

Macroscopic, Histological, and Ultrastructural Characterization of Cutaneous Papillomatosis in Buffaloes (*Bubalus bubalis*)

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Received:- 01 September 2026/ Revised:- 14 September 2026/ Accepted:- 28 September 2026/ Published: 05-10-2026

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Abstract— Macroscopic and microscopic observations were performed on papillomas located in the periauricular region of buffaloes (*Bubalus bubalis*), with comparison to the same anatomical region in buffaloes with normal skin. The macroscopic features of the papillomas in situ, as well as the microscopic features observed by light and electron microscopy, were described. The changes identified in the epidermal and dermal layers were predominantly proliferative, involved both regions, and supported a diagnosis of fibropapilloma. Degenerative changes were also observed in the epidermis, together with small virus-like particles located from the spinous layer toward the more superficial layers. Transmission electron microscopy also revealed areas of perinuclear degeneration, tonofibrils arranged around the nucleus and involved in the formation of intercellular bridges, melanin granules, and occasional cytoplasmic vacuoles. In these layers, in particular, osmiophilic virus-like particles were observed within the nucleus and in the intracytoplasmic spaces.

Keywords— buffalo, skin, papilloma, cutaneous papillomatosis.

I. INTRODUCTION

The buffalo *Bubalus bubalis* (LINNAEUS, 1758) was introduced into Brazil in 1890 from animals imported from the island of Trinidad to Marajó Island. Originating from the Asian continent, buffaloes were subsequently introduced into Europe, Oceania, and the Americas and are currently adapted to a wide variety of ecosystems worldwide (MARQUES & CARDOSO, 1997).

Buffaloes have productive aptitudes similar to those observed in cattle of European and Indian origin and can be used for milk and meat production and as draft animals. Although they are naturally more rustic, they have a high capacity for domestication, a process dating back to prehistoric times, particularly in regions of Indochina (MARQUES & CARDOSO, 1997).

Among the organs directly involved in the interaction between the organism and the environment, the skin stands out as one of the largest organs of the body and is responsible for covering the body surface. In addition to acting as a physical protective barrier against external agents, it performs several essential functions for maintaining organismal homeostasis. The skin consists of the epidermis, which is of ectodermal origin, and the dermis, which is of mesodermal origin. The epidermis is avascular, and its cells undergo progressive differentiation and keratinization, forming an important protective barrier against physical, chemical, and biological agents (JUNQUEIRA & CARNEIRO, 2013).

Warts or papillomas have long been recognized and described as disorders of the skin and mucous membranes of humans and animals. These growths are regarded as benign neoplastic lesions characterized primarily by the proliferative nature of the epithelium (FUJIMOTO & OLSON, 1966). The term papilloma came into use in the late nineteenth century to designate tumors formed by dermal papillae covered by epithelium (HELIAD, 1934). Over time, different forms of papillomatosis have been

observed in several animal species, including horses and cattle, with variations in location and morphology related to the host and the type of virus involved (OLSON, 1963; LANCASTER & OLSON, 1982).

The etiology of papillomas was debated for many years. The viral nature of the disease began to be demonstrated in the early twentieth century, when CIUFFO (1907) described the existence of a filterable agent associated with human papillomas. Subsequently, MAGALHÃES (1920), working with bovine warts, demonstrated their viral origin by intradermal inoculation or skin scarification using cell-free filtrates obtained from papilloma suspensions. CREECH (1929) confirmed the infectivity of these filtrates, demonstrating that suspensions of bovine cutaneous papillomas were capable of reproducing the disease in healthy animals and that the resulting lesions could infect new animals.

The viral nature of papillomas was subsequently reinforced by experimental studies investigating their transmission, composition, and pathological changes in different settings (KOLLER & OLSON, 1972). In 1949, electron microscopy enabled visualization of virus-like particles in papillomas, associated with intranuclear inclusions arranged in crystalline patterns (STRAUSS et al., 1949). Other ultrastructural studies contributed to characterization of the viral particles and their relationship with epithelial cells (ALMEIDA et al., 1962; CHAPMAN et al., 1963). The papillomavirus genome was characterized as a circular double-stranded DNA molecule (FUJIMOTO & OLSON, 1966).

Papillomaviruses are small, non-enveloped viruses with an icosahedrally symmetrical capsid approximately 55–60 nm in diameter. The capsid consists of 72 capsomers corresponding to pentamers of the L1 structural protein and also contains the minor capsid protein L2. Within the capsid is a circular double-stranded DNA molecule associated with cellular histones. Virions have a sedimentation coefficient ($S_{20,w}$) of approximately 300S and a buoyant density of approximately 1.34–1.35 g/cm³ in cesium chloride (ICTV, 2018).

Under negative-staining electron microscopy, papillomavirus particles can be observed in forms referred to as 'full' and 'empty,' with a rounded morphology, no envelope, and icosahedral symmetry. In bovine papillomas, CATROXO et al. (2013) demonstrated isolated or clustered particles approximately 60 nm in diameter, in which individual capsomers could be distinguished. In ultrathin sections, the same authors observed groups of rounded viral particles within the nuclei, measuring approximately 35 nm, as well as tubular structures approximately 35–39 nm in diameter. These tubular forms have been associated with alterations in the formation and maturation of viral particles (CATROXO et al., 2013).

Papillomavirus replication is closely related to epithelial cell differentiation. In proliferating cells, the viral genome is initially maintained at a low copy number; as infected cells differentiate and migrate toward the more superficial epithelial layers, viral DNA amplification and expression of the L1 and L2 capsid proteins occur, culminating in the assembly and formation of new viral particles. Thus, the productive phase of infection occurs predominantly in differentiated epithelial cells of the more superficial layers (ICTV, 2018).

In cattle, cutaneous papillomatosis is economically important because it can reduce weight gain and, in particular, damage the hides of affected animals (ROSEMBERG, 1940). The disease occurs more frequently in young animals, particularly those less than two years old, although it can also be observed in newborns (SHOPE, 1933; NJOKU & BURWASH, 1972). The interval between contact with affected animals and the appearance of papillomas may range from approximately 3.5 to 4 months. In experimental papillomatosis, this period may be reduced to three weeks to two months. Once established, the lesions may persist for one to six months before regressing (OLSON et al., 1960).

Natural papillomavirus infection is primarily associated with the skin and mucous membranes, depending on the tropism of the agent. Transmission occurs mainly through direct contact between healthy and infected animals and is facilitated by small skin lesions and abrasions. Fences, drinking troughs, feed troughs, and equipment used for restraint and handling can serve as means of dissemination. Arthropod vectors, such as ticks and other hematophagous arthropods, may also act as mechanical transmitters of the virus (SOMVANSHI et al., 1986). The appearance of numerous papillomas in areas subjected to trauma, such as tattoo sites used for cattle identification, particularly on the ears, reinforces the importance of disruptions in skin integrity in transmission of the agent (STUDDERT et al., 1988).

According to the classification presented in classic studies, bovine papillomaviruses initially comprised six distinct types associated with different lesion patterns and locations (BARTHOLD et al., 1974; CAPPELLARO et al., 1978; LANCASTER & OLSON, 1982; JARRETT et al., 1984). Among the classically described types frequently associated with lesions in cattle,

BPV-1, BPV-2, BPV-5, and BPV-6 are particularly notable. BPV-1, BPV-2, and BPV-5 are associated with fibropapilloma formation, whereas BPV-6 is mainly associated with epithelial papillomas. BPV-2 is also particularly important because of its association with proliferative and neoplastic changes in the urinary bladder, especially in cases of bovine enzootic hematuria (WOSIACKI et al., 2006; MEDEIROS-FONSECA et al., 2021).

With the development of molecular techniques and genetic sequencing, the diversity of bovine papillomaviruses was found to be considerably greater than that established by the initial classifications. FIGUEIRÊDO et al. (2020) reported 28 known BPV types at that time and demonstrated high genotypic diversity in cattle from Northeastern Brazil, including the identification of possible novel viral types. More recently, LIN et al. (2026) reported 44 bovine papillomavirus types characterized and classified into different genera, demonstrating the continuing expansion of knowledge regarding the genetic diversity of these viruses. Among the *Deltapapillomavirus*, BPV-1 and BPV-2 remain particularly important because of their association with fibropapillomas and their broader host range (FIGUEIRÊDO et al., 2020; LIN et al., 2026).

Lesion progression is also related to the host immune response. Fibromas may regress, whereas fibropapillomas remain under development for longer periods, and persistence of these lesions is associated with absent or less intense lymphocytic infiltration. The presence of precipitating antibodies does not ensure complete protection against reinfection, although animals whose papillomas are regressing show greater resistance (LEE & OLSON, 1969). These findings indicate that the immune response plays an important role in lesion regression and persistence.

Papillomaviruses, or particles morphologically compatible with these agents, have been described in different animal species. In veterinary medicine, in addition to cattle, cases have been reported in horses, pigs, goats, cervids, dogs, cats, and other species. Their occurrence in different hosts and locations demonstrates the biological diversity of these viruses and their relationship with tissue tropism. In some species, lesions are predominantly benign and regressive, whereas in others they may persist or be associated with neoplastic transformation (LANCASTER & OLSON, 1982).

Studies of papillomatosis in buffaloes (*Bubalus bubalis*) are less numerous than those in cattle. SILVESTRE et al. (2009) demonstrated the presence of BPV-1 in cutaneous, perivulvar, and vulvar fibropapillomas in buffaloes using histopathology, transmission electron microscopy, molecular techniques, and demonstration of E5 oncoprotein expression. That study confirmed that bovine papillomaviruses can also cause infection and proliferative lesions in buffaloes.

In addition to cutaneous lesions, BPV-2 has been identified in the urinary bladder of buffaloes and associated with urothelial carcinogenesis (ROPERTO et al., 2013). Subsequently, RUSSO et al. (2020) demonstrated the presence of BPV-2 in papillomatous lesions of the amniotic membrane of buffaloes, showing that infection in this species is not restricted to the skin and may involve different tissues (ROPERTO et al., 2013; RUSSO et al., 2020).

Despite these advances, studies aimed at the detailed characterization of ultrastructural changes in cutaneous papillomas in buffaloes remain scarce, particularly studies that relate cellular changes to the different epidermal and dermal layers and enable comparison with normal skin. Thus, ultrastructural examination of these lesions may contribute to a better characterization of the cellular changes associated with papillomatosis and of the relationship between viral particles and the different stages of epidermal cell differentiation.

II. OBJECTIVE

Accordingly, the present study aimed to characterize the macroscopic, microscopic, and ultrastructural features of cutaneous papillomas located in the periauricular region of buffaloes (*Bubalus bubalis*), comparing the observed changes with those found in the same anatomical region of animals with normal skin.

III. MATERIAL AND METHODS

3.1 Material

Skin from Murrah buffaloes, from the periauricular region, was collected by excision for laboratory examinations. Some samples were kept refrigerated and others in 10% formalin buffered with calcium carbonate or in 2.5% glutaraldehyde, also under refrigeration. Those fixed in formalin were directed to light microscopy study and embedded in paraffin, and those in glutaraldehyde were embedded in Spurr resin for transmission electron microscopy studies.

3.2 Methods

3.2.1 Routine Histological Technique: hematoxylin and eosin (H&E)

Organ fragments, fixed in buffered 10% formalin, were dehydrated, cleared, embedded in paraffin, and sectioned in a microtome at a thickness of 5 microns. After deparaffinization with xylene, they were stained with hematoxylin and eosin, and the slides were mounted with coverslips and Entellan and viewed under a conventional light microscope using direct light.

3.2.2 Electron Microscopy Technique: Resin embedding technique followed by positive contrasting of ultrathin sections

The skin fragment samples were processed according to the usual resin-embedding procedures, based on the methods of Gonzalez-Santander (1969). When this technique is used, freshly collected samples are fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.0, post-fixed in 2% osmium tetroxide in 0.2 M phosphate buffer, pH 7.0, stained with 0.5% uranyl acetate, dehydrated in an increasing ketone series (50 to 100%), and embedded in Spurr resin. After ultrasectioning of the blocks, the ultrathin sections obtained were positively stained by sequential treatment with uranyl acetate (WATSON, 1958) and lead citrate (REYNOLDS, 1963), before being observed under a Philips EM 208 transmission electron microscope.

IV. RESULTS

Also known as warts, these are papillary structures whose shape is determined by epidermal hyperplasia over the dermal papillae, in a process in which connective tissue may also proliferate as long extensions into the papilla; these lesions are considered fibropapillomas.

4.1 Macroscopic features

The papillomas examined were in the periauricular region of five Murrah females aged 18–20 months.

Macroscopically, they appeared as elevated, circumscribed, irregular, roughly spherical masses ranging from 0.5 to 2 cm in diameter. The surface was grayish, rough, cornified, and hairless, with elevations and depressions that gave the lesions the classic 'cauliflower' appearance (Figure 1).

4.2 Microscopic features of the epidermis

Under light microscopy, some areas showed increased hematoxylin staining intensity, suggesting a greater number of nuclei (Figure 2) and, consequently, greater cell proliferation in this region, resulting in marked irregularity of the basement membrane at this site (Figure 5). In other areas, this membrane resembled that of normal skin and consisted of a single layer of basal cells resting on a regular basement membrane.

4.2.1 Spinous layer

A very marked increase in the number of cell layers was observed in this region, with some areas containing as many as 20–30 cell layers. Marked acanthosis was therefore evident, characterized by thickening of the stratum spinosum and elongation of the epidermal ridges (Figure 2).

Among the various cellular changes, the most characteristic was ballooning degeneration, observed both in isolated cells, referred to as ballooned cells (Figure 3), and in clusters of these cells (Figure 4).

The ballooned cells appeared clear, with loss of cytoplasmic staining to varying degrees; this was less pronounced in the deeper epidermal regions and more evident in the superficial layers. These cells were enlarged and vacuolated. The vacuoles were smaller in cells located closer to the basal layer and became larger as the cells approached the granular layer (Figure 4). Neither tonofibrils nor intercellular bridges were observed in clusters of ballooned cells. The nucleus was enlarged, and the cytoplasmic vacuoles frequently displaced it (Figure 3); on other occasions, perinuclear degeneration was observed (Figure 4).

In the other cells of this region, which were smaller than the ballooned cells, the intercellular bridges were more prominent and could be visualized mainly in toluidine blue-stained sections (Figure 5). The intercellular space was visibly dilated (Figure 6).

Under electron microscopy, these cells showed areas of perinuclear degeneration (Figure 6), tonofibrils arranged around the nucleus and contributing to the formation of intercellular bridges (Figures 7 and 8), melanin granules within the cells (Figure 7), and occasional cytoplasmic vacuoles displacing the nucleus (Figure 8).

In this layer, virus-like particles could be observed in the nucleus (Figure 9) and intercellular spaces (Figure 10).

Within the nucleus, osmiophilic particles, whose boundaries were not always well defined, could be closely associated with the chromatin or not. Their mean size was 35 nm, and no clusters with a crystalline organization were found in the material examined (Figure 9). In the intercellular spaces, clusters of osmiophilic virus-like particles also lacked a crystalline organization and measured an average of 40 nm.

4.2.2 Granular layer

In the papillomas examined, light microscopy revealed an increased number of granular cell layers, generally appearing as three to five rows of cells (Figures 2 and 3). However, some areas contained a much greater number of these cells (Figure 2), whereas in other areas adjacent to clusters of ballooned cells they were not visible (Figure 4).

Under light microscopy, this layer was characterized by basophilic granules and, as in the spinous layer, contained ballooned cells readily identifiable by their clear cytoplasm (Figure 3), sometimes with a pyknotic nucleus (Figure 4) and perinuclear degeneration (Figure 3).

Under electron microscopy, the nucleus often showed marginated chromatin in the form of clumps adjacent to the nuclear membrane (Figure 11).

Some cells that were not yet markedly degenerated showed tonofibrils evenly distributed throughout the cytoplasm (Figure 11); others showed more pronounced cytoplasmic degeneration in the perinuclear region (Figure 11).

The plasma membrane in this area was thick and finely serrated (Figure 12).

Within the cytoplasm, well-defined virus-like particles occurred in clusters without forming crystals; the particles measured an average of 40 nm (Figure 12).

4.2.3 Lucid layer

This layer was observed in some regions, where it consisted of clear, eosinophilic, flattened cells with indistinct or inapparent nuclei (Figures 3 and 4).

4.2.4 Keratinized layer

The number of cells in this layer was also markedly increased relative to normal skin. Although these structures were clearly difficult to count, their number was estimated to reach 20–30 layers in some regions (Figure 2).

When present, the nucleus was sometimes basophilic, but generally it could not be observed.

Under electron microscopy, the cells could be identified by the presence of a cell membrane that individualized them, and degenerating desmosomes could be demonstrated; however, the nuclear membrane could never be visualized (Figure 13).

4.3 Microscopic features of the basement membrane

It appeared as a thin sheet following the basal portion of the basal layer. It was therefore more regular at some sites, but at most sites the boundary was irregular and more difficult to visualize; nevertheless, it was clearly visible in toluidine blue-stained semithin sections (Figure 5).

4.4 Microscopic features of the dermis

The papillomas examined also showed marked proliferation of this layer, visualized as 'glove-finger' projections accompanied at their upper portion by proliferation of the epidermal layer (Figure 2). Together, these changes gave the papilloma the classic 'cauliflower' shape.



FIGURE 1: Photograph of the papilloma with a cauliflower-like appearance located at the base of the buffalo ear.

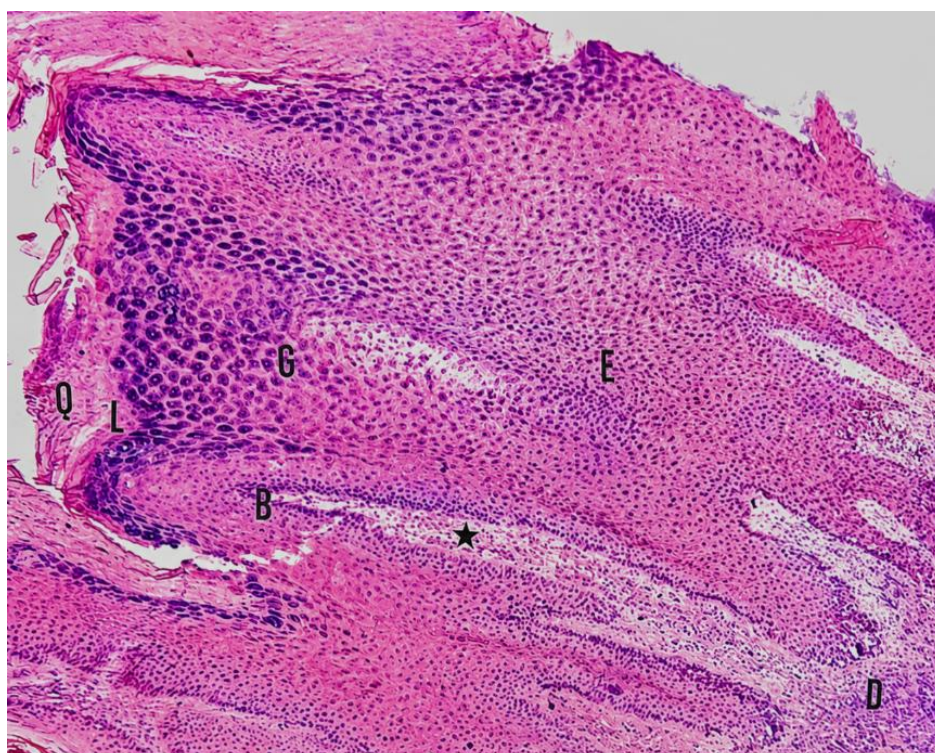


FIGURE 2: Photomicrograph of a histological section (paraffin) of a papilloma showing the overall appearance, with hyperplasia of the dermis (D) and epidermis. The dermis forms glove-finger projections (★), and the epidermis shows marked proliferation of all layers: the basal layer (B), with a greater concentration of nuclei; the spinous layer (E), with acanthosis; the granular (G), lucid (L), and keratinized (Q) layers. Stain: hematoxylin and eosin. Magnification: 80×.

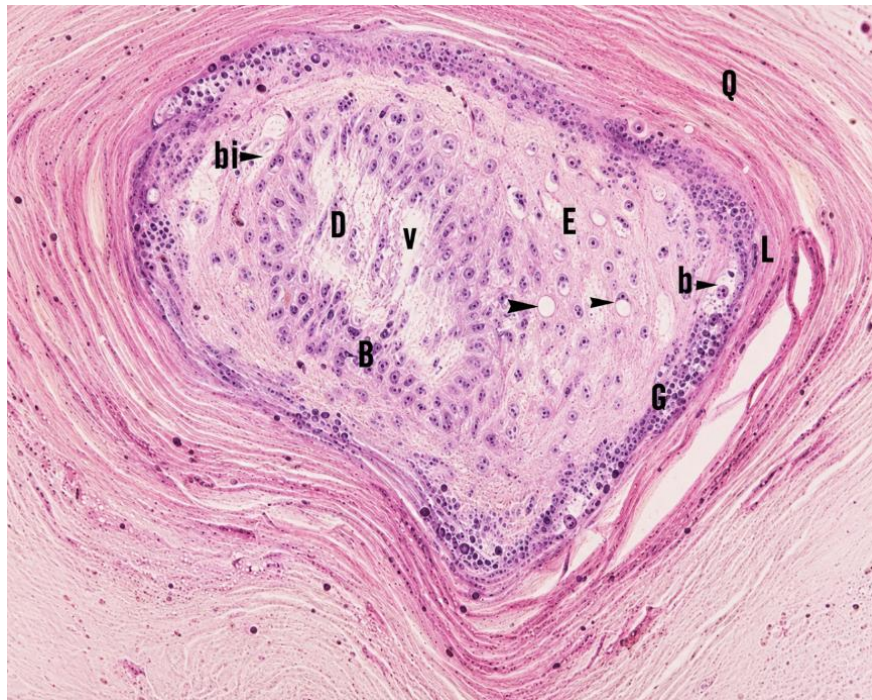


FIGURE 3: Photomicrograph of a transverse histological section (paraffin) of a papilloma, showing the dermis (D), with vessels (v), at the center. From the center toward the periphery, the following proliferated layers are observed: basal (B); spinous (E), with cells showing cytoplasmic vacuolation displacing the nucleus (►), vacuolated nuclei (►), and isolated ballooned cells (bi); granular (G), with basophilic granules and ballooned cells with pyknotic nuclei in an area of perinuclear degeneration (b); lucid (L); and keratinized (Q). Stain: hematoxylin and eosin. Magnification: 160×.

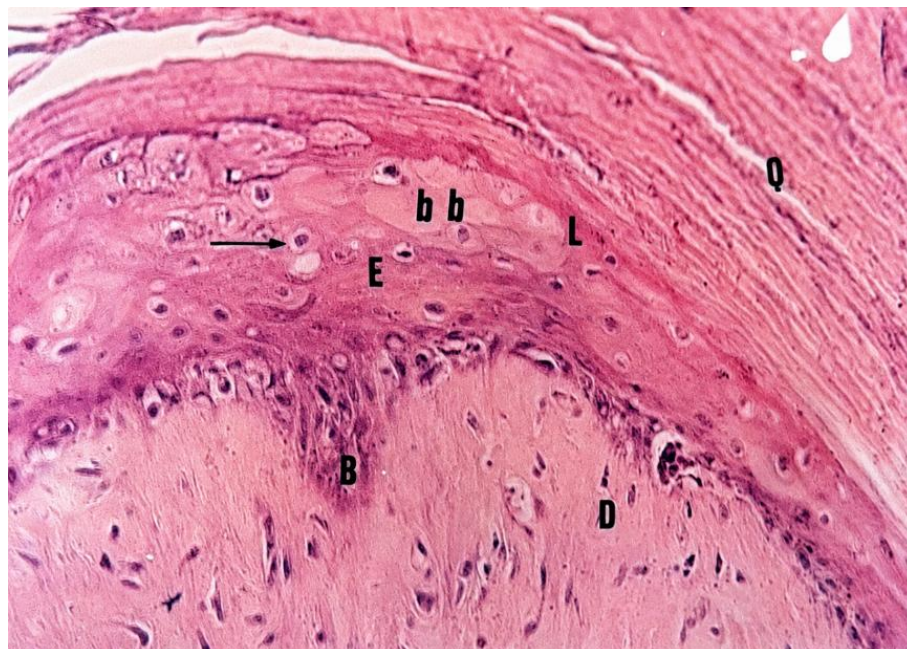


FIGURE 4: Photomicrograph of a longitudinal histological section (paraffin) of a papilloma, showing the dermis (D) and epidermis with hyperplasia of the basal (B) and spinous (E) layers, in which clusters of ballooned cells (bb) and areas of perinuclear degeneration (→) are observed; absence of the granular layer; and presence of the lucid (L) and keratinized (Q) layers. Stain: hematoxylin and eosin. Magnification: 300×.

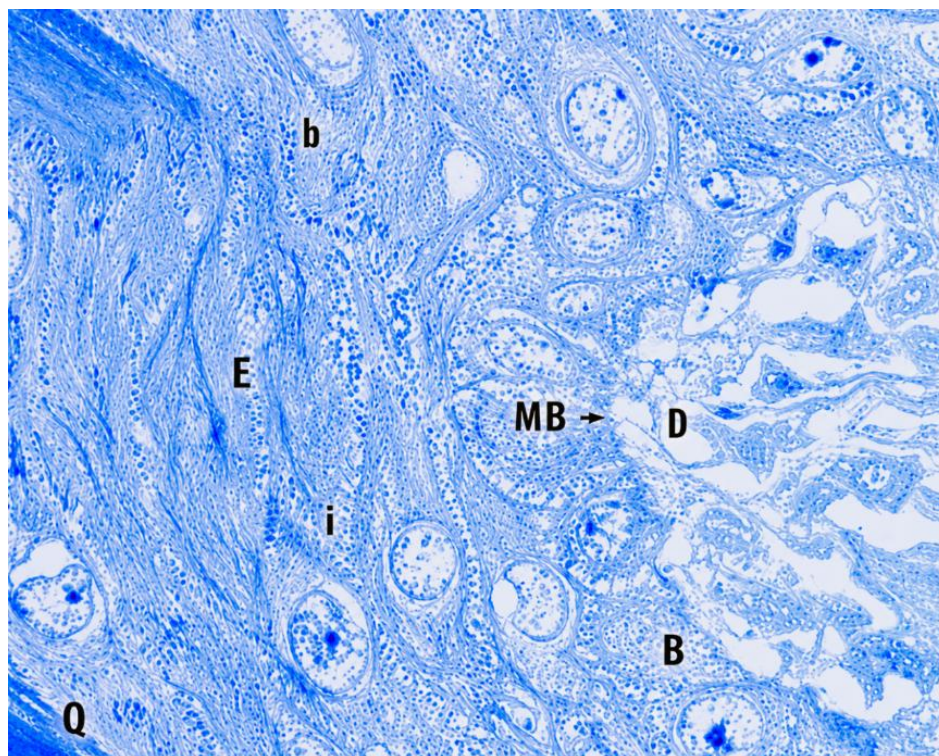


FIGURE 5: Photomicrograph of a resin-embedded histological section of a papilloma at the dermis (D)-epidermis transition zone, with an irregular basement membrane (MB). The epidermis shows prismatic cells of the basal layer (B), the spinous layer (E) with intercellular bridges (i), a ballooned cell (b), and the keratinized layer (Q). Stain: Toluidine blue. Magnification: 2,000×.

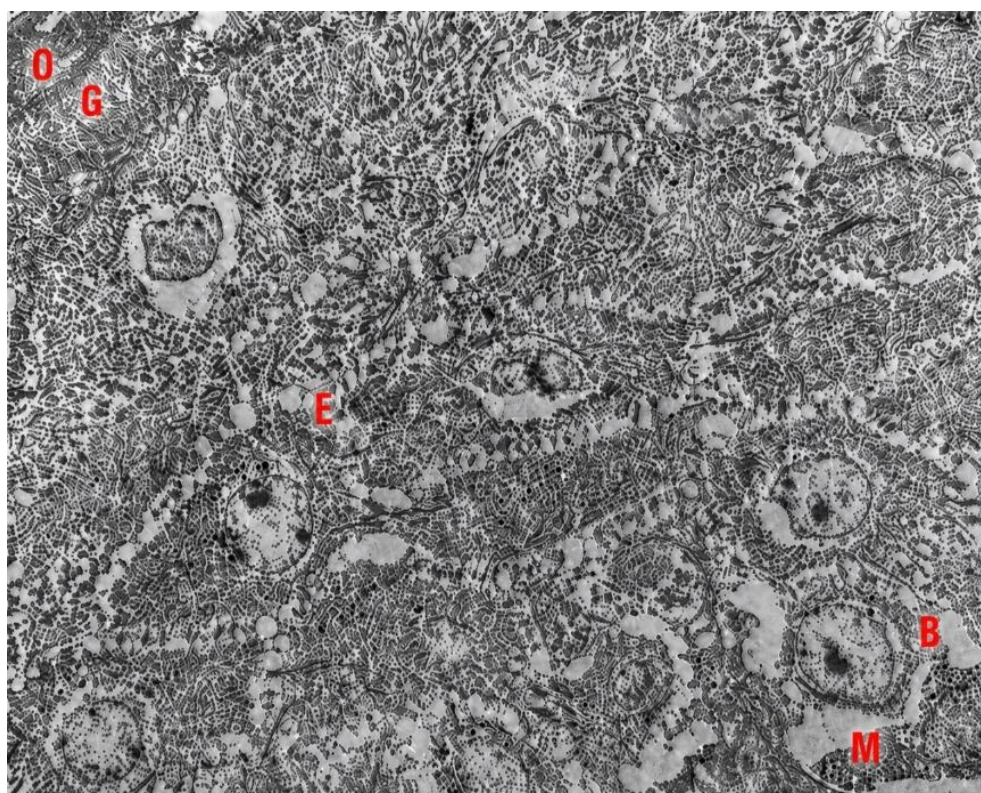


FIGURE 6: Electron micrograph of a portion of the dermis and epidermis of a papilloma. A melanocyte is observed in the transition zone (M), as well as the basal (B), spinous (E), with flattened polygonal cells, granular (G), and keratinized (Q) layers. Magnification: 2,300×.

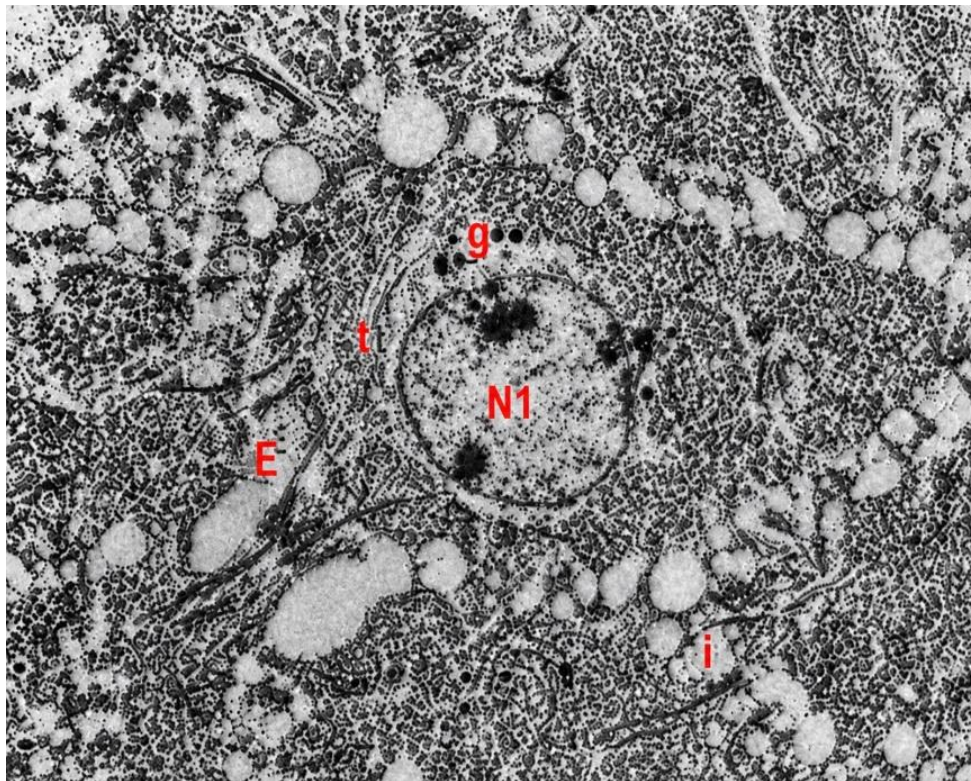


FIGURE 7: Electron micrograph of the spinous layer (E), showing cells with tonofibrils (t) around the nucleus (N) and contributing to the formation of intercellular bridges (i), melanin granules (g), and a nucleus with rarefaction (N1). Magnification: 5,700×.

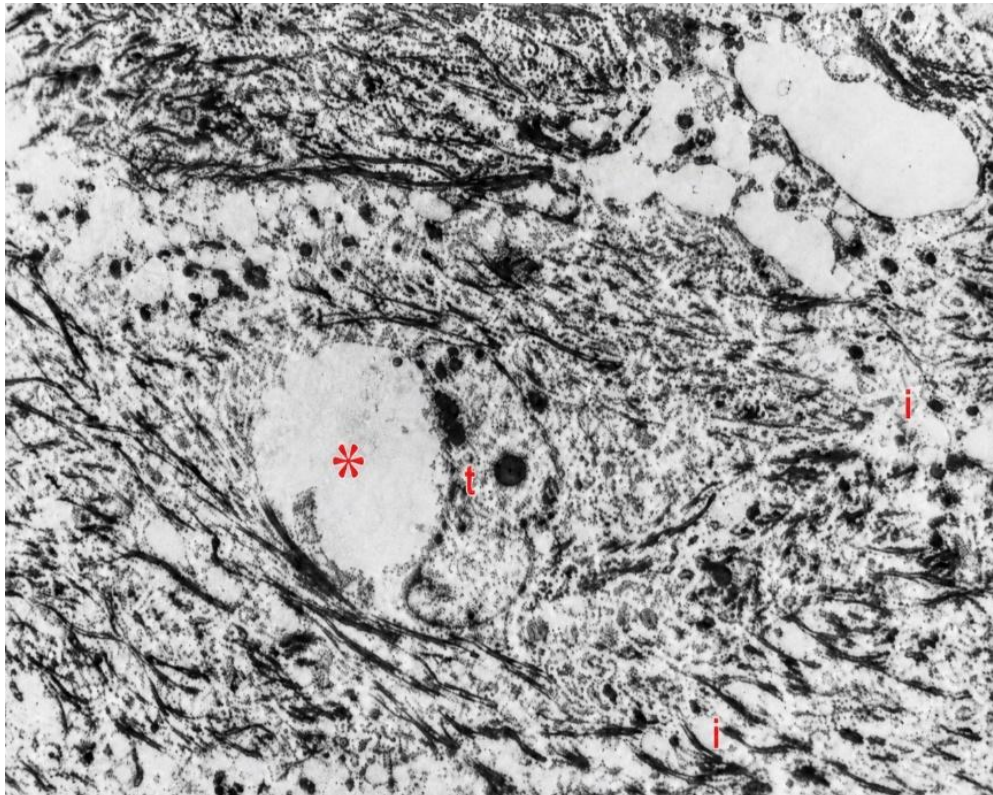


FIGURE 8: Electron micrograph of a papilloma showing a cell of the granular layer, with a vacuole (*) displacing the nucleus, preserved tonofibrils (t), and visible intercellular bridges (i). Magnification: 4,800×.

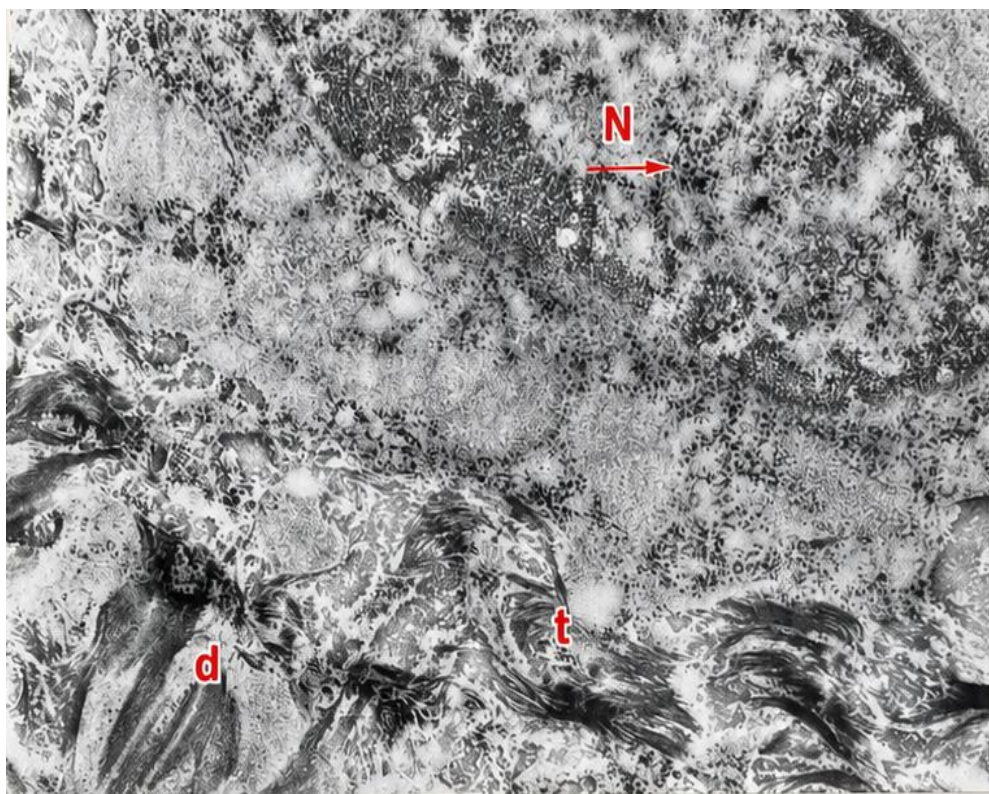


FIGURE 9: Electron micrograph of cells of the granular layer of a papilloma, showing desmosomes (d), tonofibrils (t) around the nucleus (N), and osmiophilic virus-like particles (→). Magnification: 26,500×.

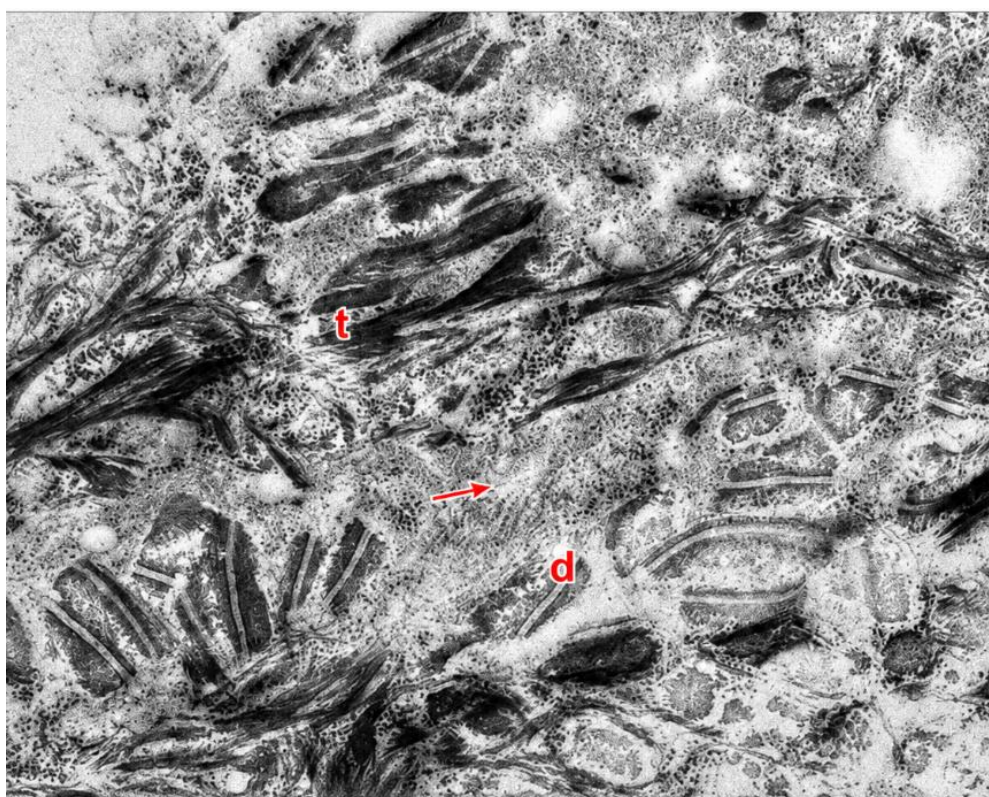


FIGURE 10: Electron micrograph of the junction between cells of the spinous layer of a papilloma, showing desmosomes (d), tonofibrils (t), and clusters of osmiophilic virus-like particles in the intercellular spaces (→). Magnification: 24,500×.

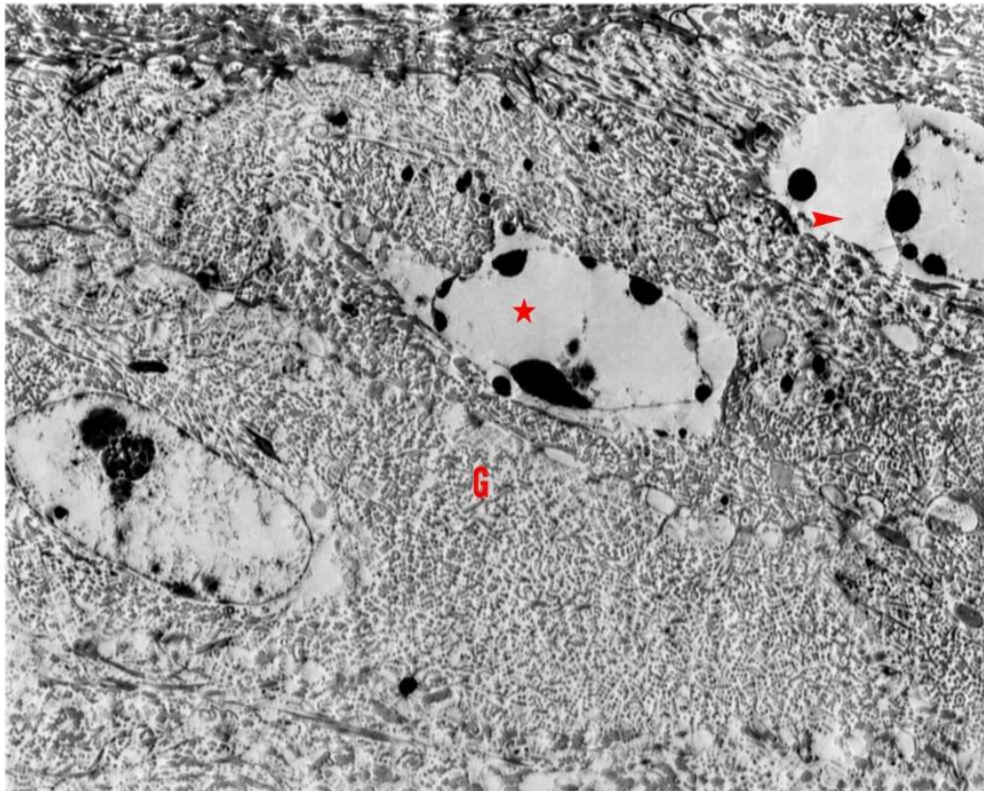


FIGURE 11: Electron micrograph of the granular layer (G), showing cells with peri- (►) and intranuclear degeneration, with margined chromatin forming clumps (★). Magnification: 6,350×.

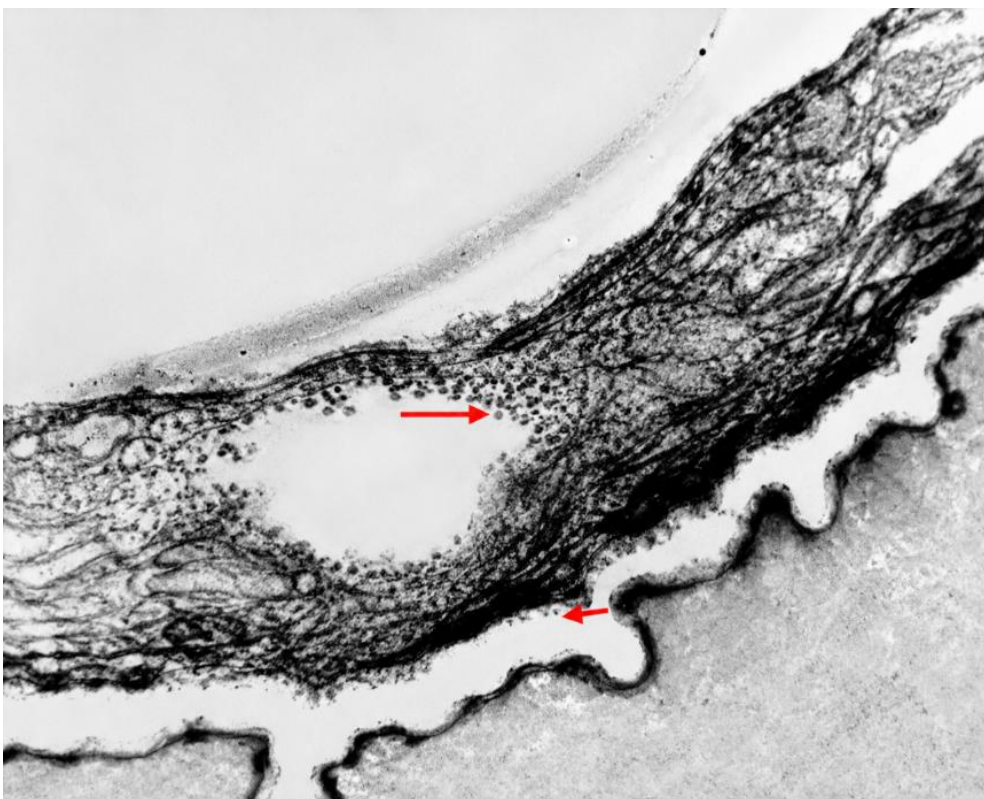


FIGURE 12: Electron micrograph of a portion of a cell from the granular layer of a papilloma, showing intracytoplasmic virus-like particles (→) and thickening of the finely serrated plasma membrane (►). Magnification: 32,000×.

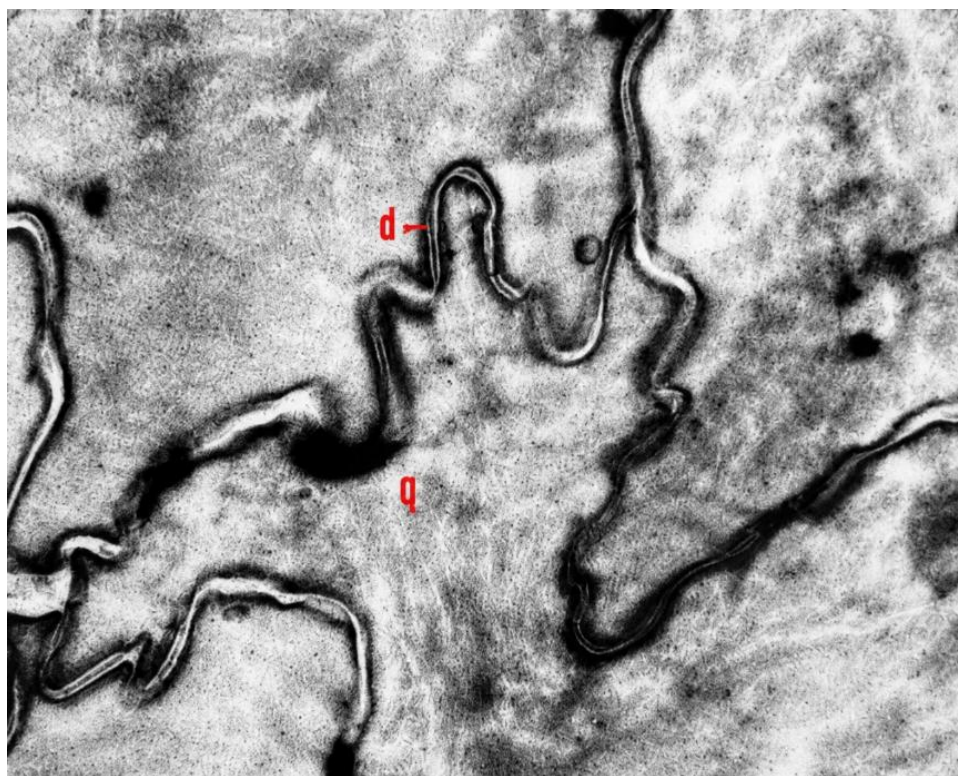


FIGURE 13: Electron micrograph of the keratinized layer (Q), showing individualization of the keratinized cells and degenerating desmosomes (d). Magnification: 32,400×.

V. DISCUSSION

Observations of normal buffalo skin were performed on fragments obtained from the periauricular region, corresponding to the same anatomical region in which the papillomas were in affected animals. This precaution was considered important to enable an appropriate comparison between normal and altered skin because the organization and thickness of the different skin layers may vary according to the body region examined (JUNQUEIRA & CARNEIRO, 2013).

Normal skin from the periauricular region showed the general characteristics described for the skin of domestic mammals and consisted of a keratinized epidermis resting on the dermis. Studies in buffaloes have demonstrated that skin structure and thickness may vary according to anatomical region, reinforcing the importance of considering the collection site in histological comparisons.

Macroscopically, the papillomas appeared as elevated, circumscribed masses measuring 0.5–2 cm in diameter, with a cornified, hairless surface and a characteristic 'cauliflower' appearance. This pattern is similar to that described in bovine cutaneous papillomas by SOUSA et al. (2025). LALRINKIMA et al. (2022) also reported bovine papillomatous lesions with a dry, rough, cauliflower-like surface, whereas CATROXO et al. (2013) observed irregular, circumscribed, cornified lesions with a macroscopic pattern like that found in the present study.

Bovine papillomas may vary in color, size, and shape and may occur as flat, pedunculated, filiform, or cauliflower-like lesions (SOUSA et al., 2025). Although certain BPV types are more frequently associated with some macroscopic presentations, lesion morphology alone does not allow reliable identification of the viral type involved. Thus, although the cauliflower-like appearance observed in the present study is compatible with the classic pattern described for cutaneous papillomatosis, it does not allow identification of the BPV type.

BPV can cause conditions ranging from asymptomatic infections to cutaneous and mucosal papillomas and fibropapillomas and is also associated with certain neoplasms. In addition to local changes, papillomatosis may have economic importance in production animals because of damage to the hide, reduced commercial value of the animals, and secondary complications such as myiasis and bacterial infections. The association between certain BPV types and chronic bracken fern ingestion is also related to the development of urinary bladder and upper digestive tract neoplasms (MUNDAY, 2018).

In the present study, the diagnosis of fibropapilloma was established not only by epidermal proliferation but also by marked proliferation of the dermal component, forming 'glove-finger' projections covered by hyperplastic epidermis. Thus, classification as fibropapilloma is supported by the concurrent involvement of the epithelial and fibroblastic components of the lesion. SOUSA et al. (2025) also histologically distinguished squamous papillomas from fibropapillomas, emphasizing the importance of dermal fibroblastic proliferation for this classification.

In the study by SOUSA et al. (2025), fibropapillomas showed epidermal proliferation accompanied by elongation of the epidermal ridges and marked proliferation of dermal fibroblasts. The authors also observed acanthosis and hyperkeratosis, as well as cellular changes suggestive of a viral cytopathic effect. A significant association was found between BPV-2 and fibropapilloma, a result consistent with the capacity of *Deltapapillomavirus* to induce proliferation of both epithelial and mesenchymal components. However, these molecular findings do not allow inference of the viral type present in the animals examined in the present study.

Histologically, epidermal proliferation in the fibropapillomas was characterized mainly by marked acanthosis, with an increased number of stratum spinosum cell layers and elongation of the epidermal ridges, associated with proliferation of the dermal component. In addition to the proliferative changes, ballooned cells, vacuolation, and degenerative changes in keratinocytes were observed and became more evident in the superficial regions of the epidermis. SOUSA et al. (2025) also reported cytopathic changes in bovine papillomas and fibropapillomas, including koilocytes and intranuclear inclusions. However, ballooned or vacuolated cells should not automatically be classified as koilocytes because this designation depends on the presence of specific morphological features.

On ultrastructural examination, the fibropapillomas showed progressive degenerative changes in keratinocytes, which were more evident in the spinous, granular, and keratinized layers. Tonofibrils, intercellular bridges, and desmosomes could still be identified in the spinous layer, although they were associated with nuclear and cytoplasmic changes. In the more superficial layers, vacuolation, peri- and intranuclear degeneration, and chromatin changes were observed; in the keratinized layer, greater cell individualization and desmosomal degeneration were present.

These findings are similar to the ultrastructural changes described in bovine fibropapillomas by FUJIMOTO & OLSON (1966), who observed cytoplasmic vacuolation, chromatin changes, and modifications of tonofibrils and desmosomes. BROBST & HINSMAN (1966) also described degenerative changes in keratinocytes that became progressively more evident toward the superficial epidermal layers. Alterations in desmosomes and in the organization of cytokeratin filaments were also reported by JELÍNEK & TACHEZY (2005), MATOLTSY (1975), and NAFFA et al. (2019). Thus, the changes observed in the present study correspond to previously described modifications of cells and adhesion structures in bovine papillomatous lesions.

Osmiophilic structures resembling viral particles were observed mainly in the more differentiated epidermal layers, both intranuclearly and, in some fields, clustered in the intercellular spaces (CATROXO et al., 2013). The occurrence of these structures in keratinocytes of the suprabasal layers is particularly relevant when the biology of papillomaviruses is considered, because their productive phase is related to keratinocyte differentiation and occurs predominantly in the more differentiated epithelial layers.

The approximate size of 35–40 nm observed in ultrathin sections corresponds to the findings of CATROXO et al. (2013), who described intranuclear particles approximately 35 nm in diameter and tubular structures measuring approximately 35–39 nm in ultrathin sections. By negative-staining electron microscopy, those authors observed larger particles measuring approximately 60 nm. Differences between the dimensions observed using the different techniques may be related to the sample preparation method and the plane of section through the particles.

Although the particles observed in the present study were isolated or clustered, no evident crystalline organization was identified. The absence of this pattern does not exclude their possible viral nature because particle arrangement may vary according to the stage of cell differentiation, the number of particles present, and the plane of section examined.

SILVESTRE et al. (2009), studying fibropapillomas in buffaloes (*Bubalus bubalis*), demonstrated the presence of papillomavirus in the lesions examined using electron microscopy combined with molecular methods and identified BPV-1. That study is particularly relevant because it demonstrates the occurrence of papillomavirus associated with fibropapillomas in the same animal species evaluated in the present study. However, identification of BPV-1 in those animals does not establish that the same viral type is involved in the lesions examined here.

Molecular, immunohistochemical, or sequencing techniques were not used in the present study to identify the viral agent. Thus, although the morphology, dimensions, and distribution of the structures observed by electron microscopy are compatible with viral particles, their nature cannot be confirmed exclusively by ultrastructural analysis. It is therefore more appropriate to refer to them as 'virus-like particles' or 'structures morphologically compatible with papillomavirus particles,' and the specific BPV type involved cannot be determined.

VI. CONCLUSION

Microscopically, proliferative and degenerative changes in keratinocytes were observed, with acanthosis, vacuolation, and changes that became progressively more evident in the superficial epidermal layers.

Ultrastructurally, nuclear and cytoplasmic changes, vacuolation, modifications of tonofibrils, desmosomes, and intercellular cohesion, and structures morphologically resembling viral particles were observed, mainly in the more differentiated epidermal layers.

These findings are compatible with the possible involvement of papillomavirus in the pathogenesis of the lesions, although confirmation of their viral nature and determination of the BPV type involved depend on the use of specific methods, such as molecular or immunohistochemical techniques.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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