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PATHOLOGY

Nasal leiomyosarcoma in a Quarter Horse

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ABSTRACT: *We described the clinical and anatomopathological findings observed in a case of nasal leiomyosarcoma in a five-year-old male Quarter Mile horse, whose main complaints were decreased sports performance and bilateral purulent nasal discharge. The nodule was observed in the nasal cavity, obstructing the left nostril and associated with purulent drainage. The nodule was of irregular shape and yellow color, measuring 19.4 cm × 6.9 cm × 4.3 cm in size, with coalescent multifocal areas that were brownish, friable, opaque, and fetid. When cut, the surface was compact, grayish-white, and smooth with yellow, friable, irregular multifocal areas, measuring 1–3.2 cm in diameter. Histopathological examination showed spindle-shaped neoplastic cells, which was negative on Masson's trichromic stain. A diagnosis of leiomyosarcoma was established based on the morphotintorial aspects of neoplastic cells and confirmed through immunohistochemistry, with positive immunostaining for antibodies 1A4, HHF35, desmin, and S100. Leiomyosarcoma primarily affects the nasal cavity of horses and should be included in the differential diagnosis of diseases that affect the nasal cavity and cause nasal obstruction associated with dyspnea.*
Key words: *nasal obstruction, dyspnea, desmin, sarcoma.*

Leiomiossarcoma nasal em equino Quarto de Milha

RESUMO: *Descreve-se os achados clínicos e anatomopatológicos observados em um caso de leiomiossarcoma nasal em um equino, Quarto de Milha, macho, de cinco anos de idade, com queixa principal de diminuição do rendimento esportivo e secreção nasal purulenta bilateral. Na cavidade nasal, observou-se um nódulo obstruindo a narina esquerda associada à secreção purulenta. O nódulo era de superfície irregular, amarelada, brilhante, fibroelástica, medindo 19,4 x 6,9 x 4,3cm de tamanho, com áreas multifocais a coalescente acastanhadas, friáveis, opacas e fétidas. Ao corte, a superfície era compacta, branco-acinzentada e lisa com áreas multifocais amarelas, friáveis e irregulares, medindo 1-3,2 cm de diâmetro. No exame histopatológico foi observado proliferação de células neoplásicas fusiformes, que foram negativas na coloração de Tricrômico de Masson. O diagnóstico de leiomiossarcoma foi estabelecido com base nos aspectos morfotintoriais das células neoplásicas e confirmado através da imuno-histoquímica, no qual houve imunomarcação positiva para os anticorpos 1A4, HHF35, Desmina e S100. O leiomiossarcoma pode afetar primariamente a cavidade nasal de equinos, devendo ser incluído no diagnóstico diferencial de doenças que afetam a cavidade nasal e que provocam quadros de obstrução nasal associado a dispneia.*
Palavras-chave: *obstrução nasal, dispneia, desmina, sarcoma.*

INTRODUCTION

Neoplasms in the nasal cavity and paranasal sinuses of horses are uncommon (LÓPEZ & MARTINSON, 2017) and can originate from the epithelial, glandular, vascular, bone, cartilaginous, and fibrous connective tissue (HEAD & DIXON, 1999). Moreover, they generally have an insidious onset with slow growth, and their main clinical signs are nasal discharge, epistaxis, and dyspnea (NICKELS, 1993).

Nasal neoplasms were usually described as sporadic cases, the most common being squamous cell carcinomas, fusiform and osteogenic sarcomas, lymphosarcoma, poorly differentiated carcinomas, adenocarcinomas, fibromas, fibropapillomas, chondromas, and osteomas (NICKELS, 1993; KNOTTENBELT et al., 2015; LÓPEZ & MARTINSON, 2017). Ethmoidal hematoma, the most common nasal proliferative lesion in horses, is not considered a true neoplasm and is a progressive syndrome that obstructs the nasal cavity in its caudal portion (WILSON, 2017).

Leiomyosarcoma is a neoplasm that originates from the smooth muscle and commonly affects the gastrointestinal tract and genital system

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of dogs and humans (COOPER & VALENTINE, 2017). In horses, this neoplasm affects the gastrointestinal tract (OREFF et al., 2018), urogenital tract (HURCOMBE et al., 2008), eyes (GROSÅS et al., 2017), ovary (PINNA et al., 2019), guttural pouch (DREW et al., 2016), lungs (ROSSDALE et al., 2004), and pelvic members (GIACCHI et al., 2020), as well as occurs in a multicentric manner (KAWABATA et al., 2016). However, there are no data on the primary involvement of the nasal cavity in equine species. Thus, this study described the clinical and pathological characteristics of primary leiomyosarcoma of the nasal cavity in horses.

MATERIALS AND METHODS

A horse with dyspnea was examined and radiographic examination of the head was performed using lateral-lateral projections.

The surgery was performed through the trepanation of the left nasal bone, in which an incision was made in the skin and periosteum, starting in the caudal nasal region at the symphysis and extending to the left at approximately 2.5 cm, continuing rostrally for 6 cm, and then turning to the median plane for 2.5 cm. Next, an osteotomy of the left nasal bone was performed using an osteotome and a hammer over the previously incised area: the bone was elevated using the osteotome, giving access to the nasal cavity. After identification and delimitation, the tumor mass was loosened through blunt dissection, and it was removed. Repositioning of the bone fragment, periosteal, and skin suture with a number 2 nylon thread was performed in a separate simple pattern.

Tissue fragments were fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E) for histological evaluation. Masson's trichrome histochemical method was also used to demonstrate the morphological characteristics of the cells.

Paraffin blocks were sent to a private laboratory for immunohistochemistry (IHC) analysis using anti-1A4, -HHF35, -desmin, -S100, -GFAP, -MyoD1, and -CD31 antibodies. Antigen recovery was performed using citrate buffers (pH 5.6) for anti-S-100, -1A4, -desmin, -HHF35, and -CD31 antibodies, and using EDTA buffer (pH 8.9) for anti-MyoD1 and anti-GFAP antibodies. The primary antibodies were incubated for 12h at 4°C. The polymer detection system (Advance Dako, Carpinteria, CA, USA) and 3,3-diaminobenzidine chromogen (Liquid DAB + Substrate-Choromogen System, Dako Carpinteria, CA, USA) with Harris hematoxylin were used.

The negative control was performed on the same section, in which it was processed without adding the primary antibody and by replacing it with PAS solution. The positive control was prepared using normal tissues indicated in the package insert of the antibody kits. For the antibodies against S100, 1A4, desmin, and HHF35 smooth muscle fragments, GFAP tissue fragment of the nervous system, MyoD1 skeletal muscle tissue fragment, and CD31 splenic tissue vessel fragments were used, respectively.

RESULTS

A five-year-old male Quarter Horse presented mainly with decreased sports performance, bilateral purulent nasal discharge that was more prominent in the left nostril, and dyspnea, which has approximately 6 months of evolution. Clinical examination revealed breathing difficulty and a slight increase in the volume of the left nostril. In the nasal cavity, a nodule obstructing the left nostril was observed, which was associated with purulent secretion. Radiography also showed an increase in radiopacity in the nasal cavity due to a structure with well-defined margins but without bone lysis (Figure 1), suggesting the presence of neoformation, granulomas, and abscesses.

Surgery was performed by trepanation of the left nasal bone. An incision was made in the skin and periosteum, starting in the nasal caudal region from the symphysis, extending to the left by approximately 2.5 cm, continuing rostrally by 6 cm, and passing to the medium plane at 2.5 cm. Then, left nasal bone osteotomy was performed over the previously incised area with the osteotome and malleus, and the bone was elevated using the osteotome, giving access to the nasal cavity. After identifying and delimiting the nodule, it was possible to release it through blunt dissection and to remove it, probably in its entirety. The nodule was attached to the left nasal cavity, affecting the dorsal, ventral, and ethmoidal shells. Next, the bone fragment was repositioned, suturing the periosteum and the skin with nylon 2 thread in a separate simple pattern. Finally, the nodule was sent for histopathological examination.

Externally, the nodule had an irregular, yellowish, shiny, fibroelastic surface, measuring 19.4 cm × 6.9 cm × 4.3 cm in size, with brownish, friable, opaque, and fetid multifocal coalescent areas (Figure 2A). When cut, the surface was compact, grayish-white, and smooth, with irregular multifocal areas that were yellow, friable, and measuring 1–3.2 cm in diameter (Figure 2B).

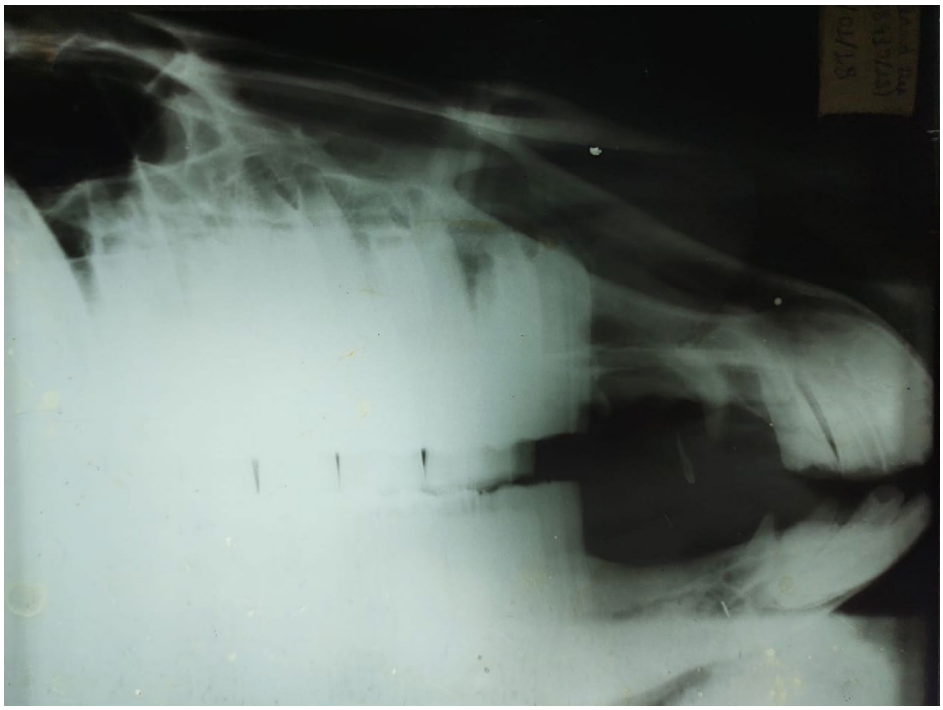

Figure 1 - Nasal leiomyosarcoma in horse. Radiography showed an increased radiopacity in the nasal cavity due to a structure with well-defined margins but without bone lysis.

Histopathological examination revealed an ulcerated, infiltrative, poorly delimited, and non-encapsulated tumor mass. This tumor mass was composed of spindle-shaped mesenchymal cells that were arranged in compact or loose bundles in various directions and were supported by a sparse stroma of fibrous connective tissues, which were associated with rare blood vessels stretching the lamina propria and dissecting the Bowman's glands. Neoplastic cells were elongated with eosinophilic cytoplasm, were homogeneous, and were poorly delimited. Nuclei were elongated, with finely granular to condensed chromatin and imperceptible nucleoli. Multinucleated cells were also observed. Pleomorphism was marked, and mitosis was moderate (1–3 per high-power field [400×] [A = 1,962.5 mm^2]) (Figure 2C and 2E). Amid the tumor mass, multifocal areas of discrete mononuclear inflammatory infiltrate were observed, consisting predominantly of lymphocytes and plasma cells, in addition to extensive areas of necrosis (Figure 2D).

The cytoplasm of neoplastic cells was stained red in Masson's trichrome staining (Figure 2F), and in the stroma, there was a discreet and multifocal marking in blue among the collagen fibers. Neoplastic cells expressed moderate and diffuse labeling for antibodies against 1A4, HHF35, and desmin, and discrete and irregular labeling for S100 (Figure 3). The cells were negative for GFAP, MyoD1, and CD31.

DISCUSSION

Leiomyosarcoma in the nasal cavity was diagnosed based on the morphological characteristics of the neoplastic cells and confirmed by IHC. In this study, observation of the morphological characteristics of the neoplastic cells together with the use of Masson's trichrome technique made it possible to directly diagnose a tumor of muscular origin (RAMOS et al., 2008). Such special staining should not be used as a reliable indicator for differentiating spindle tumors (WILSON, 2017), because poorly differentiated fibrosarcoma produces little collagen and may show little reactivity (GROSS et al., 2009). Thus, the use of IHC is essential in determining the cellular origin.

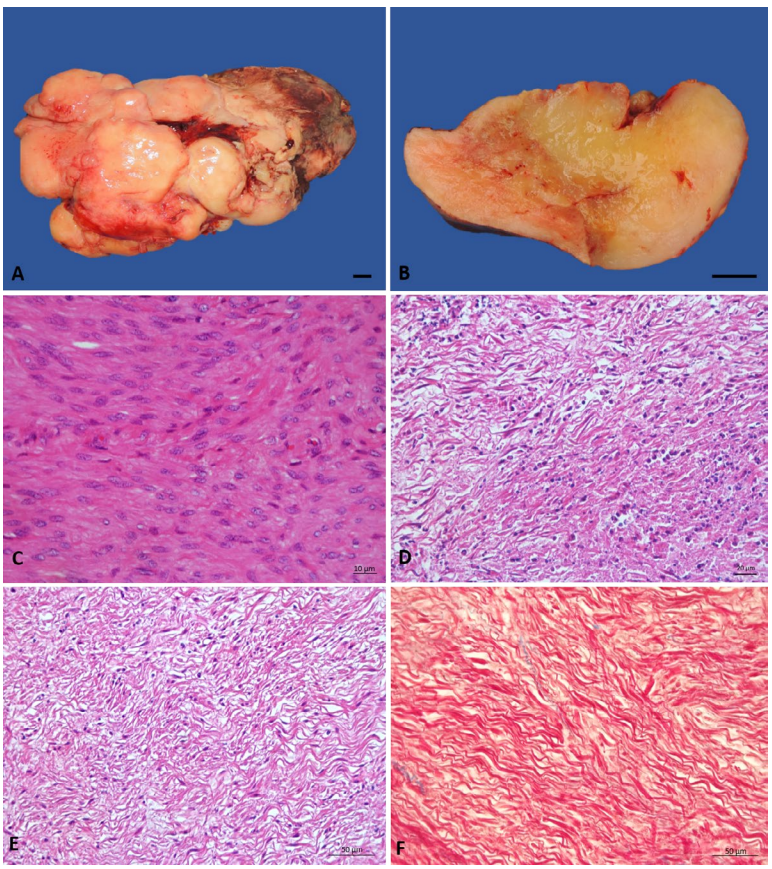

Figure 2 - Nasal leiomyosarcoma in horse. (A) Biopsy of the nasal cavity showing a multilobular nodule with a shiny yellowish surface, containing multifocal coalescent brown and friable areas. Scale bar = 1 cm. (B) Biopsy of the nasal cavity, cut surface, compact, yellowish-white and smooth. Scale bar = 1 cm. (C) Nasal cavity showing spindle-shaped neoplastic cells with an eosinophilic cytoplasm and poorly delimited borders. The nuclei are elongated with finely granular to condensed chromatin and little evident nucleoli. The cells are arranged in bundles. H&E staining, scale bar = 10 µm. (D) Nasal cavity. Amidst the neoplasm, there is an area of necrosis (lower right corner) associated with a mild infiltration of degenerated neutrophils. H&E staining, scale bar = 20 µm. (E) Nasal cavity. In other regions of the nodule, the bundles are loose compared to what is seen in (C). H&E staining, scale bar = 50 µm. (F) Neoplastic cells of the nasal cavity stained red in Masson's Trichomic stain. Scale bar = 50 µm.

IHC is an important diagnostic tool in determining the cellular origin of neoplasms because of its practicality, high sensitivity, and specificity when compared to other diagnostic methods (WILSON, 2017). In the evaluated sections, there was moderate and diffuse cytoplasmic reactivity in the neoplastic cells for the antibodies against desmin, 1A4, and HHF35, thus confirming the muscular origin and specifying the smooth muscle type using markers 1A4 and HHF35, given that the latter markers are specific for alpha-smooth muscle actin (DREW et al., 2016; GRACCHI et al., 2020).

Simultaneous positive immunostaining of neoplastic cells for smooth muscle alpha desmin and actin strongly suggested tumors of smooth muscle origin; although, striated muscle cells often express positive desmin marking, they are rarely positive for alpha smooth muscle actin (COOPER

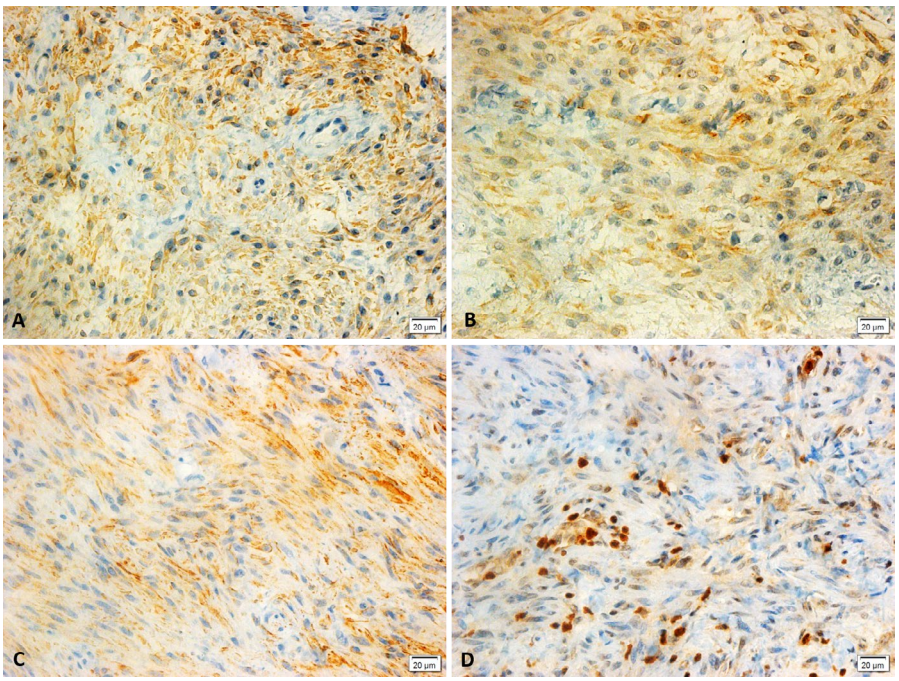

Figure 3 - Nasal leiomyosarcoma in horse. Neoplastic cells expressed labeling for 1A4 (A), HHF35 (B), desmin (C), and S100 (D) antibodies. Immunohistochemistry, scale bar = 20 µm.

& VALENTINE, 2017). In addition, the absence of immunostaining of neoplastic cells for the MyoD1 antibody reinforces the cellular origin as smooth muscle. The low reactivity of neoplastic cells with the S100 antibody was unforeseen, as observed by other authors (GROSÅS et al., 2017).

Neoplasms of the nasal cavity are uncommon in domestic animals, with the canine species being the most affected (LÓPEZ & MARTINSON, 2017). In the nasal cavity of horses, sporadic cases of mesenchymal tumors such as fibrosarcomas have been reported (SCHMOTZER et al., 1987; HULTGREN et al., 1987; KNOTTENBELT et al., 2015; LÓPEZ & MARTINSON, 2017), besides hemangiosarcomas (LÓPEZ & MARTINSON 2017), mastocytomas, osteosarcomas, osteomas, and myxomas (LÓPEZ & MARTINSON, 2017).

The main differential diagnoses include polyps of inflammatory origin (TROTTE et al., 2008), ethmoidal hematoma (TROTTE et al., 2008), amyloidosis (TROTTE et al., 2008; PORTELA et al., 2012), fibrous osteodystrophy, (LÓPEZ & MARTINSON, 2017), and rhinitis infectious diseases such as rhinosporidiosis (NICKELS, 1993; TROTTE et al., 2008), cryptococcosis (CRUZ et al., 2017), aspergillosis (GREET, 1981), and pythiosis (SOUTO et al., 2016).

In this case, leiomyosarcoma must also be differentiated from other neoplasms that have similar cellular characteristics, such as fibrosarcomas, peripheral nerve sheath tumors, rhabdomyosarcomas, undifferentiated sarcomas (WILSON, 2017), and myofibroblastic sarcoma (SILVA et al., 2012) from those in which immunohistochemical techniques must be used to determine the cellular origin of neoplastic cells.

CONCLUSION

Leiomyosarcoma can affect the nasal cavity of horses and should be included in the differential diagnosis of diseases that affect the nasal cavity, leading to the formation of large neoformations, nasal obstruction, and dyspnea.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of this manuscript. All authors critically reviewed the manuscript and approved the final version of the manuscript.

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