Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Case Report

Diffuse melanosis secondary to metastatic melanoma in a Nelore bull

Jacqueline de Jesus[1], Andrieli Bortolini[1], Gabriela Corrêa de Almeida[1], Bárbara Cardoso de Oliveira[1], Artur Bruzamarelo[2], Raquel Rubia Rech[3], Claudio Severo Lombardo de Barros[4], Carlos Eduardo Fonseca Alves[5], Fabiana Elias[1]*.

[1] Faculdade de Medicina Veterinária da Universidade Federal da Fronteira Sul (UFFS), Realeza, PR
[2] Médico Veterinário autônomo, Rua Euclides Prade 465, Bairro Santa Maria Chapecó, SC
[3] Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX 77843-4467, USA.
[4] Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS.
[5] Instituto de Ciências da Saúde, Universidade Paulista (UNIP), Bauru, SP

***Corresponding author:** Fabiana Elias. Faculdade de Medicina Veterinária da Universidade Federal da Fronteira Sul (UFFS), Realeza, PR, Brazil. E-mail: fabiana.elias@uffs.edu.br

Submitted July, 21th 2021, Accepted August, 31th 2021

Abstract

We report a case of diffuse melanosis secondary to metastatic malignant melanoma in a Nelore bull. Clinical signs included isolation from the herd, epistaxis, hyperthermia, pale ocular membranes, mucoid diarrhea and dark urine. Despite anti-inflammatory and antibiotic therapy, the bull died 45 days after the onset of the clinical signs. The most striking lesion was diffuse black discoloration to the visceral organs including liver, spleen, lungs, lymph nodes, and kidneys; all these affect organs were moderately enlarged". The urine was black. Histologically, 50-80% of the parenchyma of the liver, spleen and lymph nodes was obliterated by aggregates of melanin-loaded neoplastic melanocytes. Those neoplastic cells also occurred within capillaries of the liver, spleen, lymph nodes, urinary bladder, lungs and kidneys. Immunohistochemistry of neoplastic melanocytes was positive for Melan A and PNL2 markers. Abundant brown to black pigment was found in melanophages in the lungs, confirmed by IBA1 immunohistochemistry.

Key words: Malignant melanoma, diffuse melanosis, immunohistochemistry, diseases of cattle.

Introduction

Melanosis is hyperpigmentation of various tissues and organs not usually pigmented, resulting from overproduction and accumulation of melanin due to abnormal migration of melanocytes during embryogenesis, in this case without much clinical significance, or even, although rare, due to an association with malignant melanomas (1,4,8,10,11). Malignant melanomas are common in dogs, may constitute 10-15% of the melanocytic tumors in miniature Sinclair pigs, and are uncommon in cats. These types of neoplasms are common in gray horses; however, in most instances, they are benign (7). Depending on the amount of melanin production, they are classified as melanotic or amelanotic (7).

Melanocytic tumors are rare in cattle and represent 6% of all tumors in this species (9), some of which have included metastases (2, 19). They are usually benign and affect young stock under two years old, commonly those with black skin, with an increased prevalence in the Aberdeen Angus breed (19). They are characterized by focal masses in the oral cavity, eyes, trunk, and mandible (16).

Although melanocytic neoplasms are rarely reported in cattle, focal production of melanin from melanocytes in atypical tissues is frequently described in cattle, mainly in the lungs (18), and in other domestic mammalian species (13,14) and birds (17).. Focal melanosis is reported in commercial birds, which are frequently condemned at the slaughterhouse (16). There are, however, breeds of poultry such as the Silkie chickens that develop

 All rights reserved 2007-2021.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

extensive and diffuse melanin hyperpigmentation due to a genetic alteration. Although uniformly black, the meat of these birds is commercially appreciated (3). Interestingly, certain dysregulation in the melanin pigmentation of the skin in humans, such Mongolian spot, nevus of Ota, and incontinencia pigmenti are genetically related (5).

Diffuse melanosis secondary to melanoma has been reported in humans, but to our knowledge, not in animals. In humans, it is reported as gray to black discoloration of the skin, mucous membranes, internal organs (4), body fluids (peritoneal fluid and urine), and excretion of melanin-derived products (8). The prognosis of this condition is reserved to unfavorable, and the time of survival of affected patients after diagnosis is about four months (8).

We report a case of generalized diffuse melanosis associated with metastatic melanoma in a Nelore bull, and, as far as we can determine, is the first report of this condition in animals.

Case report

An eight-year-old, 700 kg Nelore bull (Bos taurus indicus) had presented isolation from the herd, and hyporexia for about 45 days. The bull had nose bleeding and black urine for three days prior to death. The external physical examination revealed pale ocular mucosa and a rectal temperature of 39.5°C. The presumptive clinical diagnosis was leptospirosis. The animal did not respond to the treatment based on antibiotics and anti-inflammatory drugs. He died nine days after the treatment, and a field necropsy was performed.

At necropsy, a striking diffuse black discoloration was observed in the liver, lymph nodes, spleen, lungs (Figs. 1-3), and kidneys on capsular and cut surfaces. The liver, spleen and lymph nodes were moderately and diffusely enlarged. The urine was black. Irregular white plaques (mineralization) were in the intima of the aorta. Samples of the kidneys, lungs, liver, lymph nodes, heart, spleen, testes, aorta, intestine, and urinary bladder were collected in 10% buffered formalin and routinely processed for histopathology.

Histologically, effacing and replacing 70-80% of the hepatic parenchyma were nodular, densely cellular aggregates of neoplastic melanocytes, supported and dissected by moderate bands of fibrous stroma (Fig. 4). Neoplastic cells have variably distinct cell borders, a large amount of eosinophilic cytoplasm containing abundant brown to black granular pigment (melanin), and round nuclei with finely stippled chromatin and one large prominent basophilic nucleolus. Anisocytosis is moderate to marked and anisokaryosis is moderate with occasional binucleation. The mitotic rate is 2 in ten 40x fields. The neoplastic cells infiltrate into capillaries. Similarly, the normal architecture of the lymph nodes, including the cortex and medullary sinuses was obliterated by neoplastic melanocytes (Fig. 5). Neoplastic melanocytes infiltrated the splenic parenchyma. The splenic capsule displayed multifocal areas of mineralization. Within capillaries of the alveolar septa of the lungs (Fig. 6) and less extensively, within glomerular tufts capillaries of the interstitium were heavily black-pigmented cells. Scattered melanocytes were intermingled between transitional epithelial cells of the urinary bladder and in the lumina of capillaries. Individual cardiomyocytes were necrotic and mineralized. Transmural mineralization was observed in the aorta.



Fig. 1. Hepatomegaly with diffuse melanosis. **Fig. 2.** Lymphadenomegaly with diffuse melanosis. **Fig. 3.** Diffuse pulmonary melanosis.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

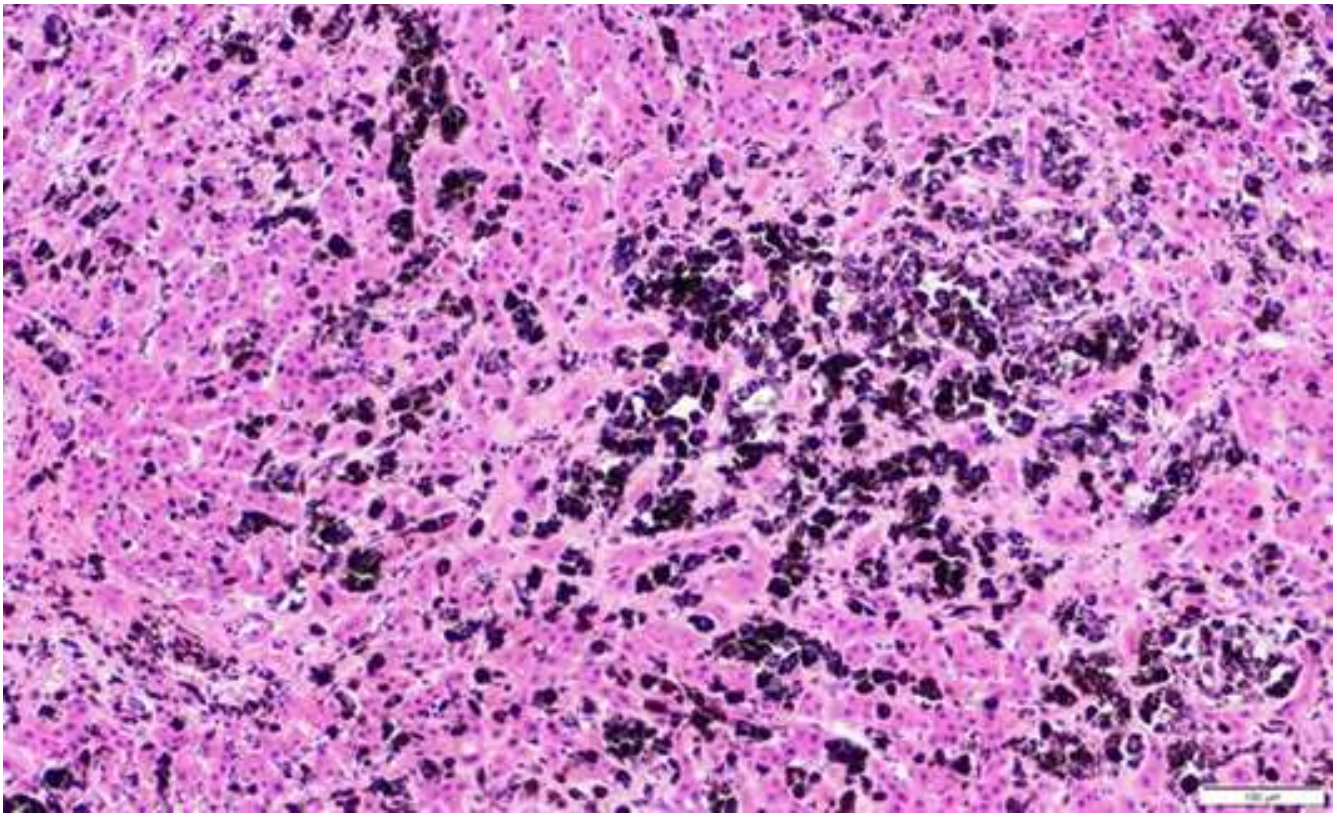


Fig. 4. Liver. Approximately 70% of the hepatic parenchyma is replaced by neoplastic cells containing intracytoplasmic black granular pigment.

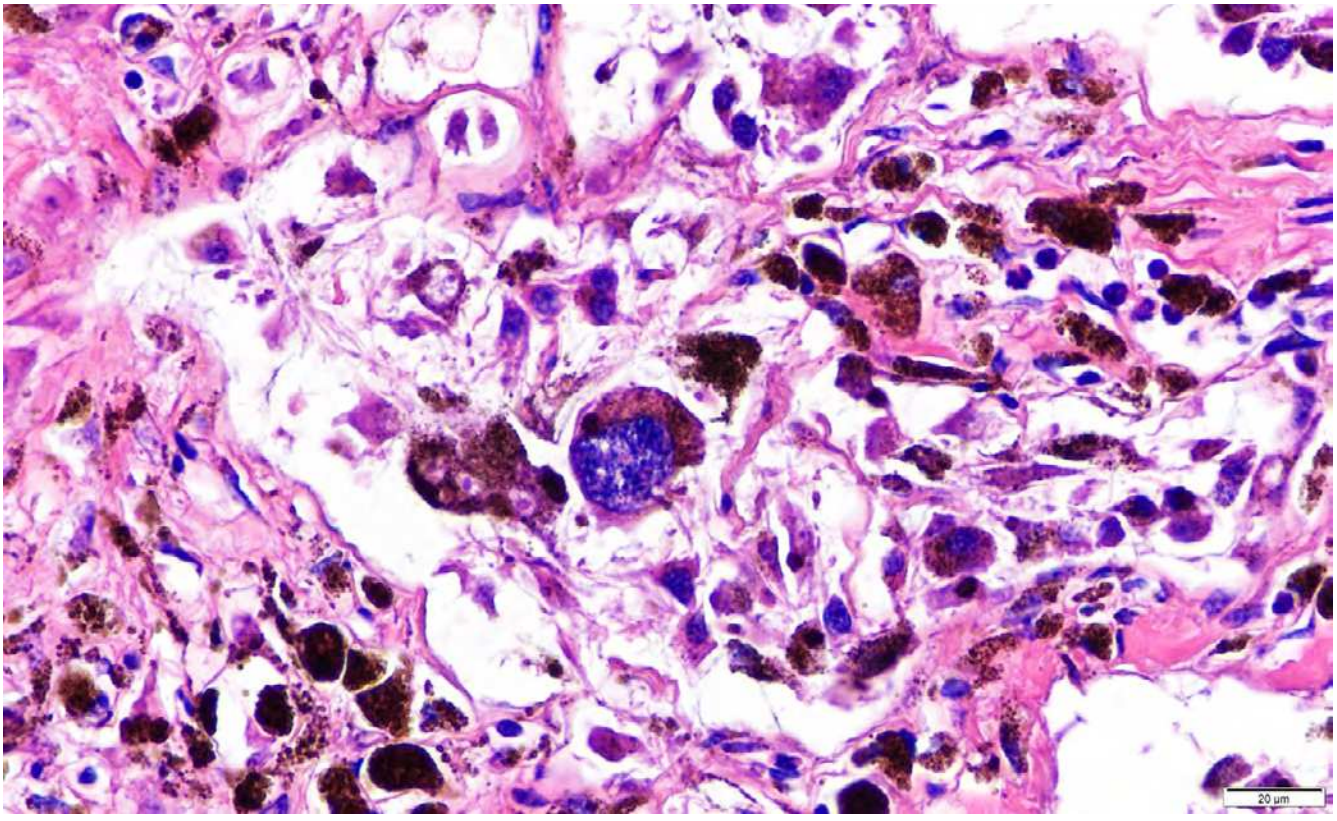


Fig. 5. Lymph node. Neoplastic cells containing intracytoplasmic brown to black pigment and marked anisocytosis and anisokaryosis. A neoplastic melanocyte is at the center of the figure.

Brazilian Journal of Veterinary Pathology. www.bjvp.org.br . All rights reserved 2007-2021.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

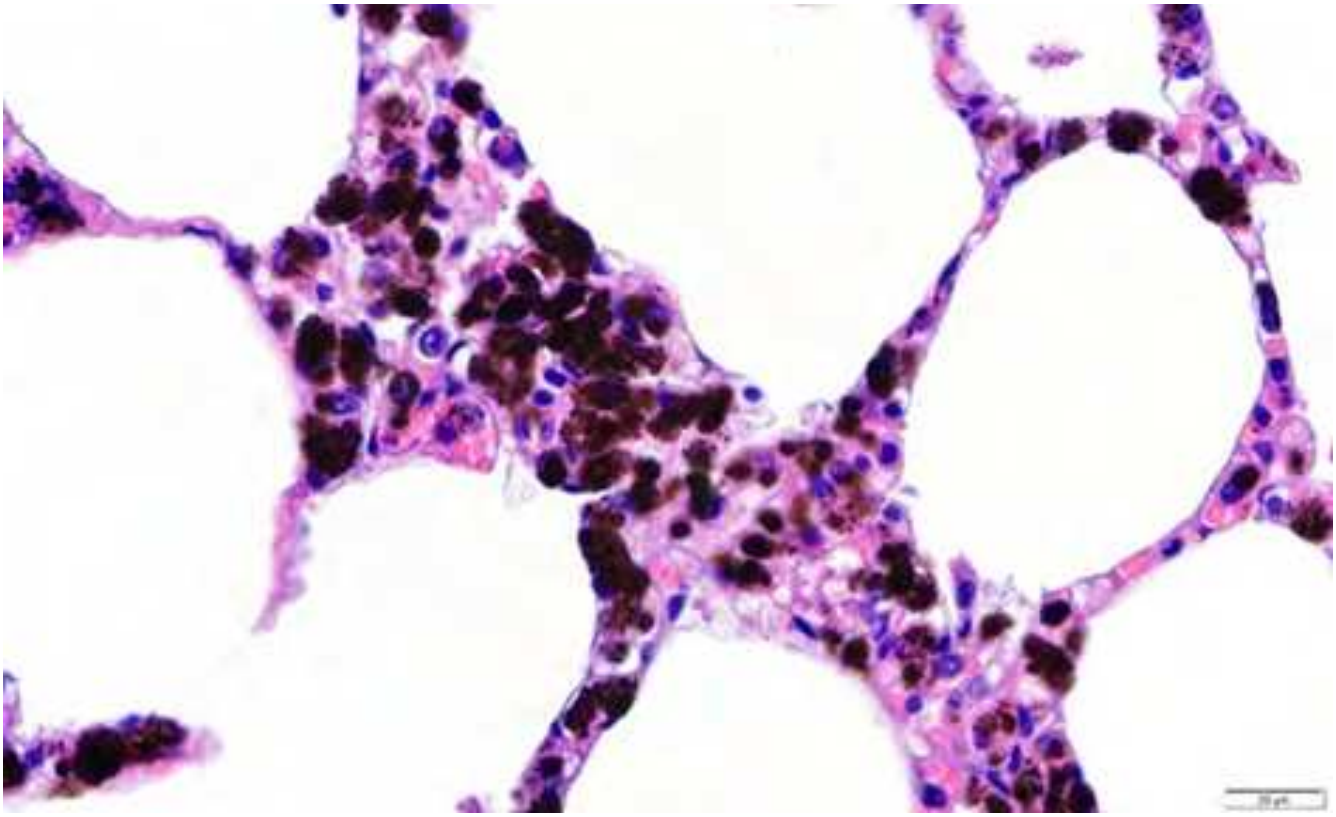


Fig. 6. Lung. Cells with intracytoplasmic black pigment expand the alveolar septa

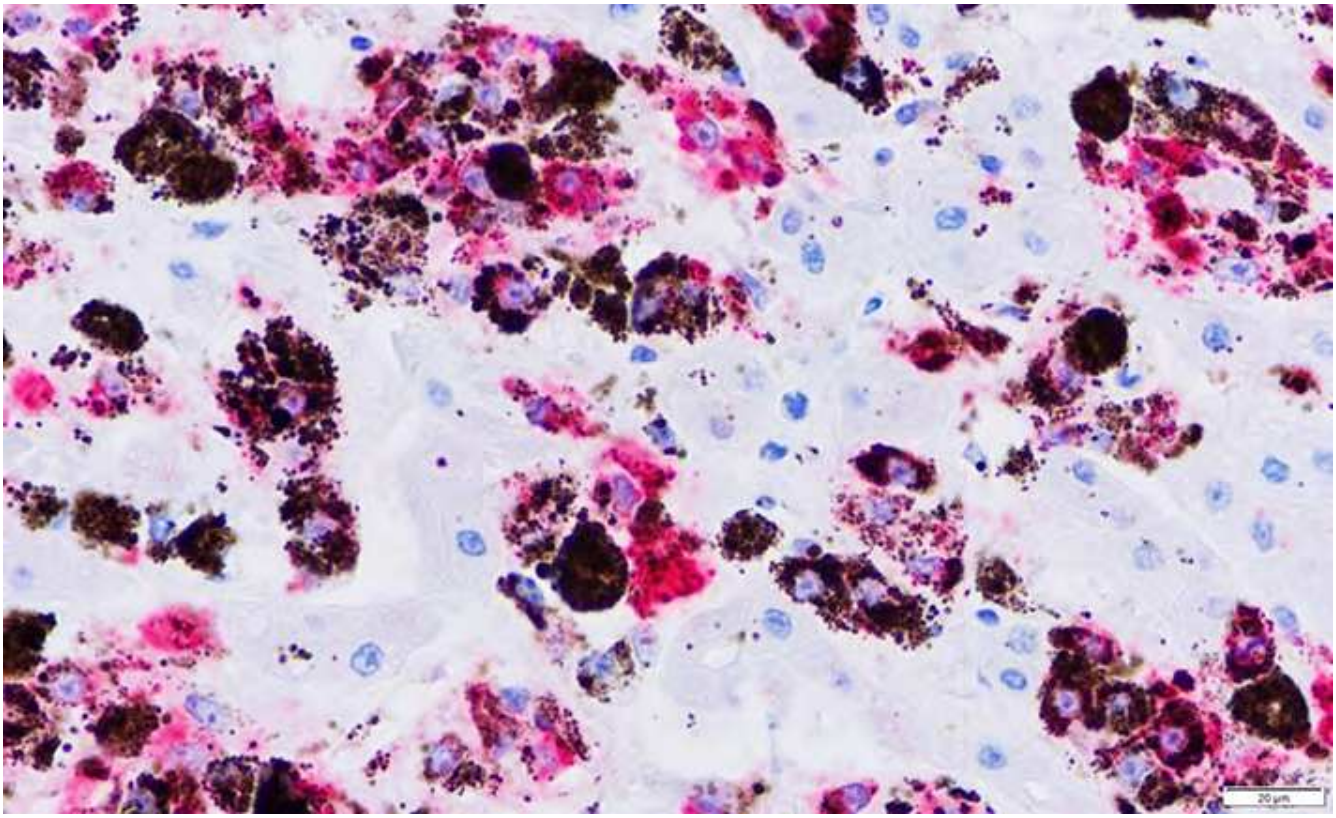


Fig. 7. Strong cytoplasmic immunolabeling of neoplastic melanocytes for PNL2 in the liver (7). Anti-PNL2 immunohistochemistry (IHC), red chromogen label, hematoxylin counter stain.

 All rights reserved 2007-2021.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

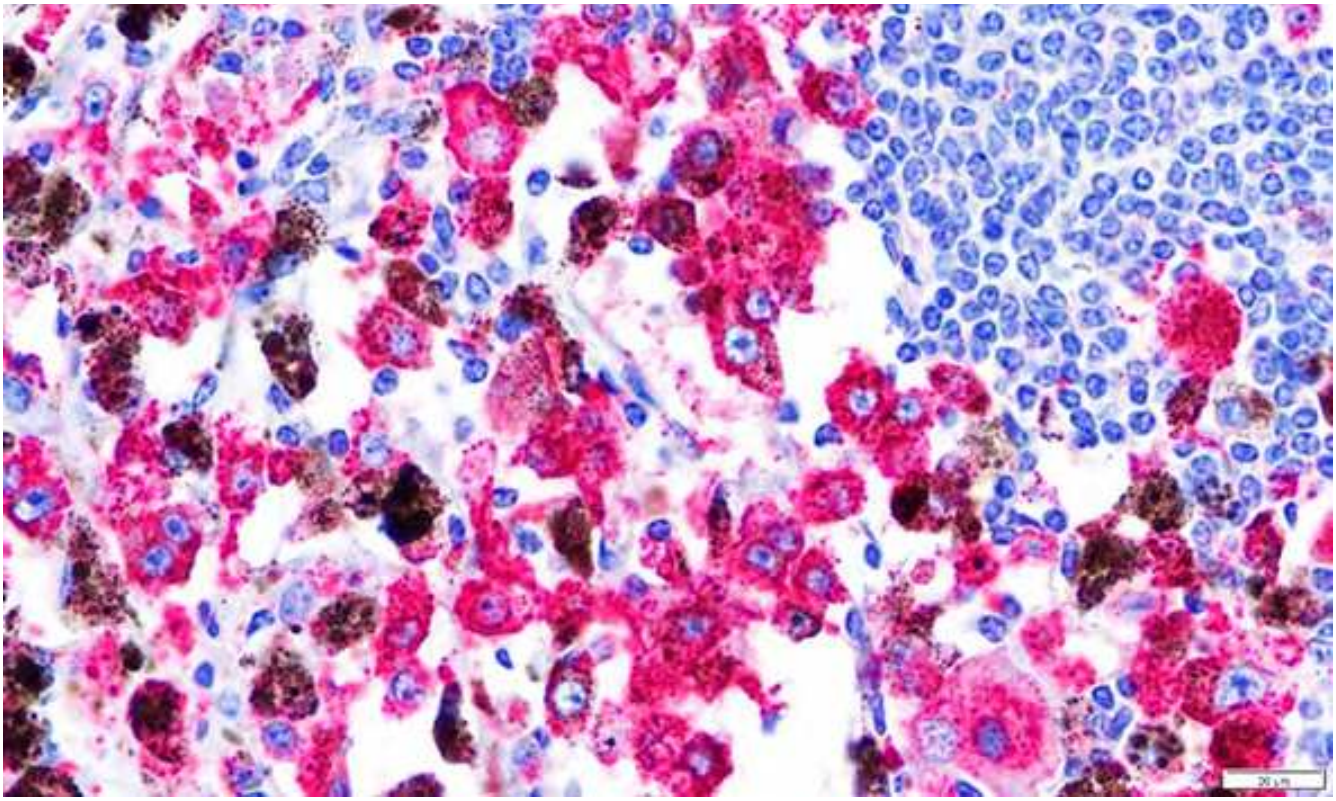


Fig. 8. Strong cytoplasmic immunolabeling of neoplastic melanocytes for PNL2 in the lymph node. Anti-PNL2 immunohistochemistry (IHC), red chromogen label, hematoxylin counter stain.

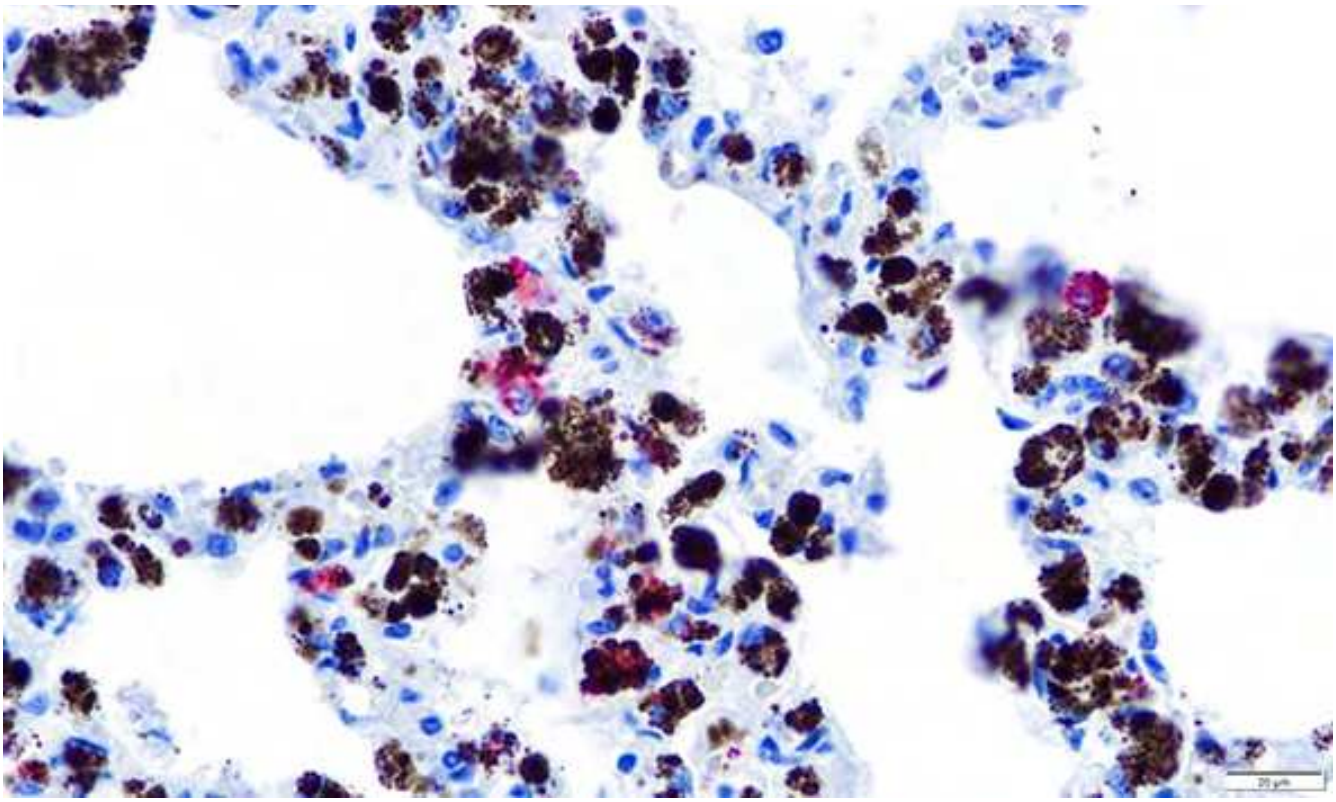


Fig. 9. Strong cytoplasmic immunolabeling of neoplastic melanocytes for PNL2 in the and lung. Anti-PNL2 immunohistochemistry (IHC), red chromogen label, hematoxylin counter stain.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

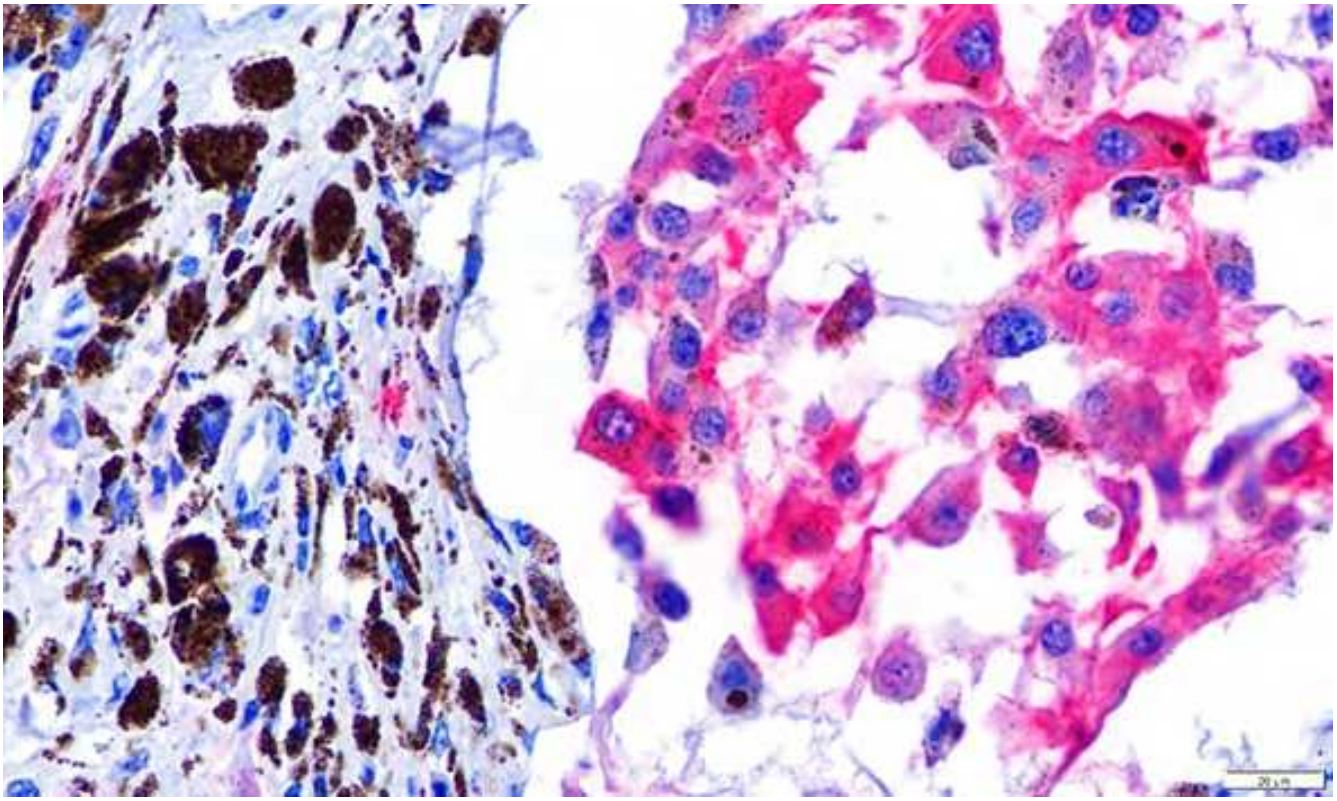


Fig. 10. Lymph node. The neoplastic melanocytes exhibit strong cytoplasmic immunolabeling. Anti-Melan A IHC, red chromogen label, hematoxylin counter stain.

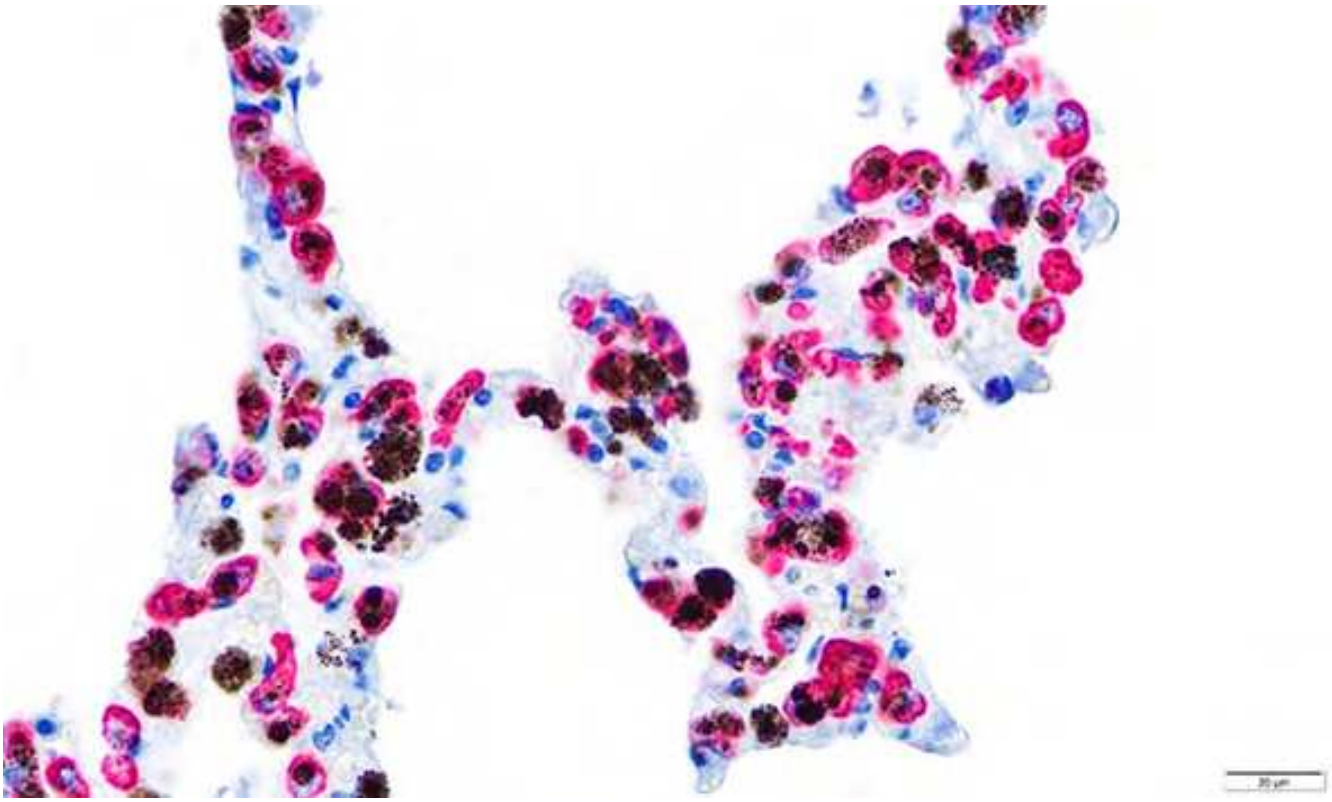


Fig. 11. Lung. Most of the cells with melanin are positive for IBA1, interpreted as melanophages. Anti-IBA1 IHC, red chromogen label, hematoxylin counter stain.

Brazilian Journal of Veterinary Pathology. www.bjvp.org.br . All rights reserved 2007-2021.

Braz J Vet Pathol, 2021, 14(3), 191 – 198
DOI: 10.24070/bjvp.1983-0246.v14i3p191-198

Table 1. Immunohistochemistry performed on multiple tissues infiltrated by cells with black intracytoplasmic pigment.

Primary antibody	Type	Company	Dilution	Antigen retrieval	Method	Tissues
Melan A	Mc (mouse antihuman)	Agilent Technologies	1:30	pH 9 for 15 min at 110°C	AP and Fast red (Warp Red) from Biocare Medical	Lymph node
PNL-2	Mc (mouse antihuman)	Cell Marque	Ready to Use	pH 9 for 15 min at 110°C	AP and Fast Red (Warp Red) from Biocare Medical	Liver, lung, lymph node
IBA-1	Pc (rabbit anti-rat)	Wako Chemicals	5,597	Citrate, 15 min at 110°C	HRP and Fast Red (Warp Red) from Biocare Medical	Lung

Mc=monoclonal; Pc=polyclonal; AP= alkaline phosphatase; HRP=horseradish peroxidase.

Immunohistochemistry using antibodies against PNL2, Melan A, and IBA1 was performed according to Table 1. Many neoplastic melanocytes were strongly positive for PNL2 within the liver (Fig. 7) and lymph node (Fig. 8), while scattered positive cells were in the alveolar septa of the lungs (Fig. 9). Neoplastic cells were also strongly positive for Melan-A in the lymph node (Fig. 10). Most of the cells with melanin in the lung (Fig. 11) were positive for IBA1 and interpreted as melanin-laden macrophages, i.e., melanophages. Thus, a morphologic diagnosis of multicentric diffuse metastatic melanotic melanoma was made.

Discussion

In humans, generalized melanosis s a rare complication of advanced metastatic melanoma (8, 10). The pathogenesis of the condition is uncertain, but probably upon lysis, neoplastic melanocytes release large amounts of melanin which are phagocytized by macrophages (4). The human syndrome, which involves diffuse cutaneous melanosis associated with metastatic melanoma, has been related to several factors. Those include the conversion of melanin precursors in the circulation or the dermal melanophages before complete oxidation into melanin; the deposition, in the skin, of pigment granules arising from the neoplasm or the sites of metastases; or even due to an unlimited propagation of melanoma cells that are capable of producing melanized melanosomes (5, 15, 16). In such cases, emaciation, black discoloration of the skin and urine, hepatomegaly, and metastases mainly in the lung and liver are described (1). In cases of diffuse cutaneous melanosis, metastases of malignant melanoma occur in the liver, lungs, lymph nodes, spleen, intestines, and heart (6). In such instances, melanuria occurs (1, 11), confirmed by the melanophages and melanin in the urine (6). The histologic and immunohistochemical findings in the current case, along with diffuse black discoloration and organomegaly in several tissues, support the diagnosis of generalized (or multicentric) melanosis secondary to metastatic melanoma in the current case. Therefore, it is likely that the dark urine, observed by the attending veterinarian who performed the necropsy of the bull and interpreted as hemoglobinuria, was, in fact, melanuria, which led him to the presumptive diagnosis of leptospirosis. Indeed, in hemolytic leptospirosis of cattle, the urine may have a coffee color. However, dark urine of the same hue associated with melanosis secondary to metastatic melanoma in humans is due to melanin in the urine (4,10,11), which must have been the case for the bull in this report. Pigment-laden cells in the glomerular tufts and capillaries of the submucosa of the urinary bladder such as the present case, allows cellular debris, including melanin granules, to reach the proximal convoluted tubules and from there enter the urine, resulting in melanuria (4). Along this same line of reasoning, it is interesting to note that the attending veterinarian reported "nose bleeding" of the bull. Although the authors cannot confirm or refute the true nature of the "nose bleeding," it is not an unlikely conjecture that the dark color of the nasal fluid is due to the same mechanism as the "black sputum" or melanoptysis described in diffuse melanosis secondary to melanoma in human beings (4). In this condition, phlegm is dark due to the presence of melanin originating from the diffuse infiltration of melanoma cells in the lungs, with secondary pigment deposition in macrophages and bronchial epithelial cells as we observed in the lungs of the bull.

Generally, malignant melanoma metastases found in different tissues originate from neoplasms located in the skin (7). However, in the present case, it was not possible to determine the primary site of the neoplasm due to its widespread dissemination and the limited number of tissues collected for histopathology. In addition, one of the characteristics of the Nelore breed standard is black or dark-colored skin; this fact, together with advanced age, may have contributed to the development of the neoplasm.

Although the skin was not analyzed in this case, the bull's clinicopathologic features in this report are very similar to cases of diffuse disseminated melanosis and melanuria associated with metastatic melanoma in humans.

Melanoma markers have variable sensitivity and specificity in domestic species. In dogs, commonly used melanocytic markers include a cocktail of antibodies against Melan A, PNL2, TRP1 and TRP2 to accurately diagnose melanocytic tumors. In horses, PNL2 is a highly sensitive and specific marker of melanocytic neoplasms; in contrast, Melan A does not react to melanomas in this species (12). Because most melanocytic tumors in cattle are benign with surgical excision generally curative, limited information on immunohistochemistry for diagnostic purposes in this species is available (19). In the bull of this report, neoplastic melanocytes were positive for Melan A and PNL2, which confirm both markers are reliable antibodies to diagnose melanocytic tumors in cattle.

References

1. Amaral ACV, Diniz LM, Lucas EA, Capeli RLA. Diffuse cutaneous melanosis: rare complication of metastatic melanoma. Ann Bras Dermatol. 2017; 92(5): 62-4.
2. Farias MB, França TN, Jabour FF, Seixas JN, Andrade GB, Oliveira LI, Peixoto PV. Metastasizing oral melanoma in a cow. Ciência Rural. 2009;39(4): 1248-52.
3. Dharmayanthi AB, Terai Y. Sulandari, S, Zein M.S.A., Akiyama T. Satta Y. The origin and evolution of fibromelanosis in domesticated chickens: Genomic comparison of Indonesian Cemani and Chinese Silkie breeds. PLoS One. 2017. 12(4):e0173147.
4. Eide J. Pathogenesis of generalized melanosis with melanuria and melanoptysis secondary to malignant melanoma. Histopathology. 1981; 5(3):285-94.
5. Fitzpatrick TB, Montgomery H, Lerner AB. Pathogenesis of generalized dermal pigmentation secondary to malignant melanoma and melanuria. J Invest Dermatol. 1954; 22(3):163-72.
6. Gambichler T, Stücker M, Kerner K, Weiner S, Waldherr R, Altmeyer P, Kreuter A. Acute kidney injury in a patient with melanuria, diffuse melanosis, and metastatic malignant melanoma. Am J Clin Dermatol. 2008; 9(4):267-70.
7. Goldschmidt MH, Goldschmidt KH. Melanocytic neoplasms. In: Meuten DJ, editor. Tumors in Domestic Animals. Ames: Wiley Blacwell;2017. p.123-31.
8. Lerner AB, Moellmann G. Two rare manifestations of melanomas: generalized cutaneous melanosis and rapid solar induction of showers of small pigmented lesions. A critical review of the literature and presentation of two additional cases. Acta Dermatol Venereol. 1993; 73:241-50.
9. Miller MA, Weaver AD, Stogsdill PL, Fischer JR, Kreeger JM, Nelson SL, Turk JR. Cutaneous melanocytomas in 10 young cattle. Vet Pathol. 1995; 32(5):479-84.
10. Perez A, Turajlic S, Szyszko T, O'Doherty M, Calonje E, Harries M, Acland K. Generalized melanosis and melanuria in a patient with metastatic melanoma. Clin Exp Dermatol. 2010; 35(3):37-9.
11. Piana S, Longo C. Diffuse Melanosis and Melanuria. N Engl J Med. 21. 2019. 380(12):1166.
12. Ramos-Vara JA, Frank CB, DuSold D, Miller MA. Immunohistochemical expression of melanocytic antigen PNL2, Melan A, S100, and PGP 9.5 in equine melanocytic neoplasms. Vet Pathol. 2014. 51(1): 61-6.
13. Rech RR, Giaretta PR, Brown C, Barros CSL, Gross and histopathological pitfalls found in the examination of 3,338 cattle brains submitted to the BSE surveillance program in Brazil. Pesq Vet Bras. 2018; 38(11):2099-2108.
14. Rech RR, Silva MC, Langohr IM, Marques MG, Pescador CA, Silva GS, Dutra MC, Brum JS, Kramer B, Bordin LC, Silva VS. Nem tudo que parece ser, é lesão: aspectos anatômicos, não lesões, artefatos, lesões sem significado clínico e alterações post mortem encontrados na necropsia de suínos domésticos e selvagens (Sus scrofa). Pesq Vet Bras. 2013; 33(10):1237-55.
15. Schuler G, Honigsmann H, Wolff K. Diffuse melanosis in metastatic melanoma: further evidence for disseminated single cell metastases in the skin. J Am Acad Dermatol. 1980; 3(4):363-9.
16. Silberberg I, Kopf AW, Gumport SL. Diffuse melanosis in malignant melanoma: report of case and of studies by light and electron microscopy. Arch Dermatol. 1968; 97(6):671-7.
17. Tamiasso, NV, Pandolfi KT, Pedrozo EA, Simões CU, Nunes LC, Boeloni JN. Multicentric melanosis in broiler: Case report. Arq Bras Med Vet Zootec. 2017; 69(2):409-14.
18. Tessele B. Lesões encontradas em bovinos abatidos para consumo (dissertation). (Santa Maria): Universidade Federal de Santa Maria; 2016. p.88.
19. Winslow CM, Wood J, Gilliam JN, Breshears MA. Congenital amelanotic melanoma in a crossbred heifer calf. J Vet Diagn Invest. 2017; 29(4):544-7.