

Comparative Pathology and Biomarker Expression in Bovine Squamous Cell Carcinoma: A Histopathological and Immunohistochemical Study from Chhattisgarh Plains, India

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ABSTRACT

Background: Bovine squamous cell carcinoma (SCC) is a common malignant epithelial tumor affecting the horn, eye, skin, and genital organs, with limited comparative studies on its pathological and biomarker profiles across anatomical sites.

Aim: To compare the clinicopathological, hematological, histopathological, and immunohistochemical characteristics of bovine SCC at different anatomical locations in the Chhattisgarh plains.

Materials and Methods: Twenty clinically suspected SCC cases from Durg, Dhamtari, and Rajnandgaon districts were examined using clinical evaluation, hematology, exfoliative cytology, histopathology, and immunohistochemistry for pancytokeratin (Pan-CK), p53, p16, and epidermal growth factor receptor (EGFR).

Results: SCC was confirmed in horn, ocular, skin, and vulvar tissues. Horn SCC predominated in aged bullocks, while ocular SCC was more frequent in females. Histopathology revealed variable differentiation from well-differentiated keratinizing tumors to poorly differentiated invasive carcinomas. Hematology showed leukocytosis, neutrophilia, monocytosis, anemia, and thrombocytopenia. Pan-CK showed strong positivity in all cases, EGFR expression increased in aggressive tumors, p53 exhibited variable nuclear positivity, and p16 expression was weak or absent, highlighting their diagnostic and prognostic significance.

Keywords: Bovine squamous cell carcinoma, horn cancer, ocular SCC, pancytokeratin, EGFR, p53, p16, immunohistochemistry.

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INTRODUCTION

Squamous cell carcinoma (SCC) is a highly aggressive, invasive, and destructive malignant neoplasm that originates from the stratified squamous epithelium and represents one of the most frequently diagnosed and clinically significant epithelial cancers across a wide variety of species of domestic animals. In bovine populations specifically, SCC frequently affects and infiltrates several critical anatomical sites, including the horn core, the globe of the eye and its associated adnexa, various regions of the skin, and the external genital tract, thereby causing considerable, widespread, and multifaceted economic losses for the livestock industry due to significantly reduced milk and meat productivity, escalating veterinary diagnostic and treatment costs, and the unfortunate necessity for the premature culling or disposal of affected livestock (Rao et al., 2014). The underlying etiology of bovine SCC is widely recognized as being remarkably complex and multifactorial in nature, typically involving a synergistic combination of chronic local tissue irritation (Kumar et al., 2025), prolonged and cumulative exposure to solar ultraviolet radiation, inherent genetic predisposition within certain lineages, a distinct lack of protective melanin pigmentation in specific breeds, and potential infections by oncogenic viral agents such as papillomaviruses.

Horn core carcinoma is particularly prevalent and endemic in *Bos indicus* cattle populations and remains a major, persistent surgical, clinical, and pathological challenge for veterinary practitioners, livestock owners, and oncology researchers across the Indian subcontinent (Tyangi and Singh, 2006). Ocular squamous cell carcinoma (OSCC), which is commonly referred to in the commercial agricultural sector and among farmers as “cancer eye,” is strongly and unequivocally associated with intense ultraviolet light exposure (Fornazari et al., 2017) and the partial or total depigmentation of the periocular tissues and eyelids in susceptible cattle breeds (Kumar et al., 2023). Detailed and systematic histopathological grading,

combined with comprehensive immunohistochemical characterization of cellular markers, are considered indispensable and essential diagnostic tools for accurately understanding the molecular mechanisms of tumor progression, the specific biological behavior of the malignancy, and the overall clinical prognosis for the individual patient. The present study aimed to meticulously evaluate, document, and compare the specific pathological alterations, morphological variations, and distinct biomarker expression patterns among various clinical forms of bovine SCC occurring in the unique environmental and geographical conditions of the Chhattisgarh plains.

MATERIALS AND METHODS

Sample Collection and Data Documentation

A comprehensive and diverse total of twenty clinical neoplastic tissue samples, all of which were strongly suspected of being Squamous Cell Carcinoma (SCC) based on preliminary physical evaluations, were meticulously collected from affected cattle and buffaloes presented for detailed examination at various government veterinary hospitals and specialized private clinics located across the Durg, Dhamtari, and Rajnandgaon districts within the state of Chhattisgarh. For every individual case included in this research study, a detailed and thorough clinical history was carefully documented, while specific biological and demographic parameters—including the animal’s exact age, specific breed, gender, and the precise anatomical location and orientation of the tumorous growths—were systematically recorded and categorized to facilitate a robust further analysis of the disease patterns.

Hematological Evaluation and Comprehensive Laboratory Assessment:

The extensive and multi-faceted diagnostic findings derived from the analysis of patient samples revealed the presence of significant neutrophilia, profound anemia, and persistent monocytosis. These specific hematological observations served as the primary clinical markers and critical focal points of

investigation throughout the duration of this study. Such well-documented and distinct alterations in the blood profile are entirely consistent with, and strongly supported by Somvanshi (1991) and Gomes et al., (2012), various earlier investigations and peer-reviewed studies previously conducted by researchers within this specialized medical field. To ensure the highest possible level of diagnostic accuracy and data reliability, detailed blood samples were systematically collected from all participating subjects and subjected to rigorous, high-precision laboratory analysis focused on a comprehensive range of clinical parameters and biochemical markers:

- Total leukocyte count (WBC) to evaluate the overall systemic immune response and detect signs of infection or inflammation.
- Differential leukocyte count to precisely identify the specific proportions and absolute numbers of various white blood cell types.

- Erythrocyte count (RBC) to thoroughly assess the oxygen-carrying capacity of the blood and the general red cell status of the patient.
- Hemoglobin concentration to accurately measure the specific protein levels responsible for effective oxygen transport throughout the body.
- Hematocrit (HCT) to determine the exact volume percentage of red blood cells relative to the total blood volume, aiding in anemia classification.
- Platelet count (PLT) to examine the blood's essential clotting functionality and monitor the overall health of the coagulation system.

Cytological Examination

Impression smears and exfoliative cytology samples were stained using:

- Giemsa stain
- Papanicolaou stain

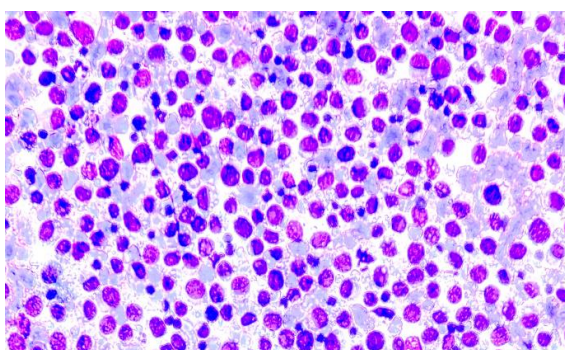


Fig. 1 Increase in cellularity (one of the early features of neoplastic growth at cellular level, Giemsa X400)

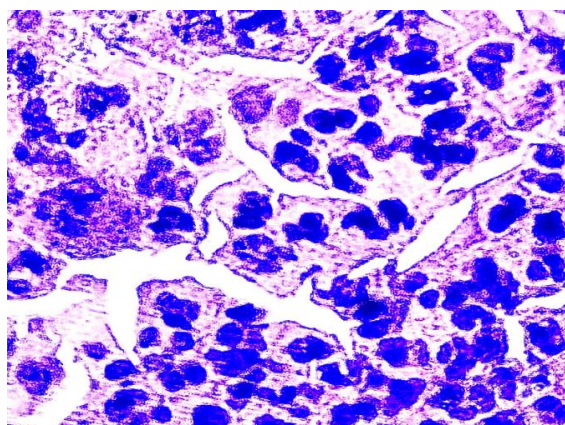


Fig. 2 Neutrophila detected in peripheral blood smear from cattle suffering from horn cancer (Giemsa X1000)

Histopathology

Formalin-fixed and paraffin-embedded (FFPE) tissue specimens were meticulously processed according to established standard routine laboratory protocols, which included sequential stages of gradual dehydration in increasing concentrations of ethanol followed by comprehensive chemical clearing using specialized agents like xylene. These prepared histological sections were subsequently stained with hematoxylin and eosin (H&E) dyes to facilitate highly detailed microscopic examination, structural visualization, and in-depth morphological analysis of the tissue architecture. The identified malignant tumors were carefully evaluated, staged, and graded by senior expert pathologists based on a rigorous and comprehensive set of the following pathological criteria: The specific degree of cellular and architectural differentiation relative to the surrounding normal tissue structures; The presence, severity, and total extent of cellular anaplasia, along with significant evidence of nuclear atypia, loss of polarity, and prominent cellular pleomorphism; The precise depth of tumor penetration through anatomical layers and the specific patterns of local tissue invasion or aggressive stromal infiltration into the surrounding microenvironment; The mitotic index, which was systematically calculated, verified, and recorded as the total number of distinct mitotic figures observed across ten representative and non-overlapping high-power fields (HPF).

Immunohistochemistry

The following essential diagnostic and prognostic biomarkers were comprehensively,

rigorously, and systematically evaluated using high-affinity, highly specific antibodies through state-of-the-art automated staining systems to ensure maximum reproducibility and standardization of results: Pancytokeratin (Pan-CK) expression was utilized to definitively and accurately confirm the epithelial origin of the identified neoplastic cells and to clearly differentiate them from various non-epithelial spindle cell lesions or mesenchymal-derived pathologies. The p53 protein expression patterns and distribution were rigorously analyzed to assess potential tumor suppressor gene mutations and overall functional status, providing deep molecular insights into the complex pathogenesis and biological behavior of the tumor. The p16 protein levels were measured as a critical and highly sensitive surrogate marker for high-risk HPV infection or significant underlying cell cycle dysregulation, both of which are vital for achieving accurate diagnostic stratification and risk assessment. Epidermal Growth Factor Receptor (EGFR) status was precisely and thoroughly determined to provide a comprehensive, evidence-based foundation for clinical targeted therapy decisions and for evaluating the patient's long-term prognosis and survival outlook. Immunoreactivity scores were systematically recorded and documented for each individual sample, being meticulously quantified and calculated according to both the specific staining intensity observed and the overall total percentage of positive-staining tumor cells identified within the representative microscopic areas examined.

RESULTS

Epidemiological Findings

Tumor Site	Predominant Species	Age Group	Sex Predilection
Horn SCC	Cattle	>7 years	Male
Ocular SCC	Cattle/Buffalo	>5 years	Female
Skin SCC	Cattle	Adult	Variable
Vulvar SCC	Cattle	Old age	Female

Gross Pathology**Horn SCC**

- Large cauliflower-like proliferative masses
- Foul-smelling discharge
- Ulceration and hemorrhage
- Deviation and loosening of horn

Ocular SCC

- Nodular hemorrhagic masses
- Corneal invasion
- Purulent conjunctivitis
- Blindness in advanced cases

Vulvar SCC

- Cauliflower-like proliferative lesions
- Ulceration and hemorrhage

Skin SCC

- Wart-like proliferative lesions
- Hyperkeratosis

Histopathology**Well Differentiated SCC**

- The tissue samples exhibit numerous and prominent keratin pearls throughout the section. There is extensive evidence of concentric keratinization within the epithelial nests. The cells show only minimal nuclear atypia, maintaining a high degree of resemblance to normal squamous epithelium (Fig. 3).

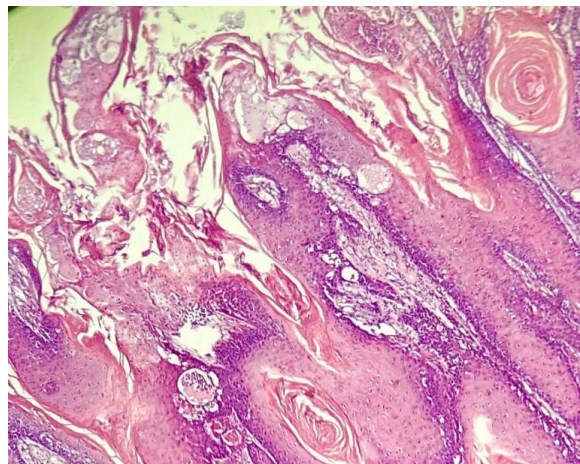


Fig. 3 Well-differentiated squamous cell carcinoma with distinctive cell nests (H&E X 100; Kumar et al., 2024)

Moderately Differentiated SCC

The tumor is characterized by the formation of irregular epithelial islands invading the underlying stroma. There is a presence of

moderate pleomorphism among the malignant cells. Furthermore, the slides demonstrate increased mitotic activity, indicating a more rapid rate of cellular proliferation (Fig. 4).

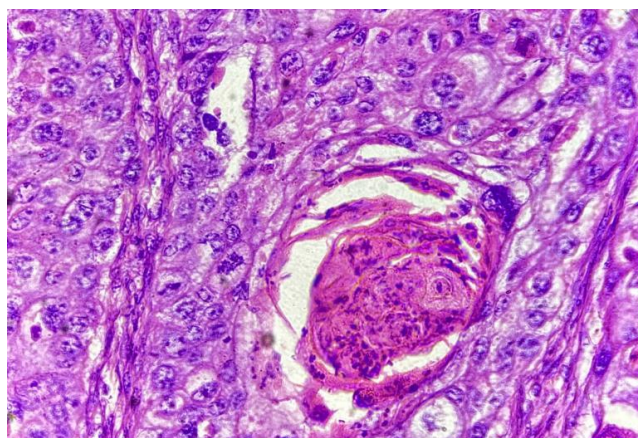


Fig. 4 Moderately differentiated squamous cell carcinoma

Poorly Differentiated SCC

This grade is marked by a complete or near-total absence of keratin pearls and organized keratinization. The malignant cells exhibit severe cellular atypia and significant

architectural distortion. There is evidence of extensive invasion into deep tissues, frequently accompanied by prominent neovascularization to support the aggressive tumor growth (Fig. 5).

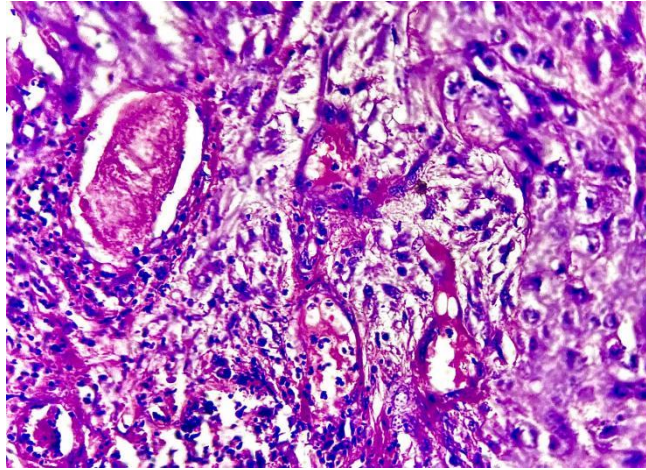


Fig.5 Poorly differentiated squamous cell carcinoma of horn (H&E X 400).

Hematological Alterations

Common findings included:

- Leukocytosis
- Neutrophilia
- Monocytosis
- Reduced RBC count
- Reduced hemoglobin
- Reduced hematocrit
- Decreased platelet count in advanced disease

Immunohistochemistry

Biomarker	Horn SCC	Ocular SCC	Vulvar/Skin SCC
Pan-CK	Strong (+++)	Strong (+++)	Positive
EGFR	Moderate-Strong	Mild-Strong	Variable
p53	Moderate	Mild-Moderate	Variable
p16	Negative	Weak/Negative	Negative

Pan-Cytokeratin: Strong and diffuse cytoplasmic immunoreactivity was consistently observed across all samples, which definitively confirmed the epithelial origin of every squamous cell carcinoma (SCC) case evaluated in this study (Fig. 6).

EGFR: Significantly higher expression levels and increased staining intensity were observed specifically in invasive regions and poorly differentiated tumors, indicating its

potential role in aggressive biological behavior and cellular proliferation (Fig. 6).

p16: Protein expression was found to be either entirely absent or notably weak in the vast majority of analyzed tumors, suggesting that this particular pathway has limited involvement or a minimal functional role in the molecular pathogenesis of bovine SCC (Fig. 6).

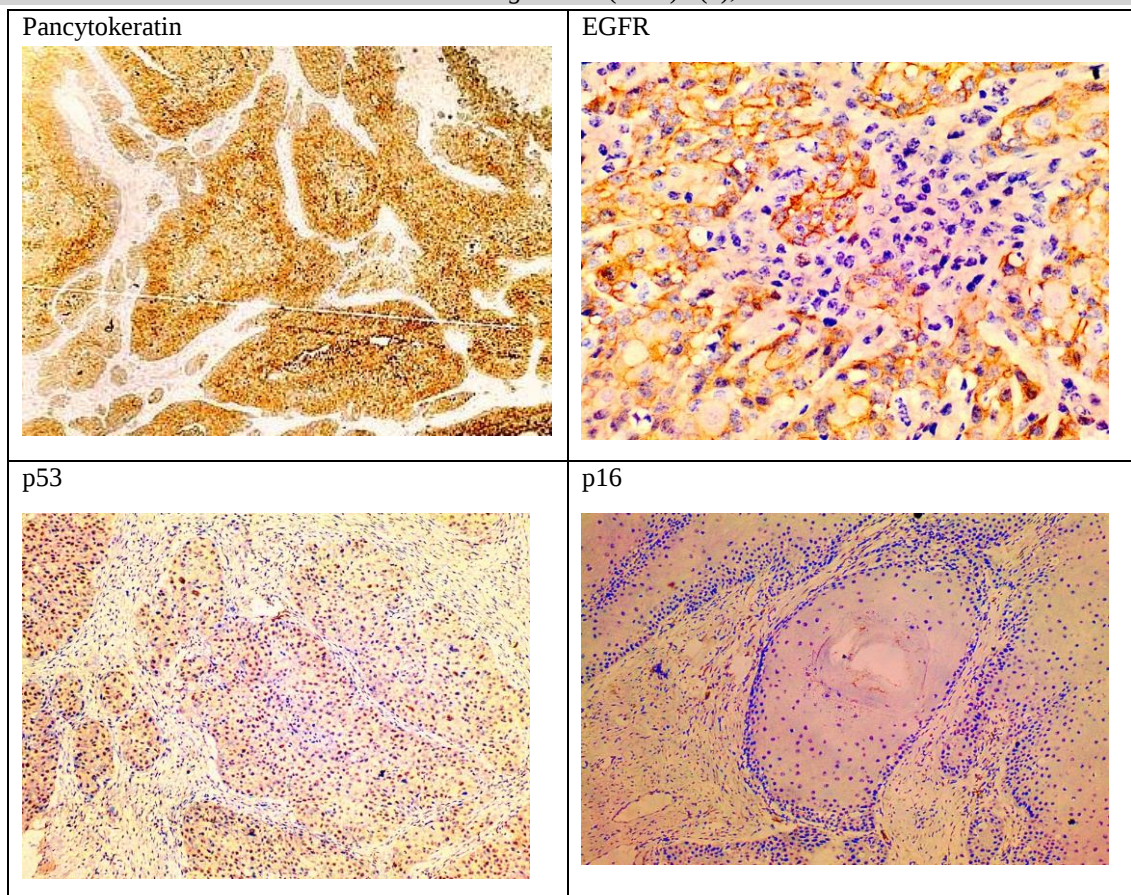


Fig. 6 Immunoexpression of Pan-CK, EGFR, p53 and p16 in bovine squamous cell carcinoma (IHC X400; IHC X 100)

DISCUSSION

The comprehensive comparative analysis revealed substantial pathological heterogeneity among various bovine squamous cell carcinomas (SCCs) occurring at distinctly different anatomical sites. Histopathological findings of this study were supported by the findings of Joshi et al., 2009; Giri et al., 2011; He et al., 2012; Jaiswal et al., 2014; Pugliese et al. (2014); Kumar et al., 2015; Filho et al., 2017; Shil et al., 2018; Kumar et al., 2020; Mathewos et al., 2020; Pazhanivel et al., 2020; Kumar et al., 2023 and Kumar et al., 2024).

Horn SCC was found to predominantly affect aged bullocks, exhibiting extensive patterns of keratinization and significant chronic inflammatory changes within the affected tissues (Sharma et al., 2020). In contrast, ocular SCC demonstrated highly aggressive local invasion into surrounding structures and a markedly higher degree of vascularization compared to other types (Lakshmi et al., 2020). Progressive

hematological alterations observed throughout the study reflected the underlying chronic inflammatory responses and complex neoplastic processes.

Strong Pan-CK expression across all examined tumors confirmed their fundamental epithelial differentiation and origin. Furthermore, significantly increased EGFR expression was closely correlated with both tumor aggressiveness and deep tissue invasiveness was in accordance with the findings of Lakshmi et al. (2020), whereas widespread p53 positivity suggested a substantial accumulation of genetic abnormalities during the stages of malignant progression. The notable absence of significant p16 expression across the samples indicates that this specific marker may have relatively limited diagnostic utility or prognostic value in the clinical evaluation of bovine SCC which was supported by the findings of Fornazari et al. (2017) and Kumar et al. (2023) and Kumar et al. (2024).

CONCLUSION

Bovine squamous cell carcinoma (SCC) exhibits significant site-specific pathological variations depending on its anatomical location, yet it consistently maintains characteristic epithelial biomarker expression patterns that define its lineage. The integration of comprehensive histopathological analysis with detailed immunohistochemical evaluation substantially improves overall diagnostic accuracy and provides deeper insight into the underlying mechanisms of tumor progression and complex biological behavior in affected cattle.

Comparative evaluation demonstrated that:

- Horn SCC is the most prevalent bovine SCC in Chhattisgarh.
- Ocular SCC exhibits greater invasive potential.

- Hematological abnormalities worsen with disease progression.
- Pan-CK is a reliable diagnostic biomarker.
- EGFR and p53 serve as useful prognostic indicators.
- p16 has limited diagnostic significance in bovine SCC.

Combined histopathology and immunohistochemistry significantly improve the diagnostic accuracy and precise classification of bovine squamous cell carcinomas, providing a more comprehensive understanding of these complex neoplastic lesions. By integrating traditional microscopic examination of tissue structures with specific molecular markers, veterinary pathologists can more effectively differentiate these tumors from other skin conditions and determine their exact pathological grade and biological behavior.

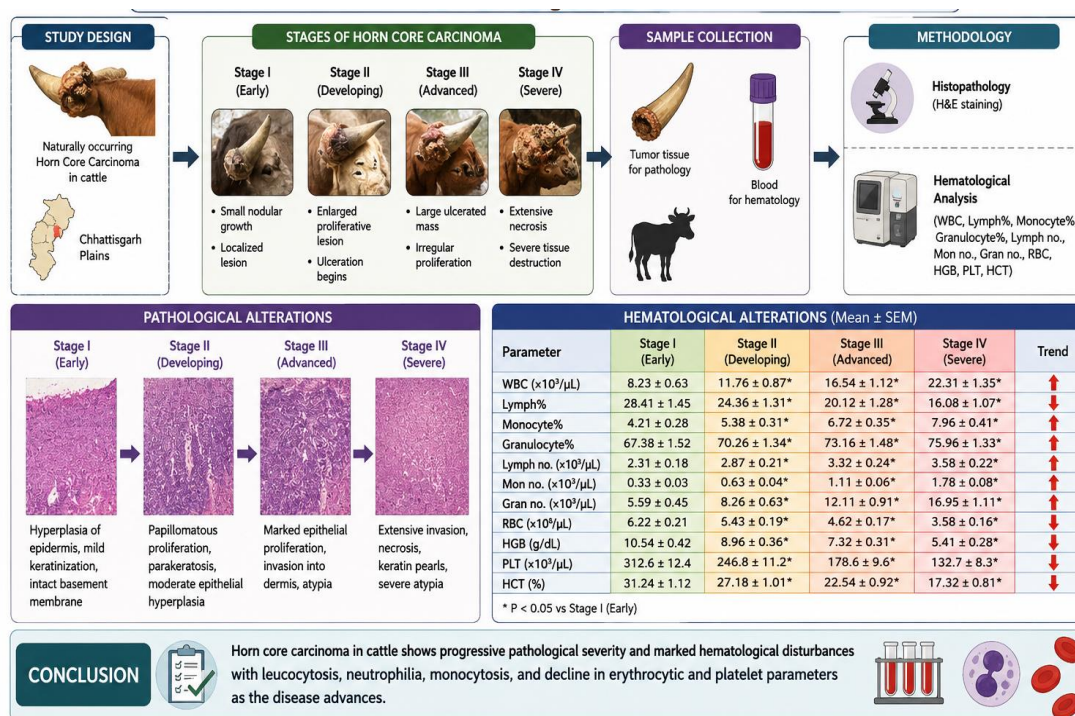


Fig. 7 Comprehensive workflow the study (AI generated).

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Conflict of Interest:

The authors declare no conflict of interest.

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