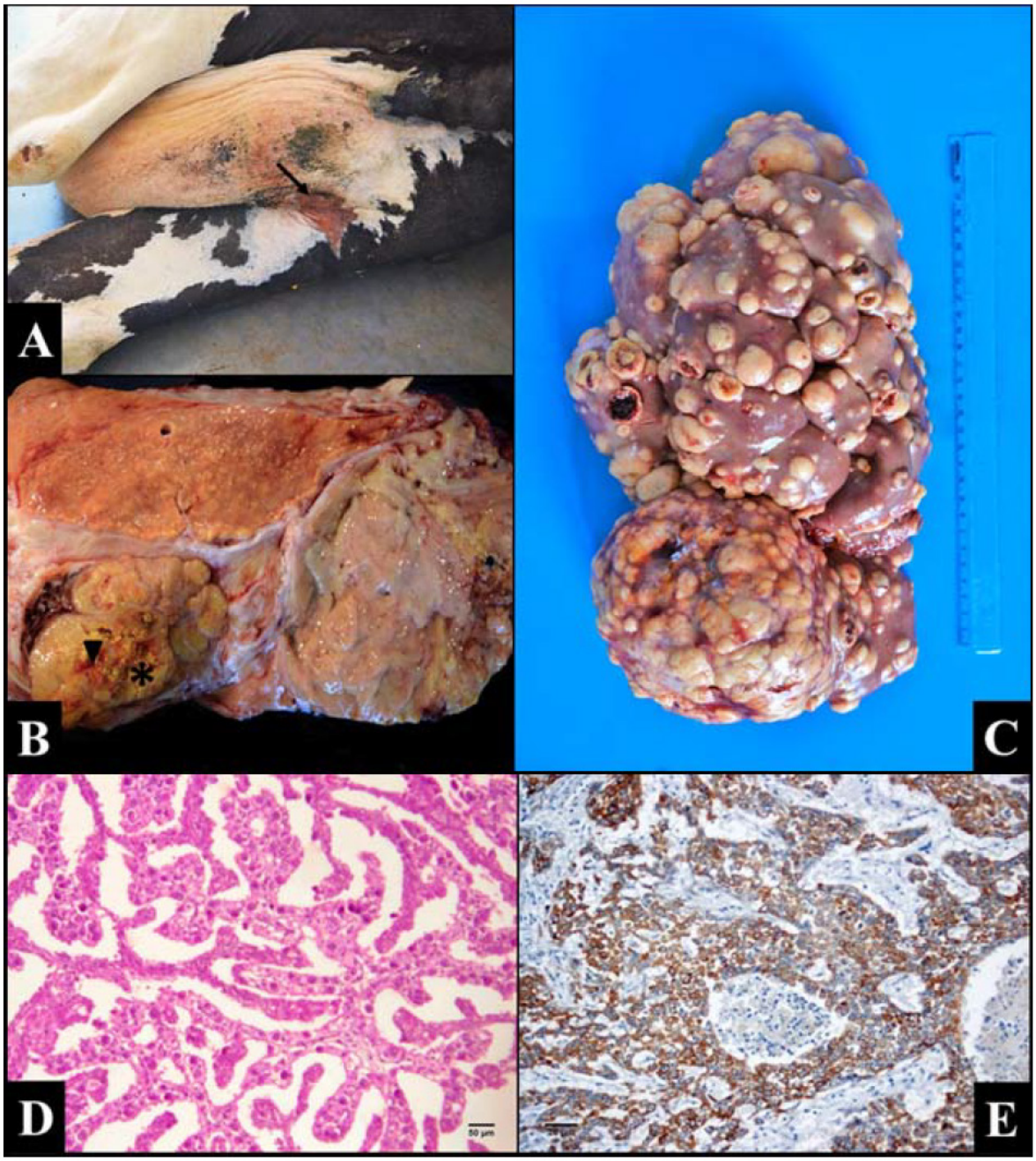

Figure 1 - A: Cow presenting a fistula in the caudal aspect of the right rear quarter of the udder (arrow). B: Cut surface of the mammary gland presenting multiple yellowish nodules. Necrotic material accumulation is observed in the center of some nodules (asterisk) as well as reddish multifocal areas of hemorrhages (arrow head). C: Kidney affected by metastases of mammary gland carcinoma. Multifocal to coalescing yellowish nodules are observed on the surface and parenchyma. D: Histopathology of mammary gland. Proliferation of epithelial cells arranged in tubules and papillae. Cells are cuboidal, with round to oval-shaped nuclei, and present abundant eosinophilic cytoplasm. HE, 100x. E: Immunohistochemistry of the mammary gland. Epithelial cells show intense positive intracytoplasmic staining for cytokeratin AE1/AE3. 200x.

material. These nodules were similar to those seen in well-differentiated squamous cell carcinomas, which produce keratin in the central cellular nests, forming "pearl like" structures (MAULDIN & KENNEDY, 2016). Additionally, the lesions in the mediastinal lymph nodes and the pulmonary nodules are grossly compatible with tuberculosis which is an important differential diagnosis that was ruled out through

histopathology as well as negative results in the Ziehl–Neelsen stain.

The histopathologic findings confirmed the neoplastic origin of the lesions, and it was possible to determine the primary origin of the tumor. Immunohistochemical evaluation was conducted in order to confirm the diagnosis. The antibodies tested were anti-cytokeratin AE1/AE3 and anti-calponin. The first one is a cocktail of antibodies that detects epithelial cytokeratins, so a positive immunostaining indicates the epithelial nature of the neoplasia. On the other hand, a negative result for anti-calponin antibody rules out the participation of myoepithelial cells in the composition of the lesion and confirms the simple type of the carcinoma (DE LAS MULAS et al., 2005). The association of immunohistochemichal and histological features of the tumor confirmed the diagnosis of simple epithelial carcinoma of mammary gland. The markers used in this case are routinely used for female dogs, since mammary tumors are rare in cattle.

The differential diagnoses of mammary tumors are described as mastitis, abscesses and hematomas. These lesions present sudden onset and easy distinction, since the animals usually show recovery after clinical treatment (MIHEVC & DOVČ, 2013).

According to the literature and the case reported here, it is concluded that mammary neoplasms are not common in cattle. Clinical development is chronic, usually accompanied by mastitis unresponsive to conventional treatment, and sometimes with metastases affecting other organs. It is unlikely to conclude this diagnosis in the clinical evaluation, so the pathological examination is frequently necessary. Cattle with chronic mastitis are usually culled, especially if they have already reached old age. Therefore, some cases of neoplasms may not receive a definitive diagnosis. It is pointed out that in cases of chronic mastitis which are unresponsive to treatment in cows older than eight years of age, mammary gland neoplasms should be considered as a differential diagnosis.

Definitive diagnosis can be usually reached through gross and histopathological examination. However, cases such as the one reported here, where many organs are affected by metastases, immunohistochemical analysis may be necessary in order to establish the primary origin of the epithelial tumor.

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