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Krushna A Jonpelliwar

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Pravin R Rathod

Associate Professor, Department of
Veterinary Pathology, College of
Veterinary and Animal Science,
Parbhani, Maharashtra, India

Govind R Gangane

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Sharad D Chepte

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Meera P Sakhare

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Pallavi D Londhe

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Mayur Khambyatkar

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Adarsh Jallela

Department of Veterinary
Pathology, College of Veterinary
and Animal Science, Parbhani,
Maharashtra, India

Corresponding Author:

Pravin R Rathod

Associate Professor, Department of
Veterinary Pathology, College of
Veterinary and Animal Science,
Parbhani, Maharashtra, India

Histopathological spectrum of squamous cell carcinoma in horn cancer of cattle

Krushna A Jonpelliwar, Pravin R Rathod, Govind R Gangane, Sharad D Chepte, Meera P Sakhare, Pallavi D Londhe, Mayur Khambyatkar and Adarsh Jallela

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Abstract

Squamous cell carcinoma (SCC) of the horn constitutes a prevalent and economically pertinent neoplasm impacting Indian cattle, particularly among indigenous breeds. This investigation sought to elucidate the histopathological spectrum and grading of SCC within bovine horn tissue, with a focus on differentiation and subtype classification. A total of 48 horn tissue samples, suspected of harboring SCC, were procured from cattle between July 2024 and January 2025 and subsequently subjected to microscopic analysis. Based on parameters including cellular morphology, keratinization, mitotic index, anaplasia, and necrosis, the neoplasms were classified into well-differentiated (G0), moderately differentiated (G1), and poorly differentiated (G2) categories. Subtypes such as verrucous, pseudovascular, and desmoplastic SCC were also discerned. G0 tumors exhibited organized keratin pearl formation alongside minimal mitotic activity, indicative of low malignancy potential. G1 tumors presented increased pleomorphism and early invasion characteristics, whereas G2 tumors were marked by pronounced cellular atypia, elevated mitotic index, and extensive necrosis, suggesting a more aggressive phenotype.

Keywords: Squamous cell carcinoma, horn cancer, cattle, histopathology, tumor grading, verrucous SCC, pseudovascular SCC, desmoplastic SCC, keratin pearls, veterinary oncology

Introduction

Squamous cell carcinoma (SCC) poses a significant health challenge within the Indian cattle population, leading to marked reductions in productivity and contributing to substantial economic losses within the agricultural and livestock sectors. As per the 20th Livestock Census (2019) ^[1], India's total livestock population stands at 535.78 million, with bovines—including Cattle, Buffalo, Mithun, and Yak—constituting 302.79 million. This represents a 1% increase from the previous census, underlining the crucial role of cattle in India's agrarian economy.

Among various neoplastic conditions affecting cattle, SCC stands out as a particularly prevalent and economically burdensome pathology, especially in indigenous Zebu cattle. The prolonged morbidity and associated mortality significantly impair the draught capabilities of affected animals. According to Udharwar *et al.* (2008) ^[8], the overall incidence of neoplasms in Indian cattle is 53.5%, with SCC comprising approximately 41.46% of these cases. A notable manifestation of SCC occurs in the horn tissue, affecting nearly 1% of the Indian cattle population and accounting for over 80% of documented tumors in this region (Mukeshbhai *et al.*, 2024) ^[6].

SCC originates from the stratified squamous epithelium of the skin and mucous membranes (Vegad, 2007) ^[10]. Though the precise etiology remains unclear, its development is understood to be multifactorial. Contributing factors include prolonged exposure to ultraviolet (UV) radiation, lack of skin pigmentation, chronic irritation due to yokes, horn painting, hormonal imbalances, sparse hair coverage, and viral agents such as papillomavirus. Compared to papillomatous tumors, SCC is more aggressive, characterized by uncontrolled cellular proliferation and infiltrative growth behavior.

Histopathologically, SCC in horn tissue reveals well-defined clusters or lobules of polyhedral squamous epithelial cells. These appear "prickly" due to prominent cell borders, with enlarged, pleomorphic nuclei. The lobules are separated by delicate connective tissue strands, and a defining microscopic feature is the presence of "epithelial pearls," which are concentrically layered keratin structures (Jaiswal *et al.*, 2014) [4].

The tumor's histological grading plays a critical role in determining its biological behavior and clinical outcomes. As described by Gharagozlou *et al.*, 2007 [3] well-differentiated SCC is marked by large islands of neoplastic epithelial cells with extensive keratinization and keratin pearl formation. Moderately differentiated SCC exhibits greater cellular pleomorphism, hyperchromatic nuclei, reduced keratinization, and early invasion. Poorly differentiated SCC is typified by pronounced cellular atypia, high mitotic activity, minimal keratin formation, and prominent inflammatory infiltration. These gradations influence tumor aggressiveness, invasiveness, and prognosis.

Despite its clinical relevance and economic impact, limited studies have explored the histopathological diversity of SCC in bovine horn cancers. A detailed investigation into the tumor grading and histological subtypes is imperative for enhancing diagnostic accuracy and refining therapeutic interventions in veterinary oncology. Accordingly, this study aims to elucidate the histopathological spectrum of SCC in horn tissue of cattle, with particular emphasis on tumor differentiation and subtype classification. Through this characterization, the study seeks to contribute valuable insights into diagnosis, prognosis, and the formulation of effective management strategies for affected animals.

Materials and Methods

Sample Collection

A total of 48 samples of horn tissue suspected of squamous cell carcinoma (SCC) were collected from cattle presented at Veterinary Clinical Complex and Nearby Veterinary dispensaries between July 2024 and January 2025. The samples were obtained from cattle of different breeds, ages, and sexes to assess the histopathological spectrum of SCC. The affected horns tissue samples were collected following surgical excision. Each sample was labelled and preserved in 10% neutral buffered formalin for histopathological evaluation.

Histopathological Processing

The fixed tissue samples were processed using standard histopathological techniques. Briefly, tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 4-5 µm thickness were cut using a rotary microtome and mounted on glass slides. The sections were then stained with Hematoxylin and Eosin (H&E) following the protocol described by Luna (1968) [5] to assess tumor architecture, cellular morphology, keratinization, and invasive patterns.

Histopathological Grading and Subtype Classification

The stained sections were examined under a light microscope at varying magnifications. SCC cases were graded into well-differentiated (G0), moderately differentiated (G1), and poorly differentiated (G2) categories based on following table:

Table 1: Histopathological grading

Criteria	Grading of SCC		
	G ₀	G ₁	G ₂
Epithelial Pearls	Small in size, comprising 4-5 layers of epithelial cells with a homogenous keratin mass at the center	Well-developed pearls of large size and irregular shape	No pearls. Extensive proliferation of squamous cells in homogenous sheets
Mitotic Figures	6-13 per hpf around keratin pearls	30-50 per hpf in proliferating cells	80-100 per hpf in anaplastic cells
Extent of Anaplasia	Minimal	Moderate	Highly anaplastic cells with large hyperchromatic nuclei and prominent nucleoli
Pleomorphism	No pleomorphism	Mild pleomorphism	Marked pleomorphism
Presence of Inflammatory Cells	+++	++	+
Necrosis	Minimal	Moderate	Severe

Adapted from Gharagozlou *et al.* (2007) [3], Van den Top *et al.* (2011) [9].

Additionally, SCC subtypes, including pseudovascular SCC, desquamated SCC, verrucous SCC, and basaloid SCC, were identified based on specific histopathological features.

Results and Discussion

Squamous cell carcinoma (SCC) exhibited distinct histopathological characteristics that varied with the degree of differentiation, influencing tumor behavior and progression. In well-differentiated SCC (G0), the neoplasm consisted of large clusters of squamous epithelial cells showing pronounced squamous differentiation. These cells formed numerous small keratin pearls comprising 4-5 layers of epithelial cells with a central homogeneous keratin core. Mitotic figures were relatively low, ranging from 6 to 13 per high-power field (hpf), primarily located around the keratin pearls. Cellular anaplasia was minimal, with cells retaining a typical squamous morphology and showing no pleomorphism. The surrounding stroma displayed a dense inflammatory

infiltrate (+++), indicating a strong host immune response, while necrosis was minimal, reflecting a less aggressive tumor phenotype (Fig 1). A distinct verrucous/papillary subtype was also identified within this group shown in Fig 2., characterized by bulbous, finger-like projections of well-differentiated squamous epithelium (Nemec *et al.*, 2012) [7].

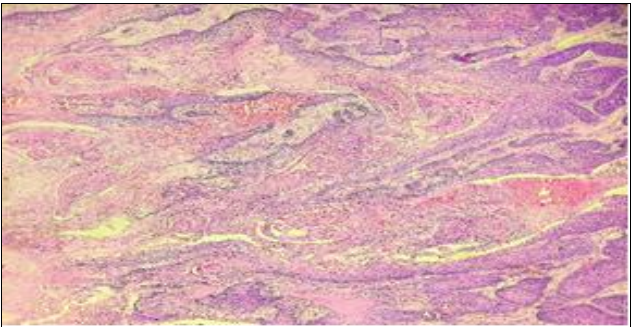


Fig 1: Well differentiated squamous cell carcinoma with macro to micro sized well developed keratin pearl (H&E, 100X)

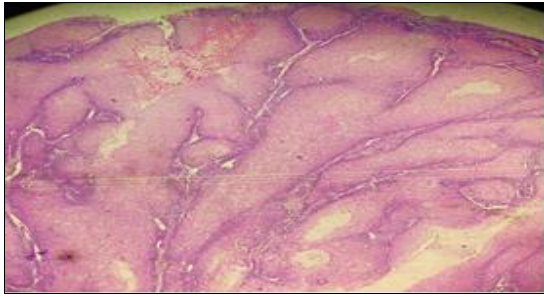


Fig 2: Well differentiated Verrucous SCC which shows bulbous and finger like sheets of well differentiated tumor cells

In moderately differentiated SCC (G1), the tumor displayed greater pleomorphism, with irregularly shaped keratin pearls and disorganized epithelial nests. Neoplastic cells exhibited hyperchromatic nuclei and partial loss of cellular polarity. Mitotic figures were significantly higher, ranging from 30 to 50 per hpf, and moderate anaplasia was evident. Mild pleomorphism was present, accompanied by a moderate inflammatory response (++). The stroma also showed noticeable fibroblast proliferation, especially around tumor clusters. Necrosis was moderate, suggesting increased tumor burden and hypoxic areas within the tissue. Early invasive behavior, including basement membrane disruption and infiltration into adjacent tissue, was observed in some regions (Fig 3). Subtype observed under moderately differentiated SCC was pseudovascular SCC. It displayed pseudo-vasculature formation, where tumor cells mimicked vascular channels, contributing to the deceptive morphology and potentially reflecting aggressive angiogenic behaviour. Fibroblast proliferation was prominent in the tumor stroma, particularly surrounding clusters of neoplastic cells, and necrosis was moderate, indicating higher tumor burden (Fig 4).

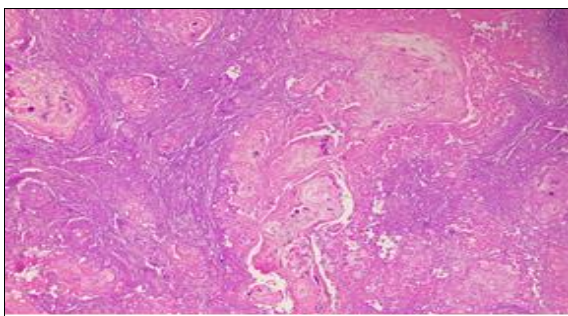


Fig 3: Moderately differentiated squamous cell carcinoma with extensive fibrous tissue proliferation and irregular shaped keratin pearl

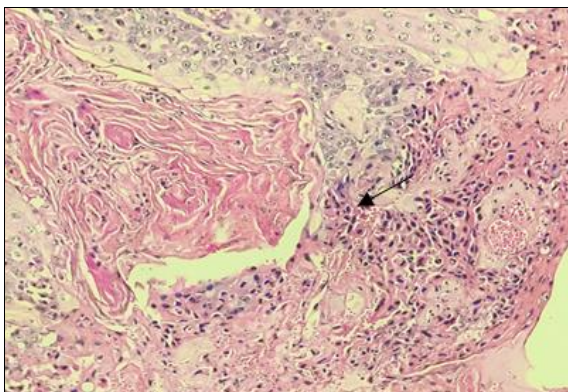


Fig 4: Moderately differentiated pseudovascular squamous cell carcinoma. Arrow indicating pseudovascularity formation.

In poorly differentiated SCC (G2), the tumor lost all signs of organized differentiation. Epithelial pearls were absent, and the tissue comprised sheets of highly anaplastic squamous cells with severe pleomorphism and prominent nuclear atypia. Neoplastic cells had large, hyperchromatic nuclei with conspicuous nucleoli and exhibited a high mitotic rate, reaching 80-100 per hpf. Dyskeratotic cells and a high nuclear-to-cytoplasmic ratio were commonly seen. The inflammatory response was minimal (+), suggesting possible immune evasion or suppression, and necrosis was extensive, indicating rapid tumor growth and tissue destruction (Fig 5). A desmoplastic variant was also observed in this group, featuring poorly differentiated tumor cells embedded within dense fibrous stroma (Fig 6).

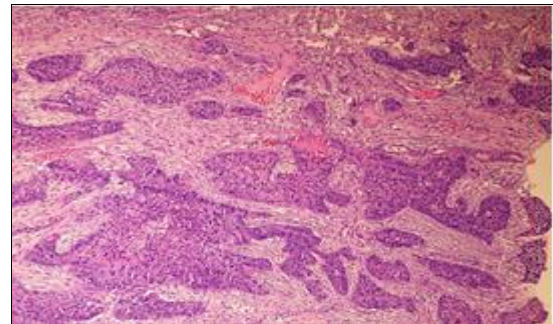


Fig 5: Poorly differentiated squamous cell carcinoma with extensive fibrous tissue proliferation (H&E, 100X)

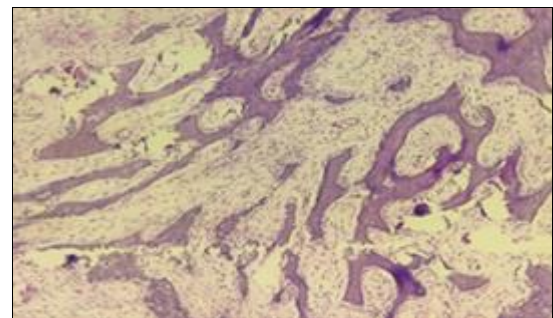


Fig 6: Poorly differentiated desmoplastic squamous cell carcinoma. (H & E, 100X)

These progressive changes from G0 to G2 underscored the increasing aggressiveness and malignant potential of SCC with decreasing differentiation. The current observations resemble with the findings of Chandatre *et al.* (2020) and Gharagozlou *et al.* (2007) ^[3] in respect of grading of horn SCC in cattle.

Conclusion

Histopathological analysis of bovine horn SCC revealed a strong correlation between tumor differentiation and aggressiveness. Well-differentiated SCC (G0) showed organized keratinization, low mitotic activity, and a strong inflammatory response, indicating a less aggressive nature. The verrucous subtype, with bulbous epithelial projections, further reflected low-grade malignancy. Moderately differentiated SCC (G1) exhibited increased pleomorphism, higher mitotic rates, and early invasion. The pseudovascular variant, mimicking vascular channels, suggested a more aggressive behavior. Poorly differentiated SCC (G2) was the most malignant, with high mitotic activity, severe pleomorphism, and extensive necrosis. The desmoplastic subtype, marked by dense fibrous stroma, indicated high invasiveness. These findings highlight the importance of

grading and subtype identification for accurate diagnosis, prognosis, and management of SCC in cattle.

Conflict of Interest

Not available.

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