

Oral papillomatosis: histopathomorphological study of gingival papilloma in a goat kid

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Abstract

A case of gingival papilloma in a 22 days old goat kid was studied. Tissue biopsy sample received from Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu for histopathological diagnosis had a description of soft, irregular, friable growth in the upper and lower gingiva. Biopsy tissue was preserved in formalin and processed as per standard histopathological technique. Histological section of the tissue revealed hyperplasia of stratified squamous epithelium which was thrown into finger-like projections supported by meagre fibrous stroma. Basal cells showed intracytoplasmic inclusion bodies with nuclear indentation. Neoplastic cells showed perinuclear halo and emperipolesis. *Stratum spinosum* showed ballooning degeneration with eosinophilic intracytoplasmic inclusion bodies. Histochemistry revealed pinkish-orange intracytoplasmic inclusion in the cells of *stratum spinosum*. Based on the histopathomorphological observations, the case was diagnosed as Oral Papilloma.

Keywords: Emperipolesis, Gingiva, Goat kid, Papilloma, Viral inclusions

Highlights

- Documenting the gingival papilloma in a goat kid
- Oral papilloma was rare in kid
- Vertical transmission was suggested in young ones
- Suckling might pose risk from affected udder of the dam

Papilloma or papillomatosis is a benign neoplasm of stratified squamous epithelium of skin and or mucosa. The exophytic proliferation of epithelial cells giving thickening of epidermis or mucosa forming rete pegs and grows deep into the dermis. The alterations grossly appear as solid papillary growth resembling finger-like projections called papilloma (Frigerio *et al.*, 2021). Multiple papillomas in a localised area of tissue is known as papillomatosis (Roperto *et al.*, 2018). Papillomatosis is a contagious neoplastic condition of animals and human beings (Frigerio *et al.*, 2021) caused by small, non-enveloped, double stranded DNA virus belonging to the family *Papilloma viridae* (Roperto *et al.*, 2018; Cutarelli *et al.*, 2023). Based on the temporal expression of translated proteins (E, early and L, late) of conserved genes, 44 genera of papilloma virus are described in bovines as bovine papilloma virus (BPV) (Dogan *et al.*, 2018; Roperto *et al.*, 2018; Cutarelli *et al.*, 2023). Further, based on the degree of nucleotide sequence diversity of the L1 gene, BPV are grouped into five genera as delta-papillomavirus, epsilon-papillomavirus, xipa-papillomavirus, dyokappa-papillomavirus and dyoxi-papillomavirus (Dogan *et al.*, 2018). Although,

papilloma viruses are species-specific, bovine delta-papillomavirus was reported to cause trans-species transmission in small ruminants (Dogan *et al.*, 2018; Roperto *et al.*, 2018). More recently, two caprine papillomavirus, *Capra hircus* papillomavirus 1 and 2 are identified and grouped under Phipapillomavirus of goats (Cutarelli *et al.*, 2023). Papilloma viruses are known to cause wart-like lesions which resulted from hyperplasia of stratified squamous epithelium of skin and mucosa (Odhah *et al.*, 2022). The occurrence is common in large ruminants and rare in other species. (Ugochukwu *et al.*, 2019; Pyrek *et al.*, 2023). Horizontal transmission is thought to be the predominant infection route, but vertical transmission is also suggested for papilloma viruses causing congenital papillomatosis in young animals (Roperto *et al.*, 2018; Ugochukwu *et al.*, 2019; Odhah *et al.*, 2022).

A male non-descript goat kid aged 22 days having greyish-pink, soft, irregular, friable mass over the left upper and lower gingival growth in the oral cavity was presented at Veterinary Clinical Complex, Veterinary College and Research Institute (VCRI), Orathanadu, Thanjavur. Biopsy sample was collected, preserved in 10% formalin and submitted to the Department of

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Veterinary Pathology, VCRI, Orathanadu, Thanjavur for histopathological diagnosis. Tissue was processed as per standard histopathological techniques with paraffin embedding. Tissue sections of 4-5 µm thickness were prepared and stained with standard hematoxylin and eosin stain (Bancroft and Layton, 2019). Duplicate sections were subjected for histochemistry for Page-Green stain for viral inclusions and Masson's trichrome and Van Gieson technique for collagen (Luna, 1968).

Grossly, the biopsy tissue was greyish pink with rough surface. Microscopically, the tissue under hematoxylin and eosin stain showed mucosal exophytic growth of stratified squamous epithelial layer with rete pegs penetrating deep into the submucosa. Mucosa revealed acanthosis and appeared as irregular folds resembling finger-like projections (Fig. 1). Epithelial projections were lined by basal cells and overlaid by cells of *stratum spinosum*. Cells of *stratum spinosum* showed hyperplasia and prominent nucleus and nucleoli. *Stratum basale* and squamous cells showed perinuclear halo (Fig. 2). Few cells showed eosinophilic intracytoplasmic inclusion bodies (Fig. 3). Hyperplastic cells of *stratum spinosum* showed ballooning degeneration. Few of these cells fused to form bullae and contained indented nucleus with eosinophilic intracytoplasmic inclusion bodies. The epithelial projections were supported by fibrous connective tissue stroma. Connective tissue stroma contained fibroblast, blood vessel and neutrophils. Fibroblast were pleomorphic with anisokaryosis (Fig. 4). Stroma showed vascular dilatation, congestion and areas of haemorrhages. Few of the neoplastic squamous cells showed cell-in-cell structures (emperipolesis). These cell inclusions are of mononuclear cell (Fig. 5). Tip of the projections displayed acute inflammation characterized by epithelial necrosis, bacterial colonies and infiltration by neutrophils. Surface epithelium on the lateral side of papilla revealed hyperkeratosis, parakeratosis and necrosis with oat cell formation. Tissue stained with Page-Green revealed pinkish-orange intracytoplasmic inclusions of variable sizes in *stratum spinosum* suggestive of viral inclusion (Fig. 6). Histochemistry with Masson's trichrome and van Gieson's technique showed meagre collagen fibres suggestive of immature fibrous stroma.

Papilloma viruses are the epitheliotropic DNA virus which causes hyperplastic proliferation of stratified squamous epithelium of skin and mucosa of oral cavity in animals (Ugochukwu *et al.*, 2019). Papilloma was found to be common in bovines and rare in other

ruminants. Papillomatosis was found to be rare in goats (Odhah *et al.*, 2022). Papilloma virus was reported to cause severe wart-like growth (Rojas-Anaya *et al.*, 2016) most commonly in skin, teat, mammary gland (cutaneous form) and rarely in the oral mucosa (non-cutaneous form) in animals (Pyrek *et al.*, 2023). Cutaneous papilloma in goat was reported over udder (Manni *et al.*, 1998), teat (Dogan *et al.*, 2018) and skin (Pavithra *et al.*, 2020). Occurrence of oral mucosal papilloma (non-cutaneous) from gingiva of left upper and lower gingiva in the present case was parallel with the earlier reports on papilloma recorded in lower lips and hard palate (Odhah *et al.*, 2022) in goats; gingiva of rostral mandibular gingiva (Roperto *et al.*, 2018) and inside of lower lip (Al-Sabaawy and Al-Sadi, 2021) in sheep; gingiva of lower premolar and molar region (Zurcher-Giovannini *et al.*, 2020) in roe deer. The histopathomorphology of present gingival papilloma was analogous with the previous reports in bovine, goat, sheep, pig and deer (Desrochers *et al.*, 1994; Vitovec *et al.*, 1999; Roperto *et al.*, 2018; Zurcher-Giovannini *et al.*, 2020; Odhah *et al.*, 2022). Multiple papillary projections of hyperplastic squamous epithelium with little fibrous tissue stroma, perinuclear halo neoplastic cells, ballooning degenerations and presence of eosinophilic intracytoplasmic inclusion in hyperplastic squamous cells were found to be characteristic of the disease (Odhah *et al.*, 2022) and was similar in the present histomorphological observation. Emperipolesis (cell-in-cell structures) observed in this study clarifies that there was interaction between cancerous cell and immune cell of the host. When cytotoxic T lymphocyte/NK cell internalize, the cancerous cell rapidly forms a vacuole around the immune cell to engulf before it destroys the target cell (Borensztejn *et al.*, 2021). This was obvious in the present observation with clear vacuolar rim around MNC within the cancerous cell. The evidence also proves that cell-mediated immunity plays a crucial role in behavior of the neoplasm. Fibrous tissue stroma composed primarily of immature fibroblast and meagre collagen observed in the present study was in contrast with previous observation by Al-Sabaawy and Al-Sadi, (2021), who reported granulation with collagenous stroma in oral papilloma of sheep. Possibly, the rate of growth and anatomical location of the neoplastic lesions might account for stromal composition. These lesions were mostly benign and very rarely transform into malignant lesions as squamous cell carcinoma (Simeone *et al.*, 2008).

Etiological evidence demonstrated by

Gingival papilloma in a goat kid

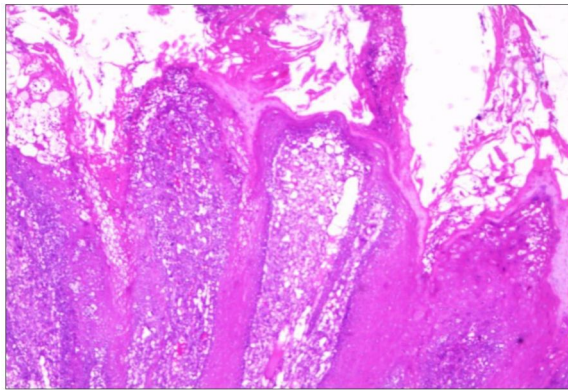


Fig.1. Squamous epithelial hyperplasia resembling finger-like projections - Gingival papilloma in a goat kid, H&E x 40

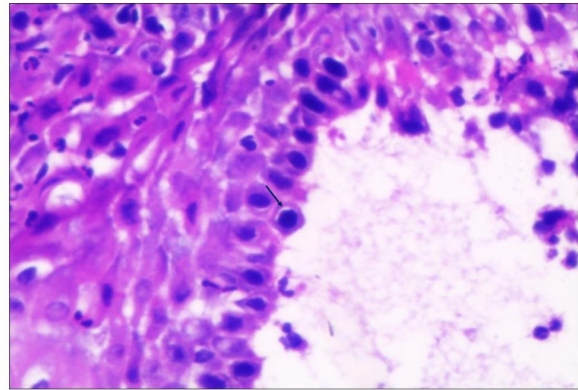


Fig. 2. Basal cell exhibiting perinuclear halo (arrow) - Gingival papilloma in a goat kid, H&E x 400

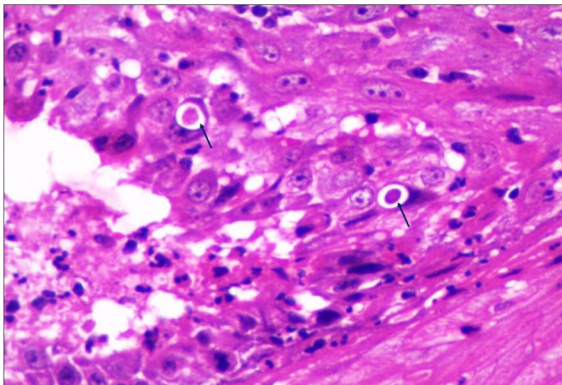


Fig. 3. Basal cells showing eosinophilic intracytoplasmic inclusion body (arrow) - Gingival papilloma in a goat kid, H&E x 400

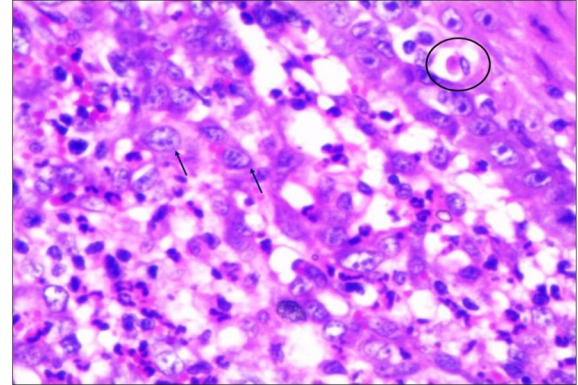


Fig. 4. Stroma with pleomorphic fibroblast (arrow), neutrophils and basal epithelial cell with eosinophilic intracytoplasmic inclusion body (circle) - Gingival papilloma in a goat kid, H&E x 400

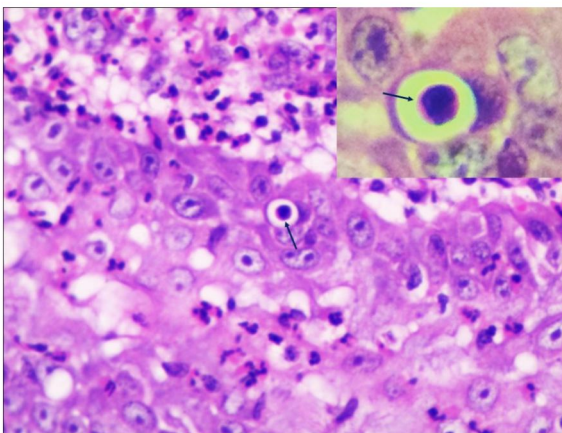


Fig. 5. Cell-in-cell Emperipolesis of mononuclear cell (arrow) within neoplastic squamous cell (inset) - Gingival papilloma in a goat kid, H&E x 400

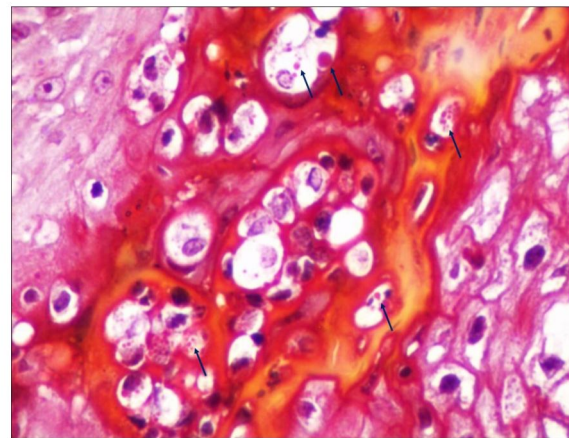


Fig. 6. Orange-red intracytoplasmic inclusion bodies (arrow) in ballooned squamous epithelial cells, Page-Green stain x 400

histochemistry by Page-Green stain for viral inclusions in the present study support the present benign growth was due to viral infection. Further we acknowledge the limitation of molecular confirmation of the virus.

Occurrence of papilloma although reported most commonly in adults, exceptionally young ones were also found to be affected. It was reported as both cutaneous and non-cutaneous form in calf, piglet, lamb and goat kid (Desrochers *et al.*, 1994; Vitovec *et al.*, 1999; Roperto *et al.*, 2018; Odhah *et al.*, 2022). Direct contact with abraded skin and less frequently by contaminated utensils, ear tagging nipper, livestock handlers and flies were found to be the cause for horizontal route of viral transmission in papilloma (Ugochukwu *et al.*, 2019). Recently, it was hypothesized that vertical transmission plays a major role in viral transmission *in utero* for papilloma in young ones (Dias *et al.*, 1955; Vitovec *et al.*, 1999; Roperto *et al.*, 2018).

The exact pathogenesis behind the vertical transmission of papillomavirus is still unclear. We proposed that occurrence of papilloma over udder or teat of lactating dam might pose risk for suckling young ones. Mucosal abrasions in oral cavity of suckling young ones might pave way for infection from affected dam. Hence, with the possible earlier documented evidences on papillomatosis in young animals, the present case was diagnosed as gingival papilloma in the presented goat kid which might have been infected either congenitally *in-utero* or direct contact from

udder/teat of affected dam.

Conflict of interest: No conflict of interest to disclose.

Author's contributions: NBP: Collected and processed the biopsy sample, interpreted the histopathological findings and prepared the manuscript; JS: Assisted in interpretation of histopathological lesions and done proof reading of the manuscript.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

Ethical statement: No ethical committee approval was required as the manuscript was prepared based on the clinical biopsy tissue collected from clinically ill animal under local anesthesia. No live animals were used for experimental purpose in the study.

ACKNOWLEDGEMENT

We extend our thanks to the Dean, Veterinary College and Research Institute, Orathanadu and Tamil Nadu Veterinary and Animal Sciences University, Chennai for the facilities provided. We extend our gratitude to the Staffs of Department of Veterinary Surgery and Radiology, Veterinary College and Research Institute, Orathanadu, Thanjavur for sample submission for the study.

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