










Intramuscular epithelioid hemangiosarcoma in a horse

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ABSTRACT: A 20-year-old, sexually intact male Dutch Warmblood horse was presented with a 5-day history of difficulty moving and non-flexion of the right pelvic limb, followed by subcutaneous swelling and permanent lateral decubitus. Despite attempts at palliative care, the horse was euthanized after clinical worsening and lack of treatment response. The main gross finding was a soft, red-to-black, lobulated mass involving the right semitendinosus, semimembranosus, and biceps femoris muscles. Soft, red nodules were also seen in all pulmonary lobes and adrenal glands. Histology revealed neoplastic proliferation of malignant polygonal cells, occasionally arranged in irregular anastomosing vascular channels filled with erythrocytes. Neoplastic cells were immunoreactive for factor VIII-related antigen, confirming the blood vascular origin. A final diagnosis of epithelioid hemangiosarcoma was made. These findings reinforced the inclusion of hemangiosarcoma as a differential diagnosis of equine muscular lesions and highlighted the histological features of the epithelioid hemangiosarcoma subtype.

Key words: endothelium, hemangiosarcoma, horse, vascular neoplasms.

Hemangiosarcoma epitelióide intramuscular em um cavalo

RESUMO: Um cavalo macho não castrado, Sela Holandesa, de 20 anos de idade apresentou histórico de dificuldade de movimentação e não flexão do membro pélvico direito, seguido por inchaço subcutâneo e decúbito lateral permanente. Apesar das tentativas de cuidados paliativos, o cavalo foi eutanasiado após piora clínica e falta de resposta ao tratamento. O principal achado macroscópico foi uma massa macia, vermelha a preta e lobulada envolvendo os músculos semitendinoso, semimembranoso e bíceps femoral direito. Nódulos macios e vermelhos também foram observados nos lobos pulmonares e adrenais. A histologia revelou proliferação neoplásica de células poligonais malignas, ocasionalmente arranjadas em estruturas vasculares irregulares preenchidas por eritrócitos. As células neoplásicas foram imunorreativas para antígeno relacionado ao fator VIII, confirmando a origem vascular sanguínea. Portanto, foi realizado o diagnóstico final de hemangiossarcoma epitelióide intramuscular. Esses achados reforçaram a inclusão do hemangiossarcoma como diagnóstico diferencial das lesões musculares equinas e destacaram as características histológicas do subtipo hemangiossarcoma epitelióide.

Palavras-chave: equino, endotélio, hemangiossarcoma, neoplasias vasculares.

Vascular neoplasms arise from blood and lymphatic endothelial cells and are classified as benign, intermediate, and malignant proliferations (KNOTTENBELT et al., 2015). A recent update in the classification system of these tumors in domestic animals describes histological subtypes based on morphological patterns (ROCCABIANCA et al., 2018). In horses, vascular neoplastic proliferations are relatively uncommon, and the classification system is confusing due to variations in diagnostic terms used over the years (KNOTTENBELT et al., 2015). Although all histological types (hemangioma, hemangioendothelioma, hemangiosarcoma, lymphangioma, and lymphangiosarcoma) have been reported in equine oncology, the details on the histological subtypes are not completely

clear (SARTIN & HODGE, 1982; GEHLEN & WOHLSEIN, 2000; SANCHEZ et al., 2002; WARREN & SUMMERS, 2007; KNOTTENBELT et al., 2015). We now described the clinical and pathological aspects of an epithelioid intramuscular hemangiosarcoma in a horse.

A 20-year-old, sexually intact male Dutch Warmblood horse was evaluated by a private veterinary hospital for a 5-days history of difficulty moving and non-flexion of the right pelvic limb. Initial physical examination revealed stiffening of the musculature in the caudal region of the right thigh, followed by severe subcutaneous swelling in the right pelvic limb four days later. Treatment was attempted with IV administration of flunixin meglumine and sulfamethoxazole, and a discrete clinical improvement

was observed. After twelve days, the horse presented permanent lateral decubitus. Additional treatment instituted at this time involved IV administration of dimethyl sulfoxide. However, the horse's clinical signs evolved to pelvic limbs non-responsive to stimuli. Due to the general poor condition, lack of antemortem diagnosis, and questionable prognosis, the horse was euthanized and sent to the Department of Veterinary Pathology at the Universidade Federal do Rio Grande do Sul for pathological examination.

A systematic gross examination revealed a good body condition and pale, pink mucous membranes. The main finding was a focal, soft increase in volume in the muscle tissue of the caudal region of the right thigh. On the cut section, the right semitendinosus, semimembranosus, and biceps femoris muscles were expanded and infiltrated by a soft, red-to-black, lobulated mass (40-cm-diameter) (Figure 1A). Frequently, the muscle mass had numerous cavitated areas filled with blood fluid and was interspersed with fibrous tissue or yellow gelatinous material. Adjacent musculature showed focally extensive hemorrhage. Soft, red nodules (0.2- to 1.5-cm-diameter) were also observed in all pulmonary lobes (Figure 1B) and the medullar region of the adrenal glands. Additional significant gross features were not observed in other organs.

Samples of several organs were collected, fixed in 10% neutral-buffered formalin, processed routinely for histopathology, and stained with hematoxylin and eosin (HE). Histologically, the muscle mass was represented by a poorly demarcated, unencapsulated, highly infiltrative, neoplastic proliferation of polygonal endothelial

cells. Neoplastic cells were arranged in irregular anastomosing vascular channels filled with erythrocytes or occasionally in solid nests, supported by mild collagenous stroma (Figure 2A). Neoplastic cells had moderate amounts of eosinophilic, indistinct cytoplasm. Nuclei were oval with vesicular chromatin and prominent nucleolus (Figure 2B). There were moderate anisocytosis and anisokaryosis and 20 mitotic figures in an area of 2.37 mm². Marked hemorrhage, fibrous tissue proliferation, and hemosiderin-laden macrophage infiltrates were observed in intratumoral and peritumoral areas. Pulmonary and adrenal nodules were composed of similar neoplastic proliferation. No significant histological findings were seen in other organs.

To confirm the blood vascular origin, sections of the muscular tumor were submitted to immunohistochemistry using a polyclonal anti-factor VIII-related antigen (von Willebrand factor) antibody (ready-to-use; DakoCytomation, Carpinteria, California, USA). Mach 4 Universal HRP Polymer Kit (Biocare Medical, Concord, California, USA), Romulin AEC Kit (Biocare Medical), and Harris's hematoxylin were used as the detection system, chromogen, and counterstain, respectively. Immunoreactivity in the endothelium of adjacent normal blood vessels was considered an internal positive control, and a canine cutaneous hemangioma was used as an external positive control. The primary antibody was replaced by a Universal Negative Control Serum (Biocare Medical) in the negative controls. Diffuse, intense immunoreactivity was observed in the cytoplasm of the neoplastic cells of the muscle mass. Therefore, a final diagnosis of

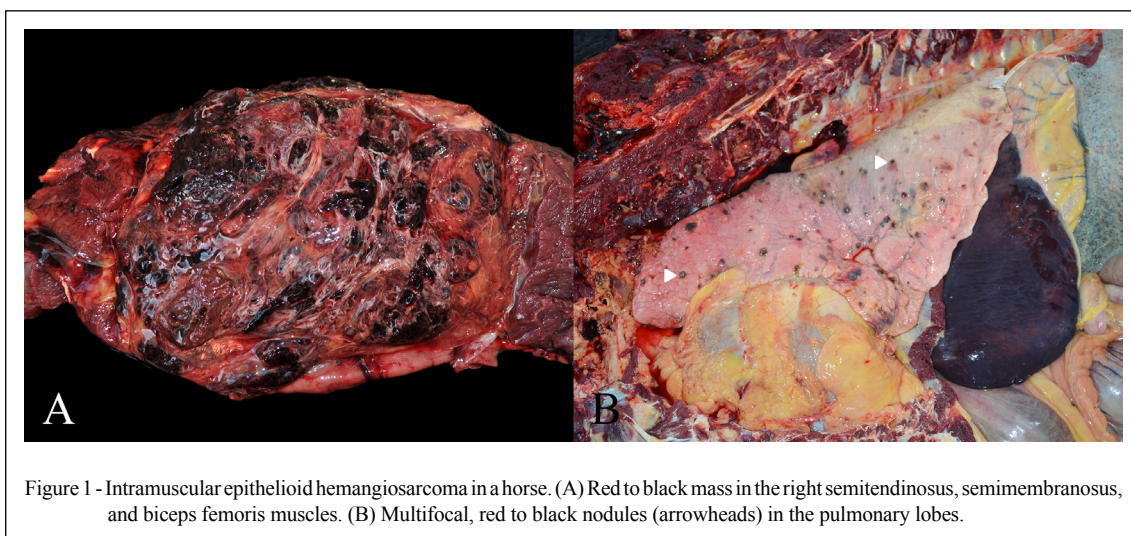


Figure 1 - Intramuscular epithelioid hemangiosarcoma in a horse. (A) Red to black mass in the right semitendinosus, semimembranosus, and biceps femoris muscles. (B) Multifocal, red to black nodules (arrowheads) in the pulmonary lobes.

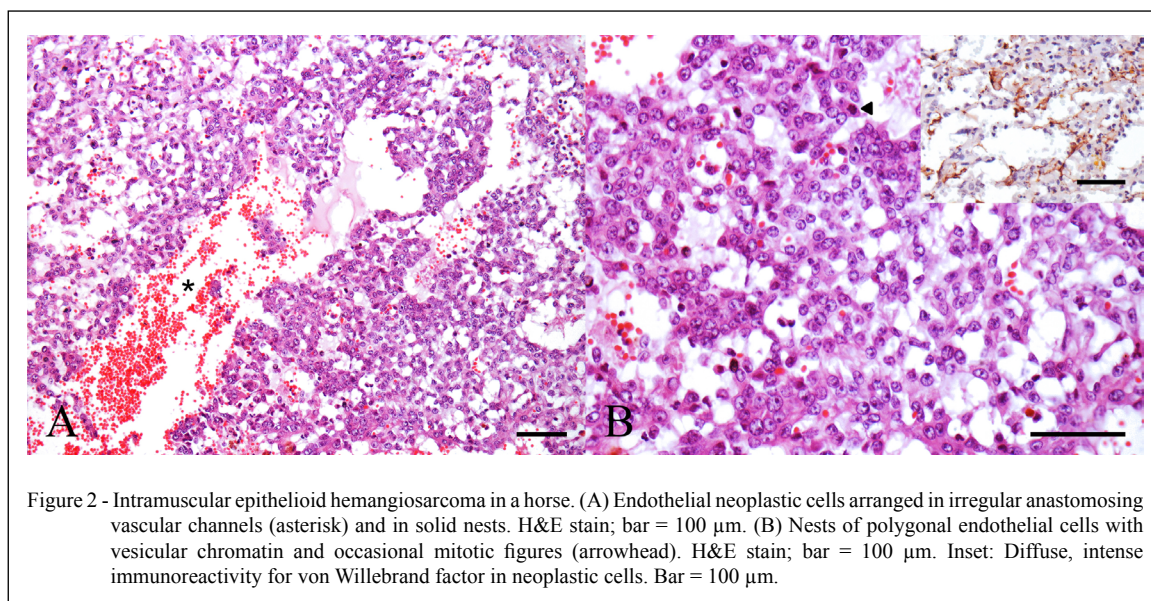


Figure 2 - Intramuscular epithelioid hemangiosarcoma in a horse. (A) Endothelial neoplastic cells arranged in irregular anastomosing vascular channels (asterisk) and in solid nests. H&E stain; bar = 100 μ m. (B) Nests of polygonal endothelial cells with vesicular chromatin and occasional mitotic figures (arrowhead). H&E stain; bar = 100 μ m. Inset: Diffuse, intense immunoreactivity for von Willebrand factor in neoplastic cells. Bar = 100 μ m.

epithelioid intramuscular hemangiosarcoma was made based on clinical, gross, histological, and immunohistochemical findings.

Hemangiosarcoma is a malignant neoplastic tumor derived from blood vascular endothelium. In horses, this neoplasm is relatively uncommon and comprises only 0.2% of postmortem diagnoses (PIERZAN et al., 2009). There is no apparent sex or breed predilection (SOUTHWOOD et al., 2000; KNOTTENBELT et al., 2015). The lesions mainly affect middle-aged to older horses; although they can be observed in foals and young animals (KNOTTENBELT et al., 2015). Disseminated tumors involving multiple systems, mainly the respiratory and musculoskeletal, is the most common pathological form observed in older animals (SOUTHWOOD et al., 2000). The primary site in these cases is related to skeletal muscle, spleen, and ovary or cannot be determined (SOUTHWOOD et al., 2000). Other frequent primary sites of hemangiosarcoma in horses include cutaneous, nasal, periocular, ocular, periarticular, and vertebral areas (ROCCABIANCA et al., 2018). In our case, based on the clinical findings and extent of lesions at the gross examination, the skeletal muscles of the right thigh were considered the primary site of the neoplasm.

Clinical findings of hemangiosarcoma in horses vary according to the affected system. In cases involving the respiratory and musculoskeletal system, the most common clinical signs are dyspnea, tachycardia, tachypnea, subcutaneous or muscular swelling, epistaxis, lameness, and pale or icteric

mucous membranes (SOUTHWOOD et al., 2000). Although the neoplasm involved the lung of the horse in the present case, respiratory clinical signs were not reported. Systemic clinical changes such as weight loss, anorexia, lethargy, and depression can also be observed. The duration of clinical signs between the first examination and dead is highly variable (SOUTHWOOD et al., 2000; JOHNS et al., 2005). Anemia, thrombocytopenia, and neutrophilic leukocytosis are the primary laboratory abnormalities seen in the affected animals (KNOTTENBELT et al., 2015). While a major challenge, the presumptive or definitive antemortem diagnosis can be attempted through several ancillary tests (eg, radiography, ultrasonography, cytology, and biopsy) (JOHNS et al., 2005; KNOTTENBELT et al., 2015). However, in some cases, the initial antemortem diagnosis of trauma is made based on the presence of hematoma and hemorrhage in the affected area (SOUTHWOOD et al., 2000). In this report, no antemortem diagnostic tests were performed.

Contrary to hemangiosarcoma in the skin, eye, and vulva, in which exposure to solar radiation may play an oncogenic role (KNOTTENBELT et al., 2015; DE NARDI et al., 2023), the risk factors for intramuscular hemangiosarcoma horses are not known. The classical gross appearance of hemangiosarcoma in domestic animals is red/brown to black, soft to firm nodules or masses of variable sizes (HENDRICK, 2017). Some tumors in horses typically resemble blood clots and have large areas of hemorrhage, mimicking

a hematoma (KNOTTENBELT et al., 2015). The intramuscular hemangiosarcoma usually infiltrates adjacent tissues and is lobulated and subdivided by thick fibrous septa (KNOTTENBELT et al., 2015). All these gross characteristics are identical to those observed in our case report. Pale mucous membranes and accumulation of blood in the abdominal and thoracic cavities may also be seen in cases of ruptured tumors (SOUTHWOOD et al., 2000; KNOTTENBELT et al., 2015). Most affected anatomical regions of primary intramuscular hemangiosarcoma in horses are the intercostal, abdominal wall, paravertebral, and thigh muscles (VALENTINE et al., 1986; COLLINS et al., 1994; TAN et al., 2014). Intramuscular hemangiosarcoma is highly aggressive and has a high capacity for metastasis, mainly to lung and spleen (COLLINS et al., 1994; TAN et al., 2014); in our case, the adrenal glands were also involved.

There are some variations in the pathological terminology used in equine vascular neoplasms (KNOTTENBELT et al., 2015). The current histological classification of hemangiosarcoma for domestic animals had three subtypes (ie, conventional, Kaposiform, and epithelioid) based on the morphological pattern of the neoplastic cells (ROCCABIANCA et al., 2018). Although histological subclassification is not entirely clear in most of the previous studies on intramuscular hemangiosarcoma in horses (VALENTINE et al., 1986; SOUTHWOOD et al., 2000; COLLINS et al., 1994), it tends to be conventional or Kaposiform (ROCCABIANCA et al., 2018). In our case, as the neoplastic vascular channels were lined by polygonal cells with vesiculated chromatin and prominent nucleolus, the diagnosis of the epithelioid subtype was made. This subtype is also described in ocular, nasal, and cutaneous locations in horses (WARREN & SUMMERS, 2007; ARENAS-GAMBOA & MANSELL, 2011; GUMBER et al., 2011). Due to the cell morphology, the epithelioid hemangiosarcoma should be histologically differentiated from undifferentiated carcinoma, epithelioid amelanotic melanoma, and epithelioid hemangioendothelioma (ROCCABIANCA et al., 2018). In the diagnostic work-up, this differentiation can be performed based on neoplastic architecture and application of immunohistochemistry for CD31 or von Willebrand factor (WARREN & SUMMERS, 2007).

Despite musculoskeletal clinical signs being common in disseminated hemangiosarcoma in horses (SOUTHWOOD et al., 2000), the history and clinical findings of this case initially suggested

a traumatic or neurologic disease (eg, rabies, equine protozoal myeloencephalitis, trypanosomiasis, and leukoencephalomalacia). Metastatic melanoma was also included as an additional differential diagnosis based on the gross findings. However, all these disorders were excluded with the histopathological and immunohistochemical analysis. The antemortem diagnosis of intramuscular hemangiosarcoma in horses, as well as in dogs, is associated with a poor prognosis (SHIU et al., 2011; TAN et al., 2014). The treatment is supportive and depends on the clinical signs. Palliative care includes nonsteroidal and steroidal anti-inflammatory drugs, furosemide, antimicrobials, and IV fluid therapy, but is generally considered unrewarding (TAN et al., 2014). As the clinical signs can deteriorate quickly, most parts of the affected animals are euthanized due to failure to respond to treatment (SOUTHWOOD et al., 2000), as was seen in this case.

In conclusion, this report provides the clinical and pathological findings of a metastatic intramuscular hemangiosarcoma in a horse. We also reinforced the inclusion of hemangiosarcoma as a differential diagnosis of equine muscular lesions and highlighted the histological features of the epithelioid hemangiosarcoma subtype.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled “Intramuscular epithelioid hemangiosarcoma in a horse” declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation of the Ethics Committee of the Universidade Federal do Rio Grande do Sul. However, we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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