

Malignant melanoma in albino water buffalo (*Bubalus bubalis*)

Melanoma maligno em búfalos (*Bubalus bubalis*) albinos

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Abstract

Two albino water buffalo affected by multiple melanocytic tumors in Southern Brazil are described. Grossly, there were multiple dark tumors within the skin, skeletal muscle, lungs, and lymph nodes. The tumor was also present in the pericardial sac, renal capsule, mediastinum and pleura. Microscopically, the tumors consisted of polyhedral epithelioid cells arranged in solid nests or interwoven fascicles supported by a thin and sparse collagenous stroma. The cytoplasm was eosinophilic and sometimes contained varying amounts of melanin pigment. The mitotic rates were low. Immunohistochemistry staining with Tyrosinase, Melan-A, Vimentin, S-100 protein, and neurofilament were positive. It is possible that the polymorphisms related to pigmentation in albino buffalo contributed with a high risk of developing melanoma as suggested in humans.

Key words: Albinism, water buffalo, immunohistochemistry, malignant melanoma

Resumo

Descrevem-se melanomas múltiplos em dois búfalos albinos da raça Murrah no Sul do Brasil. Macroscopicamente havia múltiplos tumores enegrecidos no músculo esquelético, pulmões, linfonodos, saco pericárdio, cápsula renal, mediastino e pleura. Microscopicamente foram observadas células epitelióides poliédricas dispostas em ninhos sólidos ou fascículos entrelaçados sustentadas por um estroma colagenoso fino e esparso. O citoplasma era eosinofílico e por vezes continham quantidades variáveis de pigmento de melanina. A taxa de mitose foi baixa. Na imuno-histoquímica houve imunomarcagem positiva utilizando anticorpo anti-tirosinase, anti-Melan-A, anti-vimentina, anti-proteína S-100 e anti-neurofilamento. É possível que o polimorfismo relacionado com o gene da pigmentação em búfalos albinos tenha contribuído para o maior risco de desenvolvimento de melanomas, como foi sugerido em humanos.

Palavras-chave: Albinismo, búfalos, imuno-histoquímica, melanoma

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Introduction

Melanocytic tumors are rare in cattle (MILLER et al., 1995; SMITH et al., 2002) and there are a few reports of the occurrence of this tumor in water buffaloes in India and Pakistan (EFEM et al., 2009; SABRI et al., 2010). Melanomas originate from neuroectodermal melanoblasts, which migrate at the beginning of their developmental period into the epidermal-dermal junction of the skin, follicles, and dermis (PULLEY; STANNARD, 1990). These neoplasms are common in dogs and in gray or white horses and less frequent in cats and sheep. The incidence is occasional in Duroc and Sinclair miniature swine (SMITH et al., 2002). Melanocytic tumors may also occur in other domestic species, such as cattle, sheep, and goats (BABIC et al., 2009; BRITO et al., 2009; VALA et al., 2012; FAZILI et al., 2013).

In humans, cutaneous malignant melanoma incidence continues to increase worldwide (HUNTER et al., 2013). However, there are few reports on melanomas in albino human beings (LEVINE et al., 1992).

The purpose of this report was to describe two cases of malignant melanoma (MM) in albino water buffalo in Southern Brazil.

Case Report

The disease affected two buffalo out of six albino animals from a 280-head Murrah buffalo herd located in the state of Rio Grande do Sul, southern Brazil (31°49'03" S and 52°26'25" W) during the spring of 2012. Case No. 1 was a 6-year-old female, and Case No. 2 was a 12-year-old female. Case No. 1 showed swelling in the chest and in the left forelimb extending across the abdomen and persisting for 24 days (Fig. 1A). In case No. 2, swelling in the inguinal region was observed, lasting for a period of 19 days. In the skin of case No. 1, there were numerous multifocal to coalescing black flattened macule or patch-like areas with irregular edges

primarily in the head and dorsal region of the body. In the skin of case No. 2, sparse black flattened macule areas were observed in the dorsal region of the body. Both animals were euthanized due to the poor prognosis according with the recommendation by the Conselho Federal de Medicina Veterinária.

In case No. 1, the subcutaneous tissue of the affected limb had edema and multiple black nodules 1-5 cm in diameter. The forelimb had multiple coalescent nodules interspersed in the muscle (Figure 1A). The left precrucial lymph node was enlarged (3 x 5 x 10cm), and the right precrucial lymph node showed a dark mass replacing the tissue. Small blackish nodules were also observed on the surface of the pericardial sac near the aorta, in the mediastinal lymph nodes and in the internal intercostal muscles and pleura. There was fluid in the thoracic cavity and pulmonary emphysema, primarily in the apical lobes. The other organs had no significant lesions.

Case No. 2 had severe edema in the subcutaneous tissue of the inguinal region, extending across the abdomen. At the region of the pre-femoral lymph node, there was a black mass approximately 15 x 20 x 30cm covered by a whitish capsule of connective tissue. Adjacent to this area, massive 1 x 1,5 cm blackened nodules were distributed from the inguinal to the thoracic region, where they were sparser.

In the abdominal cavity, blackened coalescent nodules covered the lymph vessels accompanying the abdominal aorta throughout its path. The capsule of the left kidney also had a black mass near the hilum.

In the thoracic cavity, multiple black nodules covered the surface of the lung and had invaded the parenchyma. Additionally, there were coalescent nodules on the surface of the aorta and small nodules on the surface of the parietal pleura. No significant injuries were observed in the other organs.

Fragments of the affected muscle and organs, including the nervous system, were fixed in 10%

formalin and embedded in paraffin and cut into 5- μ m sections. The slices were stained with hematoxylin and eosin (HE). For immunohistochemistry, the Biotin-streptavidin-peroxidase technique was used with the primary antibodies Tyrosinase (1:400, Novacastra, Newcastle Upon Tyne, UK), Melan-A (1:100, Dako, Glostrup, Denmark), Vimentin (1:200, Invitrogen, Camarillo, US), S-100 protein (1:100, Dako Glostrup, Denmark), and neurofilament (1:500, Serotec, Kidlington, UK). The chromogen was 3,3'-diaminobenzidine and 3-amine-9-ethylcarbazole chromogen (AEC, DakoCytomation, Carpinteria, CA), and the counterstain was Mayers hematoxylin. Sections of skin, tonsil, a dog melanoma and brain were used as positive controls. For negative controls, instead of the primary antibodies, a phosphatase-buffered saline solution was used.

Microscopically, all neoplastic masses were similar in character. The tumors consisted of polyhedral epithelioid cells arranged in solid nests

or interwoven fascicles supported by a thin and sparse collagenous stroma. The cytoplasm was eosinophilic and sometimes contained varying amounts of melanin pigment. The nuclei were irregularly round to oval and were vesicular with chromatin margination along the membrane; single or double nucleoli were also evident. There were few binucleate cells. The mitotic rate was low, commonly ranging from 0-2 per 5 high-power fields (40x). The skin was infiltrated by nests of melanin-containing neoplastic cells displaying junctional activity (Figure 1B) and lymphatic invasion. In sections used for immunohistochemistry staining, there was a marked specific immunostaining of melanocyte cytoplasm in tyrosinase (Figure 2A) and mild to moderate immunostaining in melan-A (Figure 2B), marked immunostaining in vimentin (Figure 2C), and moderate to marked immunostaining in S-100 (Figure 2D). There was mild immunostaining in neurofilaments and no immunostaining in cytokeratin.

Figure 1. Albino water buffalo. **A-** There is swelling from the chest to the abdomen. Inset: variably sized masses extensively replace the muscles. **B-** Skin, melanin-containing neoplastic cells displaying junctional activity. HE. Bars=50 μ m.



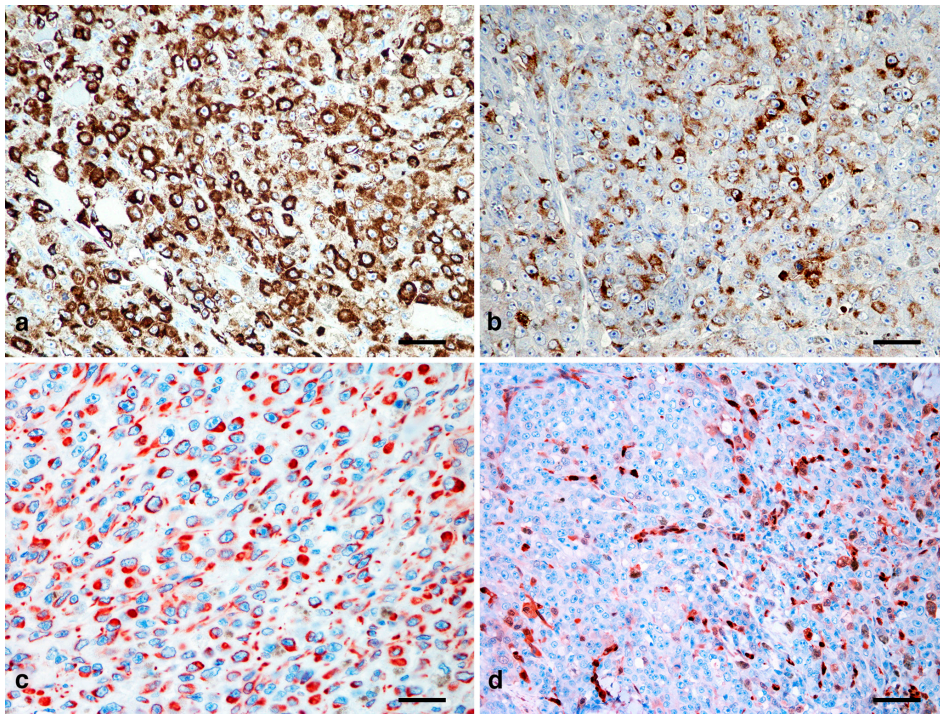
On the bases of morphologic features and immunohistochemistry results, this neoplasm was considered consistent with the MM epithelioid type.

There are a few reports of melanoma in water buffalo, with the majority of the diagnosed cases in India and Pakistan (SABRI et al., 2010). Susceptibility to the occurrence of this

tumor is most likely related to the lack of skin pigmentation. The high frequency of occurrence of MM is associated with exposure to ultraviolet (UV) radiation. The animals of this report were albino, and they were maintained in Rio Grande do Sul, where there are the highest concentration

of UV ambient due to its geographical location in Brazil (BAKOS et al., 2002). Animals with little pigmentation in regions with a higher incidence of UV radiation are highly vulnerable to the development of melanomas (GOLDSCHMIDT et al., 1998).

Figure 2. Immunohistochemical expression of **A:** tirosinase, **B:** melan-A, **C:** vimentin, and **D:** S-100 in the cytoplasm of the neoplastic cells. Bars=50µm.



In humans, the prevalence of MM in albinos remains relatively rare, and approximately 30 cases have been documented in the literature (EFEM et al., 2009; WICK, 2010). This is the first report of MM in albino water buffalo in the English-language literature to date. Buffalo albinism in this report was identified as a nonsense mutation in the tyrosinase gene (DAMÉ et al., 2012).

Macroscopically and histologically, the tumor was pigmented and had positive immunostaining for anti-tyrosinase. This showed that in these animals there is tyrosinase expression in the cells; this likely contributed to the synthesis of melanin by the

tumor. In humans, approximately 77% of patients with albinism had amelanotic lesions (LEVINE et al., 1992).

Malignant melanomas are typically vimentin-, neuron-specific enolase-, S-100 protein-, NKIC3-, and HMB-45-positive. Anti-Melan-A and anti-tyrosinase antibodies have been introduced as two other melanocytic markers (WICK, 2010). In the albino water buffaloes, the tumors were positive for S-100 protein, Melan-A and Vimentin. In addition, these tumors were positive for neurofilament protein. The association of both neuroendocrine and melanocytic proliferation with

neuroectoderm could explain why this neoplasm demonstrates immunohistological homologies with a neuroendocrine proliferation (WICK, 2010). Nevertheless, this positive reactivity could be considered aberrant; it could create diagnostic problem (WICK, 2010).

Anti-tyrosinase and Melan-A antibodies show a high level of sensitivity and virtually absolute specificity for melanoma (WICK, 2010). S-100 protein is a marker for cells of the neural crest and is widely used in the diagnosis of melanomas (SMITH et al., 2002).

Melanocytic tumors are rare in cattle (MILLER et al., 1995; SMITH et al., 2002), representing approximately 5.1% out of 2,728 diagnosed tumors in this species (RAMAKRISHNAN; MURALIMANO HAR, 1980). In cattle, melanomas are often benign and occur in dark-skinned races such as Aberdeen Angus (MILLER et al., 1995). Some studies have shown that brown or red-skinned races are predisposed to the occurrence of this tumor (MILLER et al., 1995). In a study of 20 cases, 18 animals had gray skin pigmentation and two had red skin (MILLER et al., 1995).

Recently, various genetic polymorphisms related to pigmentation have been associated with a high risk of developing melanoma in humans (GUDBJARTSSON et al., 2008). These factors combined with constant exposure to solar radiation may have contributed to the development of skin malignancies in albino water buffalo.

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