

## Pathomorphological and Immuno histochemical Findings of Different Types of Cutaneous Papillomas in Bovines

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### ABSTRACT

Bovine Papillomatosis (BP) is a cutaneous viral disease of cattle caused by Bovine Papilloma Virus (BPV). Out of 57 tumor samples, 12 were diagnosed as papillomas or warts (21.05%) of which ten were collected from cattle and two from buffaloes. The animals had either generalized or solitary cutaneous growths in different parts of the body. Grossly, the growths were solitary or multiple, pinkish to greyish-white in colour, 0.5 to 10 cm in diameter, sessile or pedunculated and hard in consistency. Histopathologically, squamous papilloma, viral papilloma (papillomatosis), fibropapilloma and angiokeratotic acanthomatous papilloma were noticed. Immuno histo chemically, PCNA expression in papilloma was observed predominantly in the cells of basal and parabasal layers of epidermis. The PCNA antibodies were very useful marker to detection of cellular proliferation in the bovine cutaneous papillomas

### INTRODUCTION

In cattle, papillomatosis is caused by bovine papilloma viruses (BPV), belonging to genus Papillomavirus, family Parovaviridae. This genus also includes viral agents causing papillomatosis in other animal species (goats, dogs, rabbits, horses, and rodents) and men. The virion has an icosahedral symmetry, size of 55 nm and contains a single two-stranded DNA molecule 7-8 kb of length (Zarkov, 2003). Twelve BPV serotypes are known – BPV 1-12, causing diseases with various clinical manifestations. Bovine papillomatosis is caused by BPV types from 1 to 10 (Vidya et.al. 2009), but BPV-1 and BPV-2 are outlined as main agents of fibropapillomatosis in cattle due to their affinity to epithelial tissue and skin (Pangty, et.al. 2010 and Singh, et.al. 2009). Under appropriate conditions, BPV-1 and BVP-2 could also cause fibroblastic papillomas in horses (Nasir and Camoi, 2008), as well as cutaneous sarcoid and non-regressing neoplasm in this species (Ragland and Spencer., 1968). BPV infection results from the replication of the virus in basal cells and subsequent formation of wart-like growths, most of which are benign and do not proliferate infinitely. The localisation of growths is different: abdominal and thoracic

wall, udder, vulva, head, neck etc. Young animals < 2 years of age are most commonly affected, although papillomatosis is also encountered in adult cattle. It is prevalent in both genders and all breeds. Heifers are more frequently affected than steers.

### MATERIALS AND METHODS

#### Source

The materials for present study included the samples suspected for neoplasia of bovine origin submitted to the Department of Veterinary Pathology, College of Veterinary Science, Tirupati by the Department of Veterinary Surgery and Radiology and Veterinary Dispensaries in and around Tirupati. Samples were also collected from animals necropsied in the Department of Veterinary Pathology. Particulars like breed, sex, location, shape, colour and consistency of growths were recorded.

#### Histopathology

Representative tissue samples collected were fixed in 10 percent neutral buffered formalin and processed routinely for histopathological examination. Sections of 4-6  $\mu$  thickness were made and stained with Haematoxylin and Eosin.

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Special staining technique with Masson's Trichrome, was employed (Luna, 1968 and Bancroft and Cook, 1994).

### IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed using Universal Dakocytomation LSAB® kit to evaluate the expression of proliferative markers namely PCNA.

#### Procedure

Sections of 5-6  $\mu$  thickness were mounted on to APES (Amino Propyl EthoxySialine) coated slides and incubated overnight at 37°C. Sections were deparaffinized and rehydrated. Sections were kept in citrate buffer (10mM, pH 6) and subjected to microwave treatment for 5 cycles each for 5 minutes at 750 watts to retrieve the antigenic sites. Sections were allowed to cool down to the room temperature. Sections were rinsed in TBS (Tris Buffer Saline). One drop of hydrogen peroxide was kept over the section for 15 minutes to block unwanted antigenic sites and to quench endogenous peroxidase activity. Sections were rinsed with TBS. Primary antibody was added onto the sections and incubated for 30 minutes. Sections were rinsed with TBS. Sections were incubated with secondary link antibody for 30 minutes. Sections were rinsed with TBS. Sections were incubated with tertiary Streptavidin peroxidase for 30 minutes. Sections were rinsed with TBS. DAB (Diaminobenzidine) was used as chromogen and the sections were incubated for 10 minutes. Sections were rinsed with distilled water. Sections were counter stained with Harris haematoxylin and mounted in DPX.

### RESULTS

Out of 57 tumor samples, 12 were diagnosed as papillomas or warts (21.05%) of which ten were collected from cattle (two from Holstein Friesian, one from Jersey, three from Hallikar and four from non-descript cattle) and the remaining two from buffaloes. Out of 11 cases of papilloma, there were nine cases of squamous papilloma and viral papilloma (papillomatosis), two cases of fibropapilloma (in a Jersey bull and a Hallikar bullock) and one case of angiokeratotic acanthomatous papilloma (in a non-descript cow). The incidence of papilloma was equal in male and female animals.

The animals had either generalized or solitary cutaneous growths in different parts of the body like head, neck, shoulder, limbs, udder and teats,

scrotum and inner aspect of thigh. Macroscopically the growths were solitary or multiple, pinkish to greyish-white in colour, 0.5 to 10 cm in diameter, sessile or pedunculated and hard in consistency. The surface was either smooth or rough and horny (Fig. 1 to 6).



**Fig1.** Squamous papilloma: HF cow: Multiple, grayish, rough and horny growths around the eye.



**Fig2.** Squamous papilloma: ND cow: Multiple gray to pink coloured warts with rough and horny surface on head, neck and shoulder region.



**Fig3.** Squamous papilloma: Cow: Small, solitary growths with smooth surface on udder and teats.



**Fig4.** Squamous papilloma: Bullock: Note multiple pinkish pedunculated growths near scrotum.



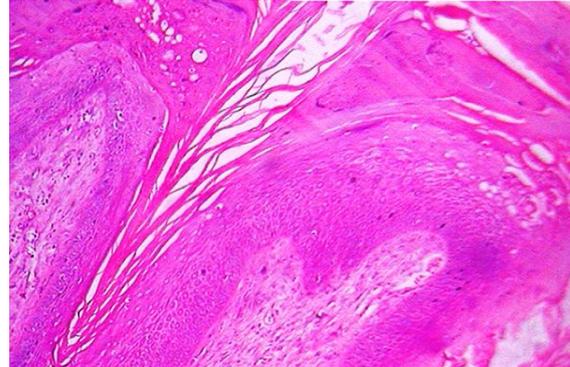
**Fig5.** Fibropapilloma: Note multiple warts which are round, sessile, dome shaped with a rough and even surface around the eye.



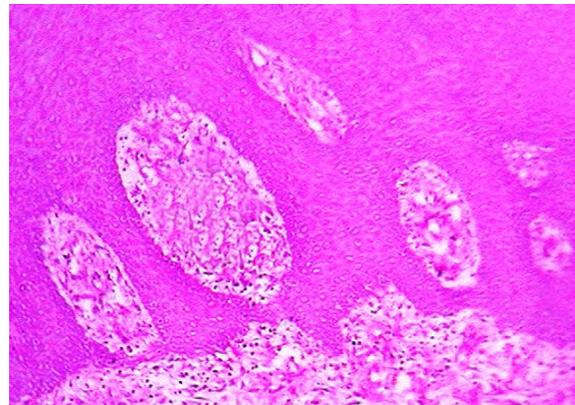
**Fig6.** Angiokeratotic papilloma: Jersey cow: Multiple, grayish pink, rough and horny growths on the ear and neck.

Histopathological examination revealed that the epidermis was thickened and hyperplastic with elongated, finger like outward projections in squamous papilloma (Fig. 7) and irregular, elongated, inward rete pegs in papillomatosis (viral papilloma) (Fig. 8) with varying degrees of parakeratosis, hyperkeratosis, acanthosis (Fig. 9) and a core of dermal fibrous connective tissue. The dermal papillae were elongated with irregular rete ridge formation deep into the dermis with lateral interconnections forming islands of dermal connective tissue surrounded by hyperplastic epidermal cells. The keratinocytes within stratum spinosum showed clear hydropic degeneration and a clear perinuclear halo with pyknotic nucleus (koilocytes). The granular cell layer with prominent basophilic keratohyaline granules was observed and the basal cell layer was hyperplastic. Occasionally melanin granules were found freely within the subepidermal and dermal melanophages. In fibropapilloma, extensive proliferation of fibroblasts and epidermal keratinocytes was noticed (Fig. 10 and 11). The dermal layer revealed proliferating cells which were large, plumb fibroblasts, arranged in haphazard whorls and fascicles. In

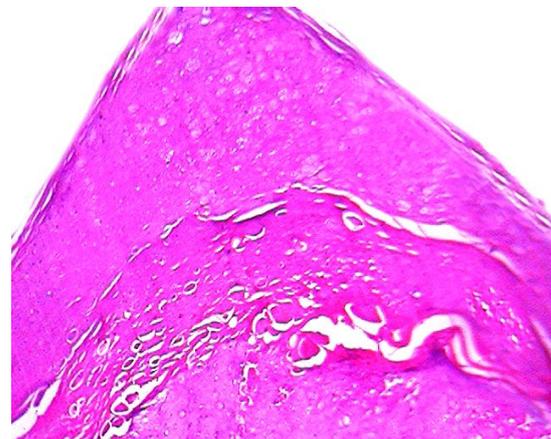
angiokeratotic papilloma, there were discrete groups of variably sized, dilated, thin walled and well differentiated vascular channels which were filled with blood and lined by a single layer of flattened endothelial cells (Fig. 12).



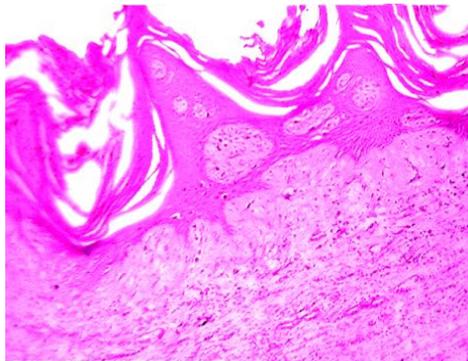
**Fig7.** Squamous papilloma: Finger like outward projections of epidermis with parakeratosis, acanthosis and a core of fibrous connective tissue. Note koilocytes with a perinuclear halo and a pyknotic nucleus. H&E: x 70.



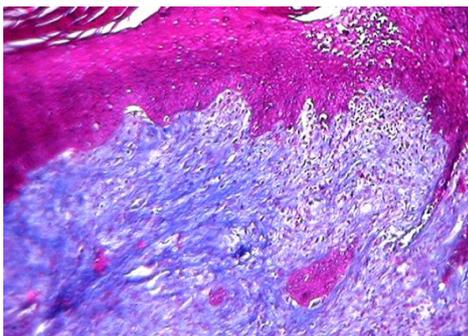
**Fig8.** Papillomatosis: Section showing irregular, elongated inward growths of rete pegs deep into the dermis with lateral interconnections. H&E: x 70.



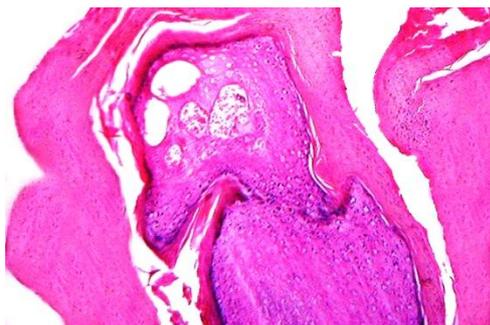
**Fig9.** Papilloma: Note finger like projection from epidermis with increased thickness, parakeratosis and hydropic degeneration of keratinocytes. H&E: x 280



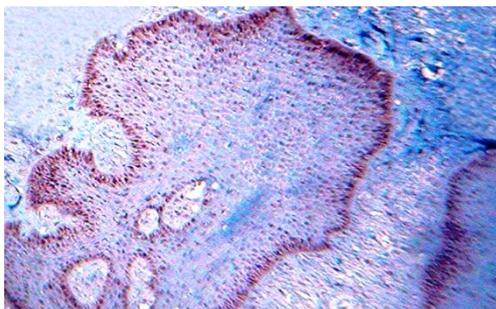
**Fig10.** Fibropapilloma: Note the finger like projection from epidermis with parakeratosis and extensive proliferation of fibroblasts in the dermis. H&E: x 70.



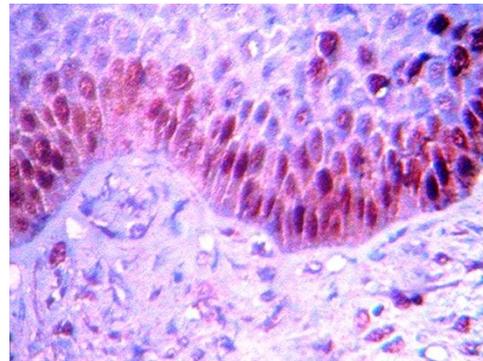
**Fig11.** Fibropapilloma: Note the characteristic thickening of epidermis with parakeratosis (red) and fibrous tissue proliferation (blue) in the dermis. Masson's Trichome stain: x 70.



**Fig12.** Angiokeratotic papilloma: Finger like projections of epidermis with groups of dilated, thin walled vascular channels filled with blood. H&E: x 70.



**Fig13.** Papilloma: IHC: Section showing positive immunostaining in the nuclei of basal, parabasal cells and connective tissue. PCNA: x 70.



**Fig14.** Papilloma: IHC: Section showing positive immunostaining in the nuclei of basal, parabasal cells and connective tissue. PCNA: x 280.

### IMMUNOHISTOCHEMISTRY

Positive expression of PCNA was observed in 90% (9/10) of tumors assessed and the protein expression was restricted to the nucleus. In papillomas, PCNA expression was detected predominantly in the basal and parabasal layer in two cases and in one case positive immunostaining was seen in the nuclei of epidermal cells and connective tissue (Fig. 13 and 14).

### DISCUSSION

In the present study, papillomas were observed in 21.05% of the cases. Sivaseelan et al. (2009) reported the incidence of papillomas in domestic animals as 4.34%. There were nine cases of squamous papilloma and papillomatosis (viral papilloma), two cases of fibropapilloma and one case of angiokeratoticacanthomatous papilloma. Similar rate incidence was observed in both male and female animals in the current study. However, Ozsoy et al. (2011) observed a slightly high incidence in males (54.9%) than in the female (45.1%) cattle. This disease might be due to the spread of BPV via direct contact, contaminated feed and equipment, castration and injections. Inheritance, nutritional and hormonal disorders, sunlight and suppressed immune system might have also played a role in the pathogenesis as reported earlier by Campo et al. (1994), Otter and Leonard (2003) and Frietas et al. (2011).

The papillomas or warts were either generalized or solitary and located in different parts of the body like head, neck, shoulder, limbs, udder and teats, scrotum and inner aspect of thigh. (Blood and Radostits, 1989; Jones et al., 1997; Veena, 2001; Goldschmidt and Hendrick, 2002; Veena and Ravi Kumar, 2002; Hatama, 2011; Tan et al., 2012). The surface was either smooth or

rough and horny. Similar findings were reported by Wadhwa et al. (1995), Veena (2001), Wangikar et al. (2001), Gulbahar et al. (2003), Sood et al. (2007), Debasis Jana and Mousumi Jana (2009), Pawan Kumar et al. (2010), Ozsoy et al. (2011), Vidya Singh et al. (2010), Tan et al. (2012) and Tozato et al. (2013).

Histopathological examination of papillomas revealed that the epidermis was thickened and hyperplastic with elongated, finger like outward projections in squamous papilloma and irregular, elongated, inward rete pegs in papillomatosis with varying degrees of parakeratosis, hyperkeratosis, acanthosis and a core of dermal fibrous connective tissue. A clear hydropic degeneration or a perinuclear halo and pyknotic nucleus was observed in keratinocytes (koilocytes). These lesions were in accordance with the observations of Wangikar et al. (2001), Vidya Singh et al. (2010), Ozsoy et al. (2011), Tan et al. (2012) and Tozato et al. (2013). There was an extensive proliferation of fibroblasts and epidermal keratinocytes in fibropapillomas. Correspondingly, Theilen et al. (1985), Abdousslam et al. (1997), Vikas (2010), Bam et al. (2012), Tan et al. (2012) and Pawan Kumar et al. (2013) observed similar findings in fibropapilloma. Discrete groups of variably sized, dilated, thin walled and well differentiated vascular channels, filled with blood and lined by a single layer of flattened endothelial cells were noticed in angiokeratotic papilloma. Similar changes were observed by Gulbahar et al. (2003), Bharath (2007) and Sood et al. (2007).

In the present study, PCNA expression in papilloma was observed predominantly in the cells of basal and parabasal layers of epidermis and in one case the expression was seen in both epidermal cells and connective tissue. These results were in conformity with the findings of Pawan Kumar et al. (2013).

In conclusion, the present study revealed that incidence of different types of cutaneous papillomas in both cattle and buffaloes. There was no sex predilection was recorded and cases were reported in cross bred and non descriptive cattle. The papillomas were noticed in different parts of the body. Histopathologically, squamous papilloma, viral papilloma (papillomatosis), fibropapilloma and angiokeratoticacanthomatous papilloma were noticed. Immunohistochemically, PCNA expression in papilloma was observed predominantly in the cells of basal and parabasal

layers of epidermis. The PCNA antibodies were very useful marker to detection of cellular proliferation in the bovine cutaneous papillomas.

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