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## Leiomyosarcoma of the Skin and Subcutaneous Tissue in a Nellore Cow

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### ABSTRACT

**Background:** Leyomiosarcomas are uncommon malignant smooth muscle tumors of hollow organs. Bovine leyomiosarcomas have been described in several sites, but smooth muscle tumors that arise from the skin are rare in domestic animals. These neoplasms may show variable histologic features depending on their grade of differentiation; therefore, diagnostic tools as special stain, electron microscopy and immunohistochemistry are essential for the definitive diagnosis. Due to the lack of reports of skin leyomiosarcomas in cattle, we described the occurrence of a leyomiosarcoma in the skin and subcutaneous tissue of a Nellore cow.

**Case:** A 10-year-old Zebu Nellore cow was admitted at the Veterinary Diagnosis Center (CEDIVET) of Pará Federal University (UFPA), Brazil, to investigate a tumor on the hip. At clinical examination the cow showed a decreased appetite and weight loss. Due to the bad prognosis the veterinarian recommended euthanasia. Representative tissue samples were submitted for histopathological and immunohistochemistry evaluation at the Department of Pathology of the Federal Rural University of Rio de Janeiro (UFRRJ). The slides were stained by Hematoxylin and Eosin (HE), Masson's trichrome and Van Gieson stains and submitted for immunohistochemistry with primary antibodies vimentin, troponin C, smooth muscle alpha actin and desmin. Grossly, the tumor appeared as a multilobular, ulcerated and firm 30 cm diameter mass with black and hemorrhagic areas on the surface. The mass was scattered throughout the subcutaneous tissue and infiltrated the adjacent muscle. On light microscopy, the neoplasm consisted of a non-encapsulated proliferation into the derma, composed of highly pleomorphic spindle to round cells arranged in broad interlacing fascicles and small islands. Metastatic lesions were found in the lungs and local lymph nodes. Masson's trichrome stain revealed collagen production and Van Gieson stains was inconsistent. Immunohistochemically, the neoplastic cells expressed vimentin diffusely and smooth Alfa actin in 10% of the cells. Desmin and troponin C failed to express immunoreactivity.

**Discussion:** The histological, histochemical and immunohistochemical features of the neoplasm were consistent with a leiomyosarcoma of the skin and subcutaneous tissue. Leiomyosarcomas are uncommon in farm animals and most of them arise from the female genital tract, especially in the vagina and within the uterus. The majority of smooth muscle tumors of the skin are benign; however, we described here a neoplasm that displayed clear signs of malignancy. Due to the high grade of differentiation, Van-Gieson stain failed to determine the origin of the neoplasm. Many sarcomas have resembling histologic features and usually display interlacing bundles of spindle shaped cells, similar to the pattern described in the present tumor. Therefore, immunohistochemistry stain with vimentin and smooth alpha actin was essential for the definitive diagnosis. We identified metastatic lesions in the local lymph nodes and lungs. This tumor tends to develop slowly and metastasize late. These findings underline the importance of diagnostic tools, such as immunohistochemistry and special stains to guide the pathologist and to establish the diagnosis of bovine smooth muscle tumors. Consequently, an association of histochemical and immunohistochemical procedures is indispensable to guide the pathologist for the establishment of the diagnosis of bovine smooth muscle tumors.

**Keywords:** cattle, smooth muscle tumor, immunohistochemistry.

## INTRODUCTION

Leiomyosarcoma is an uncommon, malignant, smooth muscle tumor of hollow organs [8]. Surveys have described bovine leiomyosarcoma in the kidneys, intestines, lungs, bladder, gall bladder, mammary gland, perianal region, uterus, vagina and soft tissue of the neck [2-4,11].

Leyomiosarcomas of the skin are reported in dogs, cats and ferrets [5,6,10], but in general smooth muscle tumors of the skin are uncommon in animals.

Grossly leiomyosarcomas appear as a minimally ulcerated, nodular to multinodular, solid and firm tumor within the dermis. Histological features are variable, depending on its grade of differentiation. These tumors may be formed by spindle cells that retain many features of normal smooth muscle cells, or be more pleomorphic, with spindle to ovoid or round cells of variable histological pattern and numerous mitoses [8].

Thereby, distinguish between leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma and other sarcomas that arise from the skin may be difficult by routine histological methods. The definitive diagnosis requires electron microscopy and immunohistochemistry to determine the cell origin. In particular the latter is the best option, given its practicability and high sensitivity and specificity [1].

Due to the lack of case reports of skin leiomyosarcoma in cattle and the need to establish tools for differential diagnosis, we described the occurrence of a leiomyosarcoma in the skin and subcutaneous tissue of a Nellore cow.

## CASE

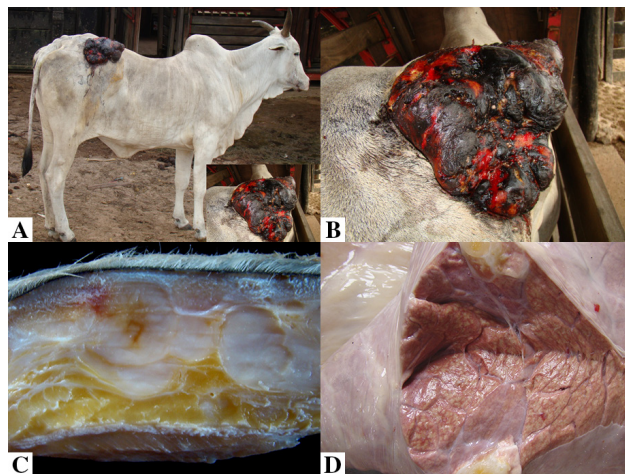
A 10-year-old Zebu Nellore cow was admitted at the Veterinary Diagnosis Center (CEDIVET) of Pará Federal University (UFPA), Brazil, to investigate a tumor on the hip, ventral to the costal tuberosity (Figure 1A). At clinical examination, the cow showed decreased appetite and weight loss. Due to the bad prognosis, the veterinarian indicated euthanasia.

Grossly, the tumor was 30 cm in diameter and appeared as a multinodular, ulcerated, infiltrative, firm mass, with black and hemorrhagic areas on the surface (Figure 1B). At cross-section, the tumor was firm and whitish in color. The mass was scattered throughout the subcutaneous tissue and infiltrated the adjacent muscle (Figure 1C). Similar masses were also seen in the lungs (Figure 1D).

Representative tissue samples were submitted for histopathological and immunohistochemistry evaluation in the Pathology Department of the Federal Rural University of Rio de Janeiro (UFRRJ). The samples were fixed in 10% buffered formalin, processed routinely, embedded in paraffin wax and stained with Hematoxylin and Eosin (HE), Masson's trichrome and Van Gieson stains.

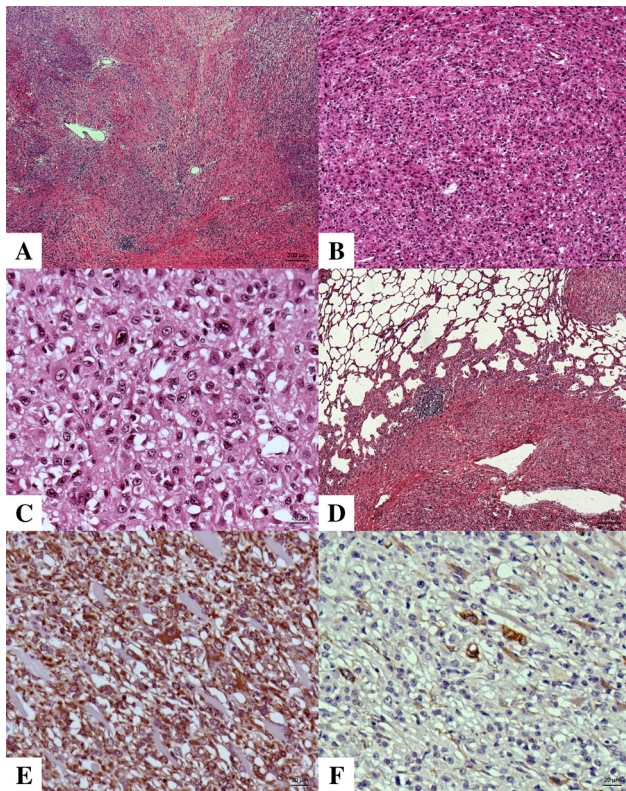
Additional formalin-fixed, paraffin wax-embedded sections were stained with primary antibodies vimentin (Monoclonal mouse antibody to human vimentin, Biosystems®, Clone V9, 1:200)<sup>1</sup>, troponin C (Lyophilized mouse monoclonal antibody troponin C, Novocastra® Clone 1A2, 1:100)<sup>2</sup>, smooth muscle alpha actin (Anti-alpha smooth muscle actin antibody abcam®, Polyclonal, 1:100)<sup>3</sup> and desmin (Anti-Desmin antibody abcam®, Clone DE-U-10, 1:100)<sup>3</sup>. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and antigen retrieval was performed with citrate buffer (pH 6.0) in a water bath set to 96°C. Non-specific reaction was blocked with 5% nonfat milk (Molico®)<sup>4</sup>. After overnight incubation, primary antibodies were detected by secondary antibodies conjugated with a biotin - free polymer - HRP marked with peroxidase (Spring Reveal polymer-HRP, mouse/rabbit®).<sup>5</sup> The reaction was developed with 3,3'-Diaminobenzidine (DAB) chromogen (Spring DAB Substrate System®)<sup>5</sup> and the preparations lightly counterstained with Harris hematoxylin.

Histologically, the tumor displayed a non-encapsulated proliferation in the derma, composed of spindle to round cells with mildly abundant eosinophilic cytoplasm (Figure 2A). The cells were arranged



**Figure 1.** A- A 10-year-old Zebu Nellore cow with a 30 cm broad tumor in the hip. B- The tumor consisted of a multinodular, ulcerated, infiltrative, firm mass with black and hemorrhagic areas on the surface. C- Details of the mass scattered throughout the subcutaneous tissue and infiltrating the adjacent muscle. D- Small whitish masses in the cross section of the lungs similar to the tumor observed in the skin.





**Figure 2.** A- A non-encapsulated neoplastic proliferation in the derma (HE, obj.5x). B- Proliferation of spindle to round cells in the derma arranged in broad interlacing fascicles (HE, obj.10x). C- High pleomorphic neoplastic cells with markedly dispersed chromatin and multiple inconspicuous nucleoli (HE, obj.40x). D- Metastasis of the leiomyosarcoma in the lung (HE, obj.10x). E- Neoplastic cells diffusely stained with vimentin (HE, obj.40x). F- Neoplastic cell stained positively for smooth alpha actin (HE, obj.40x).

in broad interlacing fascicles and small islands divided by abundant connective tissue (Figure 2B). The nuclei were round to elongated, frequently blunt-ended, with markedly dispersed chromatin and multiple inconspicuous nucleoli. The neoplastic cells were extremely pleomorphic, with highly irregular bizarre nuclei and numerous mitoses, including occasional abnormal forms (Figure 2C). Ulcerative areas and foci of lymphocytes infiltration were also seen scattered throughout the tumor. Proliferation of mesenchymal cells of the same nature were also seen in the lungs (Figure 2D) and local lymph nodes, qualifying metastatic lesions.

Masson's trichrome stain demonstrated collagen fibers encircling each cell. Van's Gieson stain was inconsistent. Immunohistochemically, the cells expressed Vimentin diffusely (Figure 2E) and smooth Alfa actin on approximately 10% of the cells (Figure 2F). Desmin and Troponin failed to express immunoreactivity.

## DISCUSSION

The histological, histochemical and immunohistochemical features of the neoplasm were consistent

with a malignant smooth muscle tumor arising from the skin and subcutaneous tissue of a Nellore cow and therefore classified as leiomyosarcoma.

Leiomyosarcomas are uncommon in farm animals and most of them arise from the female genital tract, especially in the vagina and within the uterus. In bovine genital neoplasm retrospective studies, smooth muscle accounted for 0.03% of the accessions and only one (0.0006%) had features of a leiomyosarcoma [12]. Likewise, a survey reported one vaginal leiomyosarcoma from 1,100 dairy and 1,300 beef cattle over a 10-year period [13].

The majority of smooth muscle tumors of the skin are benign and arise from the arrector pili [7]. However, we described a neoplasm that displayed clear signs of malignancy such as high pleomorphism, abnormal mitotic figures and metastatic lesions. Similar findings were reported in a leiomyosarcoma arising from the soft tissue of the neck in a cow. The authors mentioned widespread areas of necrosis and hemorrhage, high cellularity, marked cellular pleomorphism, atypical giant cells with multiple or bizarre-shaped nuclei, and numerous mitoses with occasional abnormal figures as malignancy criteria for this tumor [4].

Due to the high grade of differentiation, Van-Gieson stain failed to determine the origin of the neoplasm. Special stains such as Gomori's trichrome and Van Gieson stains may be useful in the diagnosis of well-differentiated leiomyosarcomas, but are unreliable indicators of muscle origins in poorly differentiated tumors [8].

Masson's trichrome technique showed a great collagen production by the neoplastic cells, a characteristic feature of leiomyosarcomas reported by several authors [4,9,11]. Meuten *et al.* [8] argued that the presence of fibrous connective tissue within a smooth muscle tumor does not warrant designation as a separate entity.

In the immunohistochemistry, the neoplasm had a strong diffuse reaction to vimentin and expressed Smooth Alfa actin on approximately 10% of the cells. Similar results were reported in other cases of leiomyosarcoma in cattle [2,4]. Alpha-smooth muscle actin is a specific marker for smooth muscle differentiation and can distinguish smooth muscle tumors from tumors of skeletal or cardiac origin. Nonetheless, less-differentiated tumors may have a small percentage of cells that react positively. Moreover, the author accounts

vimetin as a nonspecific marker of mesenchymal cells that is commonly more expressed in poor differentiated lesions [8]

Many sarcomas have resembling histologic features, and usually display interlacing bundles of spindle shape cells, similar to the pattern described in the present tumor. Therefore, immunohistochemistry is essential for an accurate histopathologic diagnosis [4].

We identified metastatic lesions in the local lymph nodes and lungs. This tumor overall tend to develop slowly and metastasis late. Most of the leiomyosarcomas reports failed to describe visible metastasis; however, Blair *et al.* [2] characterized a mammary leiomyosarcoma in a cow with metastasis to the lungs, adrenal and lymph nodes.

In conclusion, immunohistochemical staining with Alfa-actin and desmin proved effective for distinguish between smooth and striated muscle cells.

Especial stains such as Van Gieson and Masson's trichrome are useful in identifying myogenic cells and connective tissue; however, those techniques are usually not conclusive, especially in poor differentiated tumors. Consequently, an association of histochemical and immunohistochemical procedures is indispensable to guide the pathologist and to establish the diagnosis of bovine smooth muscle tumors.

#### MANUFACTURERS

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<sup>2</sup>Leica Biosystems. Newcastle, UK.

<sup>3</sup>abcam - Immunohistochemistry (IHC) kits and reagents. Cambridge, UK.

<sup>4</sup>Nestlé Brazil. Rio de Janeiro, RJ, Brazil.

<sup>5</sup>Spring Bioscience. Pleasanton, CA, USA.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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