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Cytological, Histopathological and Immunohistochemical Studies on Naturally Occurring Cutaneous Melanomas in Cattle in and Around Namakkal, Tamil Nadu, India

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ABSTRACT

Background: Melanomas are tumours that originate from melanoblasts. The studies on bovine tumours are of clinical concern since some melanocytic tumours are congenital and occur in young animals. The present work was carried out to study the pathomorphological characteristics of melanomas in cattle for early diagnosis and intervention.

Methods: Tumour samples were collected from suspected cases of neoplasia from four animals during the study period. Data pertaining to the animals were collected. Fine needle aspiration cytology and impression smear were stained with Giemsa stain. Tissue samples were collected in 10 per cent neutral buffered formalin for histopathological examination. Special staining with Fontana silver stain and immunohistochemistry with Melan- A and S- 100 markers for confirmatory diagnosis of melanin pigment was done.

Result: Cytology revealed neoplastic cells with vacuolation and pleomorphism. Cytoplasm contained abundant melanin pigments. Microscopically, the cytoplasm contained abundant brown, black coloured melanin pigment granules. Tissue sections stained with Fontana silver impregnation method revealed the presence of black coloured granules. Immunohistochemistry with Melan-A and S-100 revealed strong expression of brown coloured reaction in the cytoplasm of the neoplastic cells.

Key words: Cattle, Cytology, Fontana silver stain, Histopathology, Immunohistochemistry, Melan-A, Melanoma, S-100.

INTRODUCTION

Tumour incidence in cattle is increasing and bovine occupies second place next to dogs (Marofsoi *et al.,* 2009). Melanomas originate from neuroectodermal melanoblasts, which migrate at the beginning of the development period into the epidermal-dermal junction of the skin, follicles and dermis (Pulley and Stannard, 1990). Melanocytic tumours are rare in cattle and usually account for five per cent to six per cent of bovine tumours (Miller *et al.,* 1995; Smith *et al.,* 2002).

The benign forms of these tumours are referred to as “melanocytoma” in animals and nevus in humans, the malignant version is called malignant melanoma or simply “melanoma” (Smith *et al*., 2002). Melanotic tumours can be found in any location where melanocytes and blasts are present in the body such as skin, eyes, adrenal glands, meninges, endocardium and tunica intima of blood vessels (Brito *et al*., 2009). The commonest sites on skin are perineal region and base of the tail followed by head, udder, prepuce and limbs (Sharma *et al*., 2010). Some melanocytic tumours are congenital or occur in cattle younger than two years old especially those of red, gray or black skin (Slominski, 2001; Smith *et al.,* 2002).

The native cattle and buffalo breeds of India are considered to have natural immunity against many tropical disease conditions in comparison with the exotic breeds/ cross breeds (Ramesha *et al*., 2002) but there are reports of disproportionate number of melanoma cases in the subcontinent (Reddy and Subba, 1990; Pazhanivel *et al*., 2003; Sharma *et al*., 2010). Hence the present study of identification of melanomas by cytological, histopathological, special staining and immunohistochemical methods will help in early and confirmatory diagnosis of these neoplasms.

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MATERIALS AND METHODS

Sample collection

Samples for the present study were collected from four cases of neoplasia in cattle brought to Large Animal Surgical Out Patient Ward, Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, (TANUVAS) Chennai from April 2021 to August 2022. Detailed clinical examination of animals was carried out and the information regarding species, age, sex, breed, pregnancy status, housing, feed intake, presence of any tumorous growths, bleeding, ulceration and exudation from such growths were recorded. Gross pathological features of tumours such as location, shape, size, colour, consistency and texture were recorded.

Cytology

Samples for cytology were collected by fine needle aspiration cytology (FNAC) and impression smear. Cytology slides were air dried, fixed in methanol and stained with Giemsa stain and evaluated based on cytological characteristics of neoplasm.

Fig 1A: A spherical greyish black coloured mass of 10 cm in dia above the hock joint.

Fig 1B: Gross picture of mass after surgical excision of mass (Fig 1A).

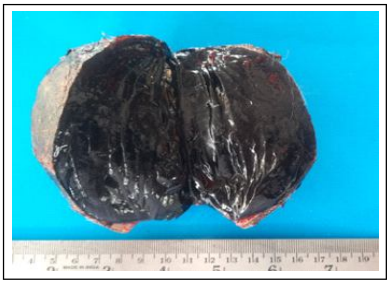

Fig 1C: Cut section of excised mass (Fig1A) showing shiny black surface.

Histopathology

The tumour tissue samples were fixed in 10% neutral buffered formalin for 24 hours and trimmed into a thickness of about 3-4 mm. Tissues were processed, embedded in paraffin, sectioned at 3-5 µm thickness and stained with haematoxylin and eosin (H&E) for histopathological examination. Special staining with Fontana silver stain was used to demonstrate melanin granules (Bancroft and Gamble, 2008).

Immunohistochemistry

The immunohistochemistry was performed using specific immunohistochemical marker of Melan-A and S-100 as per the procedure described in commercial kit.

RESULTS AND DISCUSSION

Gross pathology

During the study period of April 2021 to August 2022, out of 202 cases of cattle attended for suspected neoplasia, four cases were found to be melanomas accounting for about 0.02 per cent. Gross examination revealed the

Fig 2A: Hard, round ulcerated black coloured mass of 6 cm in dia in lateral thoracic region.

Fig 2B: Gross picture of ulcerated mass after surgical excision of mass (Fig 2A).

Fig 2C: Cut section of excised mass (Fig2A) showing shiny black surface.

presence of round greyish black coloured mass about 10 cm in diameter just above the left hock joint in one year old Jersey cross bred heifer (Fig 1 A, B). Round, ulcerated black colour firm mass about 6 cm diameter noticed in the right lateral thoracic region behind the scapula in a 7 year old pregnant Jersey cross bred cow (Fig 2 A, B). Hard, round, 15 cm diameter black colour mass found in the lateral aspect of abdominal region in a Jersey cross bred cow (Fig 3 A, B) and another lobulated soft grey colour mass about 7 cm in diameter noticed in the dorsal aspect just above the hoof of left hind limb in a 1.5 years old male Theni Malaimaadu breed cattle (Fig 4 A, B).

Cut section of all the masses revealed shiny black surface which was smooth to cut (Fig 1C,2C,3C). The melanomas were recorded in cattle in the region of brisket (Pazhanivel *et al.,* 2003), outer thorax (Pravettoni *et al.,* 2003), flank (Sharma *et al.,* 2010), skin (Miller *et al.,* 1995) in different age groups. Reports of cases of melanoma was recorded in oral cavity below the last incisor of left lower jaw region (Brito *et al.,* 2009; Chandrashekaraiah *et al.,* 2013).

In the present study, two cases of melanoma were noticed in the hindlimbs and one case in the thoracic region just behind the scapula and another in the lateral abdominal region. Whereas, Jakar *et al.* (2006) reported 11 cases of melanoma and found highest in buffaloes followed by dogs. The occurrence is more in eyes (5) followed by limbs (3), trunk (2) and neck (1). Vijayakumar *et al.* (2020) recorded melanoma in four animals, two each in Jersey cross breed and Holstein Friesian cross breed cattle aged between one year to six years at the mandible, neck, thigh and coronet region.

Cytology, Histopathology and special staining

Cytological examination of FNAC and impression smear revealed cluster of neoplastic spindle shaped cells, melanin pigment in melanophages characteristic of melanoma (Fig 5 A, B). Histopathological examination revealed single to cluster of neoplastic cells. Neoplastic cells were pleomorphic, round to polygonal in shape. The cytoplasm of the neoplastic cells contained abundant brown black coloured melanin pigment granules completely masking the cells with vacuolation. The nuclei were round to oval shaped with prominent nucleoli and irregularly clumped chromatin. Mitotic figures were also found (Fig 6 A, B). Fontana silver stained tissue sections revealed the presence of black coloured melanin granules (Fig 7 A, B). The microscopical finding of the present cases were characteristic of melanoma and in accordance with earlier reports (Baba *et al.* (1983), Miller *et al.,* 1995; Pazhanivel *et al.,* 2003; Pravettoni *et al.,* 2003; Brito *et al.,* 2009; Sharma *et al.,* 2010; Chandrashekaraiah *et al.,* 2013).

Immunohistochemistry with Melan - A and S-100

The immunohistochemical evaluation for melanoma using Melan-A and S-100 in the present cases revealed strong positive expression in the cytoplasm of neoplastic cells (Fig 8, 9 A, B) which is in agreement with earlier report

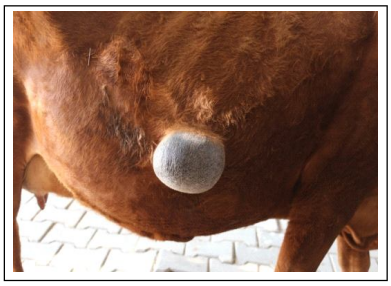

Fig 3A: Hard, round black coloured mass of 15 cm in dia noticed in right lateral abdomen.

Fig 3B: Gross picture of mass after surgical excision of mass (Fig 3A).

Fig 3C: Cut section of excised mass (Fig3A) showing shiny black surface.

Fig 4A: Lobulated soft grey coloured mass about 7 cm in dia just above the hoof (left hindlimb).

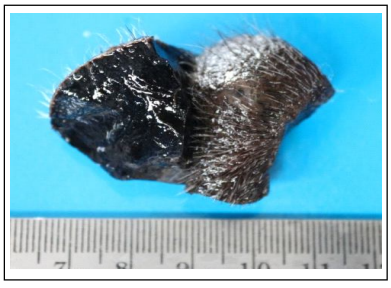

Fig 4B: Gross picture and cut section of excised mass (Fig4A) showing shiny black surface of mass after surgical excision.

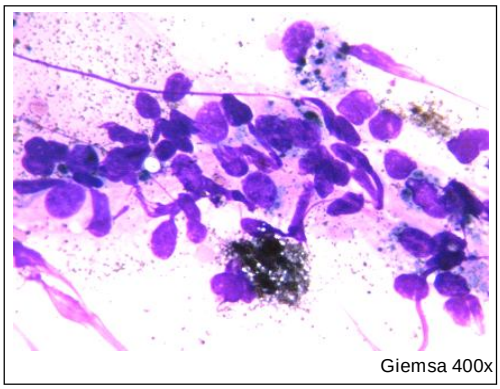

Fig 5A: Cytology-FNAC smear showing cluster of spindle cells and melanophages with melanin pigment.

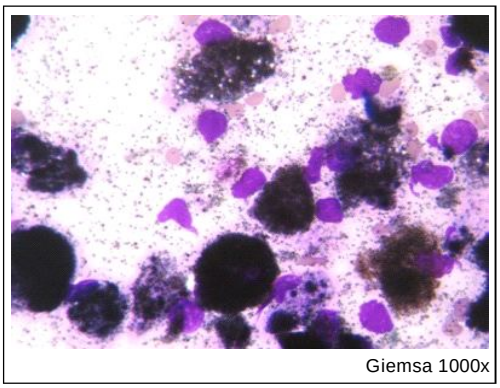

Fig 5B: Cytology- Impression smear showing melanophages with heavy deposition of melanin pigments in the cytoplasm.

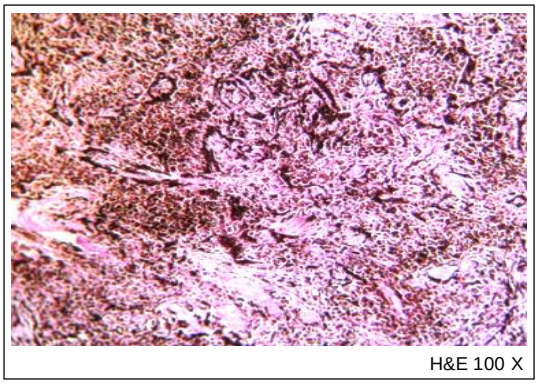

Fig 6A: Histopathological section of melanoma revealing abundant accumulation of melanocytes, melanin filled melanopahges in dermis.

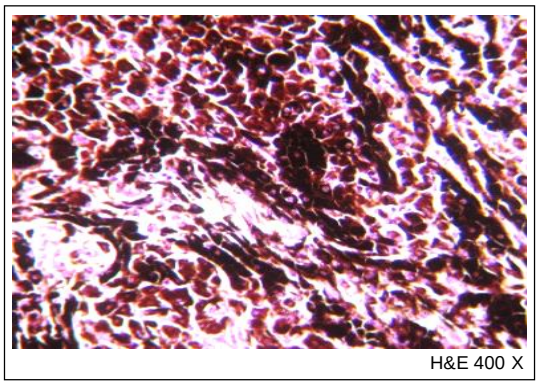

Fig 6B: Histopathological section of melanoma revealing abundant accumulation of melanocytes, melanin filled melanopahges in dermis.

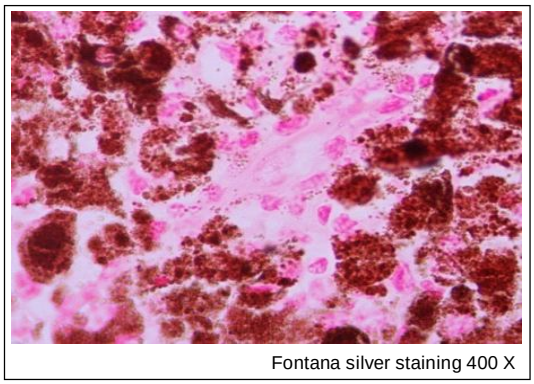

Fig 7A: Histopathological section of melanoma showing abundant melanin pigments.

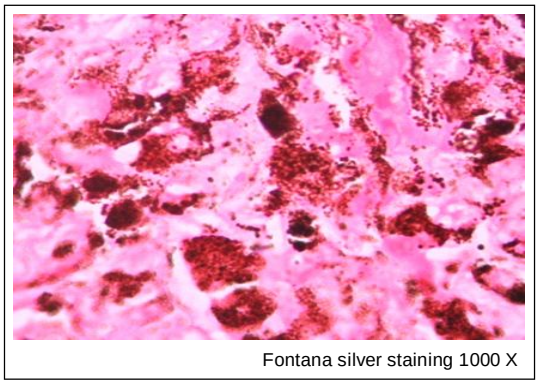

Fig 7B: Histopathological section of melanoma showing abundant melanin pigments.

(Beytut *et al.,* 2018; Pazhanivel *et al.,* 2021). In the present study, the incidence of melanoma might be due to sun exposure as recorded by Armstrong and Kricker, (2001) and Pazhanivel *et al.* (2021).

CONCLUSION

The present study indicates the occurrence of melanomas in native and cross bred cows especially the incidence was higher in Jersey cross breed cattle. Cytological and histopathological examination revealed neoplastic cells characteristic of melanoma. Demonstration of melanin granules with Fontana silver stain and strong immunolabelling with markers Melan- A and S-100 helps in confirmatory diagnosis thereby reduces economic losses in younger and productive animals by early intervention.

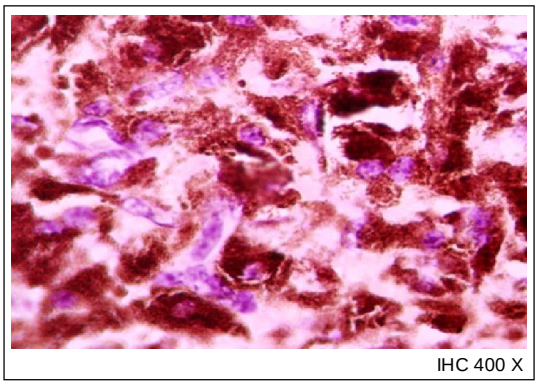

Fig 8A: Positive strong immunolabelling of Melan- A in the cytoplasm of melanocytes.

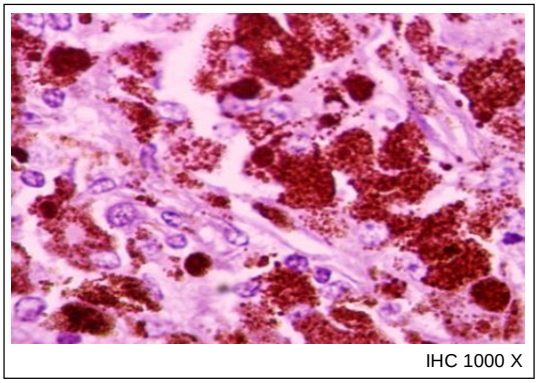

Fig 8B: Positive strong immunolabelling of Melan - A in the cytoplasm of melanocytes.

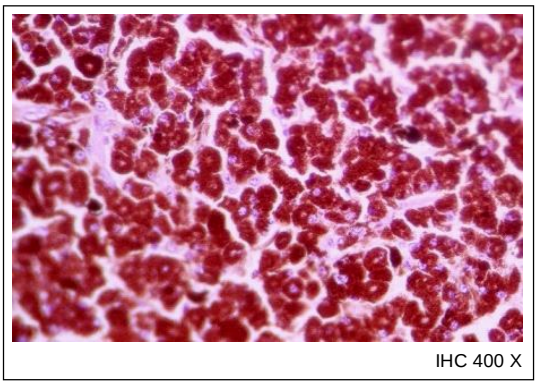

Fig 9A: Positive strong immunolabelling of S-100 in the cytoplasm of melanocytes.

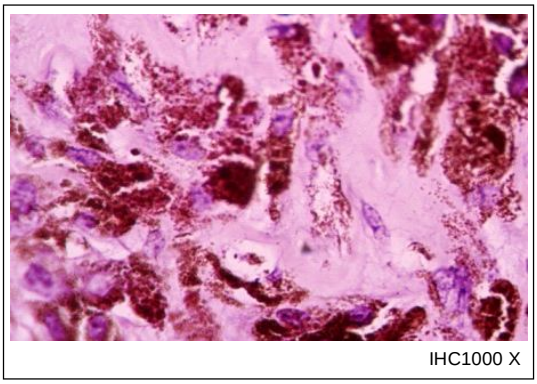

Fig 9B: Positive strong immunolabelling of S-100 in the cytoplasm of melanocytes.

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Conflict of interest

All authors declared that there is no conflict of interest.

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