

Pathomorphological and immunohistochemical studies on melanocytic tumors in cattle

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ABSTRACT

The present study was carried out to know the occurrence and pathomorphology of melanocytic tumors *viz.* melanocytoma and malignant melanoma in cattle. Out of 29 tumors of cattle collected during a period of 2 years, five melanocytic tumors (17.4%) were recorded in the present study. Grossly, the tumors were 3 to 10 cm in diameter, solitary, circumscribed to multinodular, sessile to pedunculated, soft to firm with variegated greyish white to black appearance. Cytology revealed clusters of pleomorphic melanocytes containing large nuclei and variable amounts of melanin granules in the cytoplasm. Histologically, melanocytoma revealed sheets of neoplastic melanocytes containing variable amounts of melanin granules obscuring the nuclear morphology while, malignant melanomas were characterized by the presence of nests of amelanotic to melanotic cells separated by a thin fibrovascular stroma located in the dermis. On IHC, all the melanocytic tumors were positive to S100.

Keywords: Cattle, melanocytoma, melanoma, S100

Melanocytomas and melanomas are tumors arising from neuroectodermal melanoblasts or melanocytes. Benign forms of the neoplasm are referred to as melanocytoma in animals and the malignant forms are called melanoma or malignant melanoma¹. Melanocytic neoplasms are frequently encountered within both veterinary and human medicine. Cattle develop melanocytomas infrequently but congenital tumors and tumors in young animals have been reported². Melanocytic tumors usually account for 5-6% of all tumors in surveys of bovine neoplasms and occur most commonly in the skin or subcutis and constitute 0.3% to 17% of integumentary tumors¹⁻³. Since the terminology for these tumors is not consistent in human and veterinary literature, it has become usual to use the term "melanoma" for all malignant melanocytic tumors, whereas "melanocytoma" refers to the benign forms. Descriptive terms of melanocytic neoplasms include 'junctional' that refers to the proliferation of neoplastic melanocytes often as small nests at the epidermal - dermal junction, 'compound' indicating both epidermal and dermal components to the tumor and 'dermal' indicating that the tumor is only intradermal with no epidermal component³. In a very few cases, melanomas invade deeper than subcutis or metastasized².

Cytological assessment through techniques like fine needle aspiration cytology (FNAC) and impression smears offers a quick and cost-effective way to obtain an initial diagnosis regarding the tumor's origin and malignant potential⁴. Although, there are several diagnostic techniques available, histopathological examination accompanied by histochemical staining found to be the best and most reliable method for appropriate diagnosis of cutaneous neoplasms⁵. Tumor markers such as S100 and Melan A are used to provide the gold standard in the diagnosis of malignant melanomas⁶.

The samples for the present study consisted of biopsy samples sent to the Department of Veterinary Pathology, NTR College of Veterinary Science, Gannavaram during the period from April 2023 to November 2024. The total samples screened included 29 cutaneous and ocular tumors of cattle. The

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tumor samples were examined for gross morphology and the cytological smears were prepared and subsequently stained with Leishman's stain.

Representative tissue samples were collected in 10% neutral buffered formalin. The samples were subjected to routine tissue processing by paraffin embedding technique and stained with haematoxylin and eosin method⁷.

The duplicate paraffin tissue sections were immuno stained using ready to use antibodies and super sensitive polymer-HRP detection system (Bio Genex, USA). Four microns thick tissue sections were mounted on positively charged slides and baked for 1 hour at 60°C prior to test. The sections were dewaxed



Fig. 1. Melanocytoma - A black, spherical, pedunculated tumor mass on the face of a cattle; **Fig. 2.** Melanocytoma - Impression smear showing amelanotic melanocytes amidst brownish black melanin granules (Leishman's stain X1000); **Fig. 3.** Melanocytoma - Section showing sheets of oval to polygonal melanocytes with abundant melanin pigment arranged around a blood vessel (H&E X400); **Fig. 4.** Malignant melanoma - A solitary, round tumor mass on the fetlock region of a cattle.

in xylene, hydrated through a graded series of ethanol solutions and washed three times in 0.1 M phosphate buffered saline (PBS; pH 7.4). Antigen retrieval was undertaken by heat treating sections in 0.01M citrate buffer at pH 6.0 in a pressure cooker for 20 min and were allowed to cool down to room temperature. Sections were rinsed in PBS for 10 min. The sections were placed in a humidifying chamber to carry out further steps at room temperature. Peroxide block™ (HK111-5K, Bio Genex), containing 3% hydrogen peroxide was applied to cover the sections and incubated for 20 min for quenching endogenous peroxidase activity. The sections were then washed in PBS three times. Power block™ (HK083-5K, Bio Genex), a protein aceous blocking agent containing casein was applied to the sections and incubated for 10 min to prevent nonspecific binding of antibodies to highly charged sites. The power block was then tipped off and incubated with S100 primary antibody (S100B/1012 rabbit polyclonal antibodies) for 60 min. The sections were rinsed in PBS and Super enhancer™ (HK518-06K, Bio Genex) was added to the slides and incubated for 20 min to enhance the signals. The sections were then

rinsed in PBS buffer and were covered with Polymer-HRP reagent (HK519-06K, Bio Genex) containing anti mouse and anti-rabbit IgG labelled with enzyme polymer in phosphate buffered saline for 30 min. Sections were rinsed in PBS buffer. The sections were then incubated with solution prepared by adding one drop of liquid DAB chromogen i.e. 3,3'-Diamino benzidine mixed with 1 ml of DAB buffer for 5 min. Sections were rinsed with PBS, washed with distilled water, counter stained with Harri's Haematoxylin and mounted with DPX mountant. Negative control sections were also included in the staining procedure by omitting the primary antibody⁸.

Out of 29 cutaneous and ocular tumors of cattle, 5 melanocytic tumors were recorded accounting to an occurrence of 17.24%. The occurrence of melanocytic tumors was recorded in Ongole and non-descript breeds of cattle in the age group of 2 - 7 years. The melanocytic tumors included two cases of melanocytomas and three cases of malignant melanomas.

In the present study, the melanocytomas were located on the thigh and face of cattle (Fig. 1). Grossly,

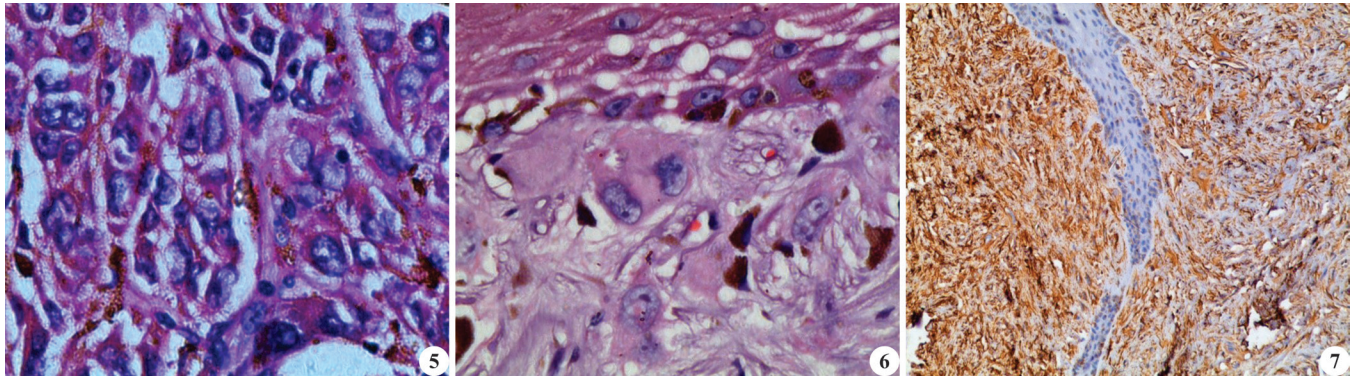


Fig. 5. Malignant melanoma - Section showing amelanotic to melanotic neoplastic cells with vesicular nuclei and prominent nucleoli (H&E X400); **Fig. 6.** Malignant melanoma - Section showing mononucleated to binucleated giant cells with abundant eosinophilic cytoplasm, vesicular nuclei and prominent nucleoli along with junctional activity (H&E X400); **Fig. 7.** Malignant melanoma: IHC-S-100 - Note intense cytoplasmic expression in the melanocytes invading the dermis X100.

the tumors were pedunculated to sessile, spherical to irregular in shape, brownish black in color and firm in consistency measuring about 6-10 cm in diameter. Cut sections revealed fleshy, dark brown to black colored surfaces. Cytological studies revealed the presence of clusters of round to oval and spindle shaped melanocytes with intracytoplasmic golden yellow melanin granules and round vesicular nuclei (Fig. 2). Histologically, melanocytomas were characterized by sheets of neoplastic melanocytes in the dermis. The cells were moderately pleomorphic, spindle to epithelioid in shape with variable amounts of melanin granules in the cytoplasm, obscuring the nuclear morphology. The tumor showed significant neovascularization with sheets of melanocytes encircling the capillaries (Fig. 3).

The malignant melanomas in the present study were located on the fetlock region (Fig. 4) and eyelids of cattle. Grossly, the tumors were solitary, circumscribed to multinodular, measuring about 3-5 cm in diameter with a variegated appearance. Cut sections were fleshy to firm and greyish white in color. Cytology revealed clusters of pleomorphic melanocytes containing large nuclei. The cells were amelanotic or contained variable amounts of melanin granules in the cytoplasm. Histologically, malignant melanomas were characterized by junctional activity and presence of nests of melanocytes separated by a thin fibrovascular stroma located in the dermis. The neoplastic melanocytes were epithelioid to spindloid and exhibited high nuclear to cytoplasmic ratio, scant cytoplasm that was frequently amelanotic and the nuclei were vesicular with prominent nucleoli (Fig. 5). The tumor also revealed a large number of giant cells with abundant eosinophilic cytoplasm, marked anisokaryosis, nuclear molding, single to multiple nuclei and nucleoli (Fig. 6).

On immunohistochemistry, melanocytomas and melanomas revealed intense cytoplasmic positivity to S-100 (Fig. 7).

In the present study, melanocytomas occurred on the thigh and face while malignant melanomas were located on fetlock and eyelids of cattle. In the previous studies, melanocytic tumors were reported on jaw, scapular, dewlap, trunk, limbs and conjunctiva of cattle similar to the earlier reports^{3,9-15}. Causes for the development of melanoma are unknown. Excessive exposure to sunlight is noted to be risk factor in humans¹⁶. UV photons can affect the DNA integrity, cell and tissue homeostasis, and induce mutations or affect expression of a plethora of genes including oncogenes and tumor suppressor genes¹⁷. In the present study, melanocytic tumors occurred in the age group of 2-7 years in accordance to the findings of a previous study². However, they have also reported congenital melanomas in their study. The gross findings of the present study are in line with the earlier findings^{10,13-15} viz. brownish black melanocytomas and greyish white to variegated malignant melanomas. In the present study, cytology of melanocytic tumors revealed presence of a large number of pleomorphic, polyhedral to spindle shaped cells with intracytoplasmic golden yellow melanin granules and round vesicular nuclei consistent to the findings in earlier studies^{10,15,16}. In the present study, benign melanocytomas were composed of sheets of neoplastic melanocytes in the dermis with moderate cellular pleomorphism and variable amounts of melanin obscuring the nuclear morphology. These findings corresponded with the results of earlier reports^{6,9,10,16,18}. Histologically, malignant melanomas in the present study were characterized by the presence of nests of pleomorphic, melanotic to amelanotic melanocytes invading the dermis. The presence of amelanotic melanocytes indicate that the cells are poorly differentiated, a feature of anaplasia. The melanocytes were epithelioid and appearance of giant cells was frequent similar to the previous findings^{12,14}. In the present study, immunohistochemistry revealed intense cytoplasmic immunopositivity to S-100 in melanotic to amelanotic melanocytes corroborating

earlier findings^{6,13}. S-100 protein has a remarkable role in melanocytic tumor diagnosis especially for differential diagnosis of amelanotic melanomas⁶. The S-100 protein family contains a large number of related calcium binding proteins, and are largely found in skin melanocytes and immunoreactivity for such proteins have been carried out as a diagnostic criterion for melanoma in humans and lab animals⁶.

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