Pathomorphological studies on mesenchymal and melanocytic neoplasms of cattle

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Abstract
The study aimed to establish the occurrence of cutaneous mesenchymal and melanocytic neoplasm’s from various breeds of cattle belonging to different geographical locations of Puducherry and Tamil Nadu, India. Out of 100 cases with cutaneous growths studied, 89 cases were diagnosed as neoplastic and 11 as non-neoplastic conditions based on gross, histological and histochemical features. Out of 89 cutaneous neoplasms, 5 (5.61%) cases were of mesenchymal and 4 (4.49%) were of melanocytic in origin. Grossly, the lesions varied in size, greyish-white to dark in colour, solitary to multiple, sessile to pedunculated, cauliflower to rice grain-like, soft to hard in consistency and occasionally were ulcerated. Histopathologically, proliferating spindle-shaped fibroblasts with oval normochromatic nuclei and abundant collagen arranged in whorls were the features in all the mesenchymal neoplasms and were diagnosed as fibroma. The melanocytic tumours revealed proliferating melanocytes were plump to fusiform with granular hyper-pigmented cytoplasm comprising of melanin and often arranged as nests were accordingly classified as melanoma.

Keywords: Cattle; cutaneous neoplasms; fibroma; melanoma; gross and histopathology.

1. Introduction
The neoplastic conditions affecting the skin must be given emphasis as they may lead to poor body condition, decreased production, culling of animals and mortality. Cattle and buffalo in India are commonly affected by a variety of neoplasms including horn cancer, eye cancer, lymphosarcoma, ethmoid carcinoma, urinary bladder cancer and cutaneous warts. Incidence of skin neoplasms has relatively increased in cattle, occupying the second position, next to dogs (Madewell and Theilen, 1978).

More than 25 morphologically distinct cutaneous neoplasms have been described in cattle, which may arise from epithelial elements (epidermis, hair follicles, sweat and sebaceous glands), mesenchymal tissues (including round and spindle cells of the skin), and melanin producing cells (Goldschmidt and Goldschmidt, 2017). Skin tumours may also arise from cells of haemopoietic system, including cells of macrophage/histiocytic lineage, plasma cells and lymphoid cells which normally pass through the dermis. In addition, neoplasms of non-cutaneous origin may metastasize to the skin. In general neoplasms of epithelial origin account for 48% and those of mesenchymal origin account for 52% (Baba et al., 2007).

Various external and internal risk factors are reported for skin neoplasms. The most frequently reported external factors responsible for initiation and development of neoplastic process include ultraviolet radiation, oncogenic viruses and chemical carcinogens. The internal influences that may support development of neoplasm include dietetic and hormonal influences, genetic predisposition and age (Institute of Medicine, 2002).

With increased use of vaccines and antibiotics in domestic animals, death due to infectious diseases has decreased in last two decades but consequently, the neoplasia has become of greater importance to the practicing veterinarians and more of their time and skill is now employed in the diagnosis, prognosis and treatment of tumours. Information on the prevalence and distribution of individual cutaneous
tumours helps veterinary practitioners for early diagnosis, proper prognosis and to determine appropriate therapy. A major advantage of standard surgical excision of skin tumour is completeness of surgery, which can only be determined by histopathological examination.

Although, mesenchymal and melanocytic neoplasms are common in dogs and cats (Goldschmidt and Hendrick, 2000), they are uncommon in large animals (Scott, 1988). The paucity of information on spontaneously occurring neoplasms of cattle has encouraged designing of the present study with an objective to study the various histological types of mesenchymal and melanocytic neoplasms of cattle based on histopathological and histochemical examination.

2. Materials and Methods

The study comprised of 100 clinical cases of cutaneous growths collected from cattle of various breeds. The samples were collected from different geographical locations, which included 16 samples from the Teaching Veterinary Clinical Complex (TVCC), RIVER and Veterinary dispensaries in Puducherry and 84 samples from various parts of Tamil Nadu [Rasipuram (31), Namakkal (25), Erode (23), Salem (4) and Attur (1)], India. The samples were collected either by excisional biopsy or by punch biopsy techniques. A detailed gross examination of the lesions with respect to its size, shape, color and consistency was carried out and subsequently, the samples were immediately fixed in 10% neutral buffered formalin.

The tissue samples fixed in 10% neutral buffered formalin were processed for histopathological examination by routine paraffin embedding technique. Five-micron thick sections were stained by H&E for detailed histopathological study and classification of tumours. Histochemical staining which included toluidine blue for mast cells, Fontana's Masson's silver impregnation technique for melanin and Masson's trichrome for collagen were carried out wherever required as per standard procedures (Luna, 1968).

The hematoxylin & eosin stained (H&E) sections were used to categorize cutaneous tumours into mesenchymal and melanocytic types (Scott et al., 2001) and were further sub-typed into benign and malignant types.

3. Results and discussion

Cutaneous neoplasms in cattle are rare entities and exceptionally life-threatening conditions. However, these pathological conditions can cause severe production and economic loss through reduced productivity by decreased weight gain, milk yield and poor hide quality. Skin affections are generally considered a minor entity when compared to diseases causing significant mortality. Studies have been conducted in various parts of the world on the occurrence and categorization of various cutaneous lesions in cattle (Moulton, 1990; Jubb et al., 1993; Misderop, 2002; Smith et al., 2002; Scott, 2007; Marosfoi et al. 2008; Hassannein & Mahmoud, 2009; Priya, 2012; Meuten, 2017; Shruthi et al., 2018). In the present study, out of 100 cases with cutaneous growths studied, 89 cases were diagnosed as neoplasms. Results from our study corroborated with previous report from other geographical area, Jammu, India (Sharma et al., 2019). However, in a study from Romania, Marosfoi et al. (2008) reported 7 (10.44%) cases of cutaneous neoplasms out of 67 slaughtered cattle. The occurrence of skin tumours in clinical cases of cattle at various regions of southern India was reported to be 33.33% (Priya, 2012). Shruthi et al. (2018) studied a total of 59 bovine samples suspected for neoplasia and recorded tumors of epithelial and mesenchymal origin, mixed tumors and miscellaneous tumors, which constituted 54.38%, 42.09%, 1.75% and 1.75% respectively. The variation in the occurrence of cutaneous tumours could be due to differences in the methodology.

Among the cutaneous neoplasms recorded, the percent occurrence of mesenchymal and melanocytic neoplasms was 5.61% and 4.49% respectively. However, a higher percent occurrence (42.09%) of mesenchymal tumours has been reported in bovines (Shruthi et al., 2018). In this study, the method described by Goldschmidt and Shofer (1992) was adopted for histological classification and categorization of the cutaneous neoplasms. Interestingly, all the mesenchmal tumours were benign and fibroblastic in origin (Fibroma). The details of the cases and gross features are presented in Table 1.

Fibromas were recorded in 5 animals belonging to Jersey cross breed (JCB) aged between 9 months to 7 years at the neck, shoulder, thigh, tail and perineal region. These neoplasms were small to medium sized, solitary to multiple, nodular and occasionally ulcerated growths (Fig. 1, 4).
Histopathological features of all the cases revealed proliferating spindle-shaped fibroblasts separated from each other by thin collagenous stroma. The neoplastic cells were arranged as ill-defined interwoven bundles, often running in all directions and occasionally forming a whorl-like pattern (Fig. 2, 3).

The nuclei of the neoplastic fibroblasts were small and oval shaped with inconspicuous nucleoli (Fig. 5). Masson’s trichrome staining confirmed the presence of fibroblast and collagen. These histopathological features were in accordance with the finding of Moulton (1990); Jubb et al. (1993) and Scott (2007).

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Benign mesenchymal tumors can often be diagnosed based on their histological appearance (Goldschmidt and Hendrick, 2000). Though cutaneous fibromas are common in dogs, however, these tumours have also been reported to occur in other animals (Goldschmidt et al., 2012). There are a few reports concerning fibroma in cattle. Fibromas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Breed</th>
<th>Sex</th>
<th>Gross features</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>9 months</td>
<td>JCB</td>
<td>Female</td>
<td>Solitary, small, growth near the right shoulder region</td>
</tr>
<tr>
<td>97</td>
<td>3 years</td>
<td>JCB</td>
<td>Female</td>
<td>Multiple, nodular and ulcerated growths in the perineal region and on the ventral aspect of the tail</td>
</tr>
<tr>
<td>38</td>
<td>4 years</td>
<td>JCB</td>
<td>Female</td>
<td>Solitary, hard, nodular, ulcerated growth in the right thigh region</td>
</tr>
<tr>
<td>37</td>
<td>6.5 years</td>
<td>JCB</td>
<td>Female</td>
<td>Solitary, small, growth on the neck</td>
</tr>
<tr>
<td>45</td>
<td>7 years</td>
<td>JCB</td>
<td>Female</td>
<td>Solitary, nodular growth in neck</td>
</tr>
</tbody>
</table>
and fibrosarcomas occur in adult and aged cattle, with no breed or sex predilection (Sundberg et al., 1980). Solitary cases of fibromas have been reported in cattle. Schamber et al. (1982) reported a case of fibroma in calf. There is also a report of multiple perianal fibroma in a cross-bred cow (Gahlod et al., 1998). A case of cutaneous fibroma has also been reported as a solitary mass (1.5 x 1.5 by 3.0 x 3.0 cm) in the skin of the right hind limb of 1-year old male Korean indigenous cattle (Jang et al., 2008). A case of fibroma has also been reported in a 7-year-old cross-breed cow (Yeruham and Perl, 2001). Movassaghi and Mohammadi (2009) also reported a case of fibroma as large subcutaneous mass measuring 32×45cm at the level of left pectoral region in a 22-month-old freemartin Holstein heifer.

Based on the special staining methods results, the cutaneous growths were confirmed as dermal fibroma resulting from an increase in collagen-producing fibrocytes in the dermis. Melanoma and sarcoma were excluded because no muscle cells or melanin were identified on histochemical staining with the van Gieson and Fontana-Masson methods, respectively. Based on the gross warty appearance of the dermal nodules, fibropapilloma was also considered in the differential diagnosis. According to a previous report (Yager and Scott, 1993), a fibropapilloma should show features of acanthosis, hyperkeratosis, the down-growth of rete-ridges, and the dermal proliferation of plump fibroblasts microscopically. However, we could not detect any proliferation disorder of the epidermis in the dermal nodules.

Fibroma is reported to be associated with papilloma viruses in all domestic animals, except the cat (Yager and Scott, 1993). A few papillomaviruses (Deer fibroma virus and Bovine papilloma virus) produce tumors of fibrous connective tissue rather than papillomas (Jones et al., 1997). Fibropapilloma is a virus-induced, transmissible tumor of the vagina and vulva of young heifers and the penis of young bulls (Campo, 1997). Papilloma virus was consistently detected using rabbit antiserum against papillomavirus group-specific antigen in all mule deer fibromas and bovine fibropapillomas (Sundberg et al., 1985). However, in the present case, antigen of papilloma virus in the tumor masses were not studied, so we cannot exclude the possibility of a papillomavirus infection in the affected animals.

Melanoma was recorded in 4 animals belonging to 2 each of Jersey cross breed (JCB) and Holstein Friesian cross breed (HFCB) aged between 1 year to 6 years at the mandible, neck, thigh and coronet region (Fig.6).

Microscopically, the epidermis showed mild hyperplastic changes (acanthosis). The superficial to deep dermis showed focal aggregates to diffuse deposition of melanin pigment. The proliferating melanocytes were plump to fusiform with granular hyperpigmented cytoplasm often arranged as nests (Fig.7).

In 3 cases, variable amounts of collagen rich fibres were observed interspersed with the melanocytes (Fig.8).
In addition, perivascular mononuclear infiltration was also observed. Masson’s trichrome staining of the parallel sections demonstrated presence of blue coloured collagen amidst proliferating abundant brownish deposits of melanoblast (Fig. 9).

Cattle develop melanocytoma infrequently (Meuten, 2017) and usually account for 5-6% of all tumors in surveys of bovine neoplasms and occur most commonly in the skin (Miller et al., 1995; Smith et al., 2002). Study conducted by Shruthi et al. 2018 reported 3.51% of melanocytic tumours among the various breeds in cattle in and around the region of Tirupati, India. A disproportionate number of reported cases have occurred in India, (Degloorkar et al., 1992; Pazhanivel et al., 2003; Sharma et al., 2010; Chandrashekaraiah et al., 2013; Shruthi et al., 2018), where cattle of all breeds were affected in different parts of the country.

Most reported melanocytic tumors have occurred on the limbs (Head, 1965; Miller et al., 1995) which was also a common site in our study. In addition, melanoma was recorded in mandible and neck regions corroborating with the earlier finding of occurrence of these tumours also at numerous sites on the trunk, neck, and head (Reddy and Subba, 1990; Scott and Anderson, 1992; Miller et al., 1995; Sharma et al., 2010; Chandrashekaraiyah et al., 2013; Javanbakht et al., 2014; Shruthi et al., 2018).

The gross and histopathological features observed in this study corresponded with earlier reports (Baba et al., 1983; Moulton, 1990; Jubb et al., 1993; Pazhanivel et al., 2003; Pravettioni et al., 2003; Scott, 2007; Babic et al., 2009; Brito et al., 2009; Sharma et al., 2010; Chandrashekaraiyah et al., 2013).

Most of melanocytic tumors histologically represented intradermal pattern with no epidermal component. Dermal melanocytomas might be less cellular. Neoplastic cells are often small spindle cells with melanin granules. Melanin granules are often difficult to identify within the cytoplasm of large round cell of melanocytoma, but will stain positive with the Fontana-Masson staining for melanin (Goldschmidt et al., 1998; Meuten, 2017). Pigmentation of bovine melanocytic tumors facilitates clinical diagnosis and treatment.

4. Conclusion

Bovine cutaneous neoplasms are challenging for both clinicians and pathologists because the neoplasms are difficult to fully characterize; thus, their behaviour and prognosis are difficult to predict. 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 neoplasms.

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Conflict of interest: The authors declare that there is no conflict of interest.

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