



## Pathological and Diagnostic Aspects of Squamous Cell Carcinoma in Bovines

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### Introduction:

Livestock in India plays a crucial role in the development of rural economy by supplementing family income and with generation of employment in the rural sector, particularly among the landless labourers, small and marginal farmers, and women. Bovines are the key constituent species of livestock. They are raised as livestock for dairy products (milk), meat purpose and as draught animals (pulling carts, ploughing etc.). Bovines in India mainly consist of cattle and buffaloes. According to the 20<sup>th</sup> Livestock census (2019), the total Livestock population shown a significant increase of 4.6% over Livestock Census 2012 with a total of 535.78 million in India.

Tumours are responsible for the loss of production in bovines resulting in substantial financial losses. Bovine tumours may cause economic losses due to negative impact on productivity, animal health and thus may reduce profits to individual farmers and dairy industry. Tumours, also referred as neoplasms (Greek word) Neo means new, plasma means formation, Neoplasms are broadly classified as benign and malignant. Benign neoplasms are mainly localized, single, showing slow and limited growth and do not show recurrence whereas malignant neoplasms are single/ multiple showing rapid and unlimited growth and recurrence after removal. Malignant tumours metastasize which means they can spread from primary site to distant secondary site within the host body. Malignant tumours grow by expansion, and invade or infiltrate adjacent tissues by growing between cells along the tissue spaces (Udharwar *et al.*, 2008).

The term tumour meaning swelling is currently restricted to neoplasm. Malignant tumours are indicated with the term cancer. Tumours are sub classified on the basis of origin. Tumours of epithelial origin involving stratified squamous epithelium are: papilloma (benign) and squamous cell carcinoma (malignant).

- Papillomas of cutaneous or mucosal epithelia in cattle they grow exophytically (outward projections). The papillomas are benign tumours which generally regress, but occasionally persist and transforms into malignant squamous cell carcinoma. Papillomas are commonly occurring tumours of epithelial origin in domestic animals.
- A malignant tumour of the stratified squamous epithelium of either the skin, or a mucous membrane, is called a squamous cell carcinoma (SCC) and it occurs mostly in older animals (Vegad, 2007). Squamous cell carcinoma (SCC) is one of the most common malignant neoplasm which is capable of metastatic spread and is observed in various forms across many animals (Yan *et al.*, 2011 and Tsujita *et al.*, 2010).

Squamous cell carcinoma of horn and eye are most commonly observed cancers in bovines and this article is focused on horn core carcinoma and ocular squamous cell carcinoma.

### Squamous Cell Carcinoma of Horn:

Squamous cell carcinoma of horn, also known as horn cancer, is a prevailing type of cancer in cattle especially *Bos indicus* (Bhatia *et al.*, 2020).

Horn cancer is generally unilateral (occurs at base of one horn either left or right side) and is encountered in cattle between 5-10 years of age (Tyagi and Singh, 2006).

In India, horn cancer affects approximately 1% of the cattle population and constitutes about 83.34% of total tumours reported (Singh *et al.* 2005).

It is one of the most commonly encountered neoplastic conditions of economic importance in zebu bullock (Somvanshi, 1991 and Kumar and Thilagar, 2000).

Exact etiology of the condition is not known, predisposing factors like irritation due to tying of a rope to the horn, trauma (rubbing, sudden fall against hard object, fighting etc.) and chronic irritation caused by yoke (Shastry, 2001), painting of horn, solar radiations, exposure to actinic rays or viruses etc. are observed.

### Ocular Form of Squamous Cell Carcinoma:

Another important form of SCC is ocular squamous cell carcinoma (OSCC). OSCC is one of the most common neoplasms of the eye. The incidence of eye cancer in cattle was observed to be high in animals above 5 years followed by 2-5 years of age (Heeney and Valli 1985). Squamous cell carcinoma is by far the most common tumour afflicting the bovine eye, as well as the most frequently diagnosed cancerous tumour in bovines. Hereditary factors, environmental factors, lack of eyelid pigmentation, age and dietary habits have all been recognized to play a role in the aetiopathogenesis of bovine ocular squamous cell carcinoma. It is a primary neoplasm of epithelial origin and occurs at the site of ocular and periocular tissues including the palpebral skin, epithelial surfaces of the cornea and conjunctiva, third eyelid and limbus (Fornazari *et al.*, 2017). Early recognition is critical due to the malignant tendencies of this tumour. In addition, in bovines the aetiology has been linked to a number of viral agents, especially bovine papillomavirus (BPV) (Ford *et al.*, 1982; Rutten *et al.*, 1992) and bovine herpes virus type 1 (BHV-1) (Taylor and Hanks, 1969) and 5 (BHV-5) (Anson *et al.*, 1982).

### Significance:

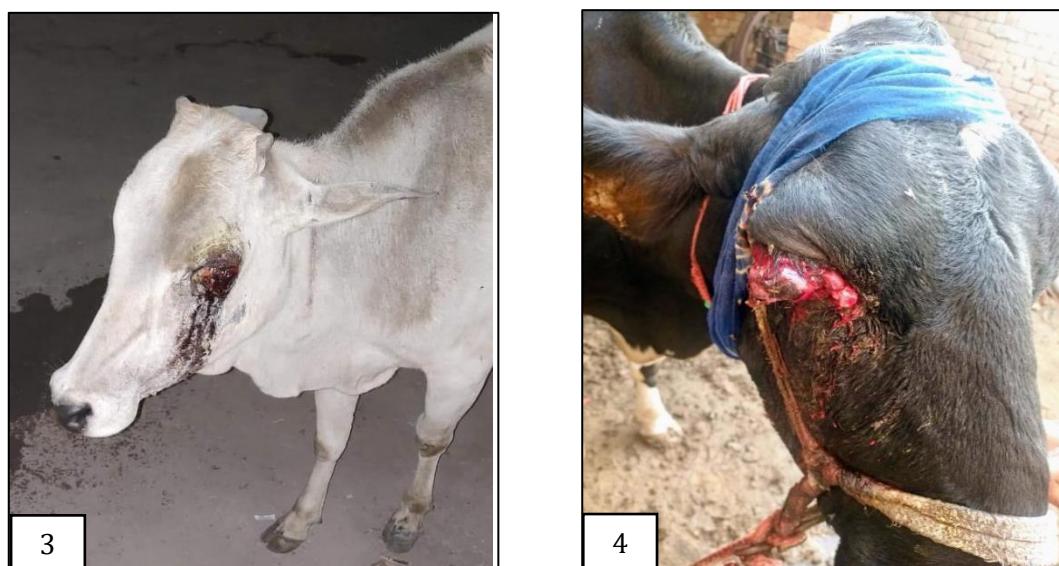
- Squamous cell carcinoma of horn is one of the most common cancer which is capable of spreading metastatically and is observed in various forms across many animals and humans.
- Horn cancer is a sporadic, malignant neoplasm affecting the horn core epithelium and predominantly seen in aged zebu bullocks and rarely observed in buffaloes.
- Horn cancer is one of the serious conditions in bullocks. The bullocks are highly susceptible as compared to bulls and cows.
- The malignant tendencies of this neoplasm make early recognition critical.

### Clinical Signs:

- Frequent head shaking, tilting at the affected side, bending of affected horn and increased nasal discharge on the affected side in advance cases
- Grossly, cancerous growth observed with spongy texture, pink cauliflower like tumour having rough and verrucous (wart like) surface which is friable and bleed easily (Reddy *et al.*, 2017).
- Cauliflower like ulcerated growth with bleeding at the base of the horn invading deep into underlying tissue (Sharma *et al.*, 2020).
- Sometimes gradual bending of horn with fowl smelling, purulent discharge due to secondary bacterial infection in cancerous growth is also observed.
- Later on infection of the tumourous growth by bacteria occurs and the suffering animal may spread and transmit infection via contact or rubbing of affected horn with other animal.



**Fig.-1&2:** Tumourous growth resembling a cauliflower at the base of Left Horn (Fig.1) and Right Horn (Fig. 2)



**Fig.-3&4:** Neoplastic growth at nictitating membrane of Left Eye (Fig.3) and Right Eye (Fig. 4).

## Susceptibility:

- Horn cancer is a type of SCC with poorly-defined genetic landscape, which arise from pseudo stratified columnar epithelium of the horn core mucosa, reported only in indigenous cattle (*Bos indicus*).
- Breed variations have also been seen with higher frequency of squamous cell carcinoma of horn in Kankrej breed than other zebu cattle, non-descript or cross-bred cattle, purebreds have been observed more susceptible as compared to cross-bred cattle (Carvalho *et al.*, 2005 and Gharagozlou *et al.*, 2007).
- The breed wise incidence of eye cancer in cattle revealed highest incidence in Hereford (exotic breed of cattle and HF crossbred followed by jersey crossbred cows and non-descript breeds. Ocular squamous cell carcinoma was also reported in Indian buffaloes but is very rarely observed.

## Diagnosis:

### 1. Histopathological Examination:

Histopathological examination is most commonly used in diagnosis of horn cancer. Histopathologically, horn cancer is either well differentiated with presence of cell nests (keratin pearls) or poorly differentiated with absence of keratin pearls (Joshi *et al.*, 2009). Typical concentric layers of keratin forming “epithelial pearls” were observed under microscope. Some histopathological studies are suggestive of anaplastic changes shown by tumour cells such as hyperchromatic nuclei (darkly stained nuclei) and mitotic figures (dividing cells mainly found in abnormal tissues such as cancers).

Anaplasia is one of the characteristic features of malignant neoplasms, also observed in horn cancer. Anaplasia means reversion of cells to a more primitive or undifferentiated or towards

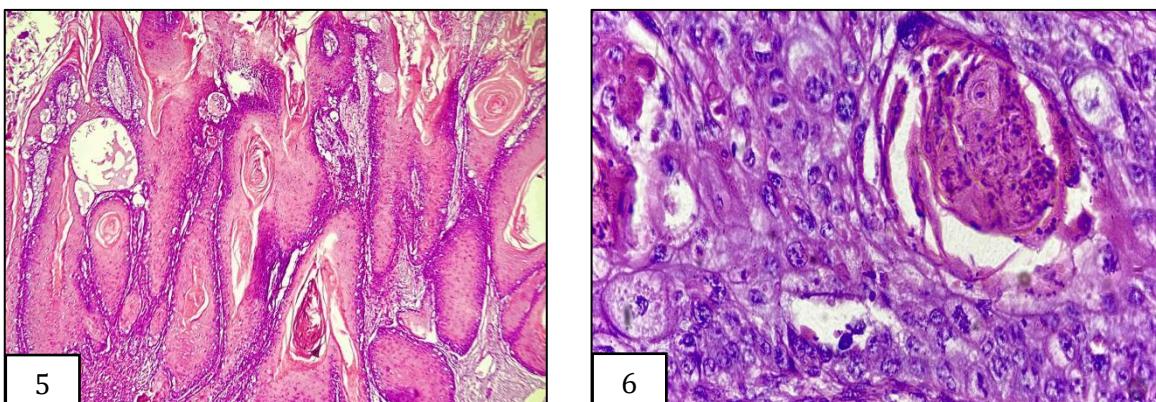
embryonic form. It indicates de-differentiation, or loss of the structural and functional differentiation of normal cells (Carvalho *et al.*, 2005). Hemorrhages and inflammatory cells such as lymphocytes, plasma cells and neutrophils are seen in local stromal and deep dermal connective tissue.

Well differentiated tumours are characterised by whorls (pearls) with intensely eosinophilic keratinized centers (Pugliese *et al.*, 2014). The neoplastic epithelial cells are arranged in islands of squamous neoplastic cells; some of these islands contained keratin pearls (Kumar *et al.*, 2023). Nuclear and cellular pleomorphism is shown by neoplastic cells, elevated mitotic index with numerous atypical mitotic figures; severe necrosis, hemorrhages are commonly observed under the microscope.

### 2. Cytological Examination:

- For tumour diagnosis a non-traumatizing technique, carried out by sampling in order to examine tumour cells, scraping of accessible mucosae and skin or other easily accessible tissues provide the cytologist with the samples necessary for a microscopic examination in order to establish the tumour nature.

- Condensation of nuclear chromatin, prominent nucleoli, severely dyskaryotic cells with irregular hyperchromatic nuclei, anisonucleosis (variation in size of cell nuclei, most pronounced in dysplasia and malignancy), bizarre mitoses, multinucleation and increased nucleocytoplasmic ratios, are mainly observed in smears prepared from histologically confirmed squamous cell carcinoma (Hoffmann *et al.*, 1978).
- Cytological examinations are countered by the difficulty of interpretation; negative diagnosis frequently occurs in the case of neoplasms that will subsequently be diagnosed histologically.



**Fig.-5&6:** Microscopic examination revealing squamous cell carcinoma with well differentiated epithelial pearls.

(Fig. 5 H&E X 100; Fig. 6 H&E X 400).

### 3. Immunohistochemistry:

Confirmatory diagnosis of SCC in bovines is done on the basis of detection of biomarkers or tumour markers by immunohistochemistry and immunohistochemical findings. Immunohistochemical evaluation of cases suspected of squamous cell carcinoma in bovines serve as a vital diagnostic technique. Immunohistochemistry as per National Cancer Institute (NCI) is defined as a laboratory method in which antibodies are used to check for certain antigens (markers) in a tissue sample. Antibodies linked to an enzyme or fluorescent dye bind to antigen in the tissue sample resulting into activation of enzyme/ dye and antigen can be visualized under microscope.

#### Significant Biomarkers Used for The Detection of SCC By Immunohistochemistry:

- Tumour markers are the unique attributes that may reflect the neoplastic process by their high / low level of expression as compared to that of normal cells, offering a putative use in the diagnosis, prognosis and tumourogenesis of cancer (Sharma *et al.*, 2020).
- Various biomarkers aids in the diagnosis of squamous cell carcinoma (both horn and eye) in the studies conducted in the past, such as: **p53, keratins, cytokeratins (pancytokeratin), p16, EGFR (Epidermal Growth Factor Receptor), VEGF (Vascular Endothelial Growth Factor), interleukins** etc.
- Immunohistochemical studies involves immunoexpression of these biomarkers in stained tissue

samples examined under microscope.

### 1. Tumour suppressor genes:

- p53 gene makes protein that is found inside cell's nucleus and plays a key role in controlling cell division and cell death. Mutations in the p53 gene may cause cancer cells to grow and spread in the body, these mutations have been found in many types of cancer (Sharma *et al.*, 2020).
- Squamous cell carcinomas commonly have mutations in p53, and positive immunolabeling for p53 has been reported in animals especially in SCCs of non-pigmented skin secondary to exposure to UV radiation (Kumar *et al.*, 2023).
- The tumour suppressor gene p16 has gained widespread importance in cancer, frequent mutations and deletions of p16 in human cancer cell lines first suggested an important role for p16 in carcinogenesis (Fornazari *et al.*, 2017 and Liggett *et al.*, 1998).
- SCCs have been shown to express p16 through immunolabeling. Antibodies targeting p53 and p16 have been used as prognostic factors in SCCs (Fornazari *et al.*, 2017).

### 2. Cytokeratin:

- Cytokeratin is one of the most important tumour markers for diagnosis of epithelial tumours such as horn cancer, high variations in expression patterns of cytokeratin have been correlated to different pathways of epithelial differentiation leading to the accurate diagnosis and classification of tumours of epithelial origin into different subtypes by immunohistochemistry (Sharma *et al.*, 2020).
- Pan- Cytokeratin expression under microscope shown by tumour cells with a distinct reddish brown cytoplasmic staining were considered positive.
- Cytokeratin expression similar to that in normal epidermal keratinocytes is conserved in well-differentiated horn cancer, but the change in expression of cytokeratins is observed during progression to malignant transformation.
- The expression of simple epithelial or non-cornifying stratified squamous epithelial cytokeratins in cutaneous tumours mainly in horn cancer is a marker for their capability of invasion and metastatic potential (Panchal *et al.*, 2020).
- Earlier studies on SCC revealed keratins and interleukins (cytokines expressed and secreted by leucocytes and other body cells, plays essential role in differentiation of immune cells in pro-inflammatory and inflammatory processes).
- Upregulation of keratin supports metastasis of tumour via cell proliferation, migration and effecting cell stability, while downregulation of interleukins deprives the immune response to tumour posing a clear pathway for metastasis of horn cancer.

### 3. EGFR:

Epidermal Growth Factor Receptor (EGFR) is a key factor in malignant tumours of epithelial origin, and its activity enhances tumour growth, invasion and metastasis by induction of angiogenesis (formation of new

blood vessels, commonly seen in malignant neoplasms which is responsible for their rapid growth) (Lakshmi *et al.*, 2020).

#### 4. VEGF:

Vascular Endothelial Growth Factor (VEGF) is a potent angiogenic factor, produced by variety of cells such as: keratinocytes, epithelial cells, macrophages, mast cells, fibroblasts etc. It is involved in several types of tumours, shown influence in both tumour neovascularization and dissemination (Lakshmi *et al.*, 2020). Scientific studies suggested a possible role of VEGF in development of eye cancer in bovines through angiogenesis, with immunoexpression of VEGF in bovine OSCC.

#### Conclusions:

- Cytological examination of tumours suspected of SCC by is characterised by variation in anisocytosis and poikilocytosis with altered nuclear details.
- SCC of horn is generally unilateral and characterised by large cauliflower like ulcerated growth with bleeding at the base of the horn with rough and verrucous surface.
- Ocular neoplastic growths vary from soft to hard in consistency. Firm cauliflower like mass, extending deep into the frontal and nasal sinus, congested with verrucous surface is also seen in OSCC.
- Cell nests or keratin pearls with high degree of keratinization and layered pattern of keratinization are characteristic of squamous cell carcinoma.
- Immunohistochemistry of tissues using tumour markers like Pan-CK, p53, EGFR, p16 and VEGF aids in confirmatory diagnosis of neoplasms of epithelial origin.
- Pan-Cytokeratin immunoreactivity confirms the tumour of epithelial origin and EGFR immunoexpression is confirmatory for malignancy and degree of metastasis.
- Tumour suppressor genes like p53, p16 plays a crucial role in control of cell division and cell death. Mutations in these genes may cause cancer cells to grow and spread in the body, these mutations are responsible for malignant transformation of cells.

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