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Pathological and immunohistochemical evaluation of skin and teat papillomas in cattle

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Abstract: Bovine skin and teat papillomas were examined for immunohistochemical expression of bovine papillomavirus (BPV), proliferating antigens (PCNA, Ki-67), $CD3^+$ T lymphocytes, and some of the type III intermediate filaments. Papillomas showed epidermal hyperplasia, koilocytes, and hyperkeratosis with prominent dermal connective tissue. Immunohistochemistry found the presence of BPV antigens in the nuclei of granulosa cells. PCNA and Ki-67 revealed positive nuclear staining in the dermis and epidermis. Perivascular cuffing in the dermis predominantly consisted of $CD3^+$ T cells. Immunoreaction to vimentin was strong in the connective tissue of the dermis. αSMA positivity was mainly in the media of vessels. Smooth muscle cells of the dermal blood vessels showed an immunopositive reaction for desmin. Dermal connective tissue and squamous epithelia were negative for S-100 protein. This work revealed that BPV proliferates predominantly in the stratum granulosum cells and that in the regression of warts cell-mediated immune response has a key role. Vimentin was a very useful marker to show the contribution of fibrotic response to viral replication.

Key words: Bovine papillomavirus, cattle, immunohistochemistry, papilloma, type III intermediate filaments

1. Introduction

Bovine papillomatosis is a viral disease characterized by the development of multiple warts. The disease is regarded as either hyperproliferative lesion or benign neoplasms since they do not metastasize (1–5). Papillomatosis is considered to be contagious in cattle, in which it naturally occurs, and in some cattle extensive papillomas might develop, resulting in debilitated animals that may succumb (1,6). Cattle with warts are usually less than 2 years old and the masses regress spontaneously within 1–14 months (2,5,7,8). Bovine papilloma virus (BPV) is common in cattle herds around the worldwide and is recognized as the cause of the disease associated with many forms of benign tumors, e.g., skin fibropapillomas, genital fibromas, and tumors of the urinary bladder, as well as benign fibroplasia in teats and the esophagus (3,4,8). Bovine papillomas are caused by different types of PVs, infecting both the epithelial cells and the underlying dermis (6). To date, at least 13 different types of BPV have been detected on the basis of DNA sequence analyses. However, six types of the virus have been well characterized. BPV 1, BPV 2, and BPV 5 commonly cause cutaneous fibropapillomas, while BPV 3, BPV 4, and BPV 6 induce squamous papillomas in both the skin and upper gastrointestinal system (2,4,5,7–9). Teat papillomatosis has often been identified in dairy herds as a cattle health problem resulting in economic losses. Benign fibroplasia of teats in cattle is caused by different BPV types, but BPV 6 has often been identified in this location (8,10). Teat papillomas have commonly been divided into three types based upon macroscopic appearance: flat-and-round, rice-grain, and frond epithelial types (2,7,8,10). To date, bovine warts have particularly been studied to establish PV types and their pathological features. However, immunohistochemical expression of type III intermediate filaments has not been evaluated in warts. This work was undertaken to investigate the immunohistochemical expression of BPV, proliferating antigens (PCNA, Ki-67), $CD3^+$ T lymphocytes, and some of the type III intermediate filaments (vimentin, alpha-smooth muscle actin, desmin, S-100) with pathomorphological findings in skin and teat warts of cattle.

2. Materials and methods

2.1. Animals and histopathology

In this work, 450 cattle of various breeds, sex, and ages slaughtered in a local abattoir were examined for skin and teat warts and 19 animal were found to have proliferative lesions on the skin (n = 7) or teats (n = 12). Additionally, solid masses on the external genitalia in 3 cattle were evaluated. Following gross examinations, tissue samples from all growths were fixed in 10% phosphate-buffered formalin solution and routinely processed. Paraffin sections

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of 4 μm in thickness were stained with hematoxylin and eosin (H&E) for histopathological evaluation.

2.2. Immunohistochemistry

Tissue sections were stained with the avidin-biotin-peroxidase complex (ABC) technique (11) for BPV, cell proliferation markers (PCNA, Ki-67), $CD3^+$ T lymphocytes, and some of the type III intermediate filament proteins (vimentin, alpha-smooth muscle actin-αSMA, desmin, S-100). Details of the primary antibodies used are given in Table 1. Serial sections of 4 μm in thickness were deparaffinized and hydrated through graded alcohols. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 20 min. Sections were placed in citrate buffer saline (pH 6.0) in a microwave oven for 20 min for antigen retrieval. Polyclonal rabbit antipapillomavirus (Dako, Carpinteria, CA, USA) and polyclonal rabbit antihuman CD3 antibodies were used with the LSAB2 system (Dako) for the detection of BPV and for differentiation of T lymphocytes. Mouse monoclonal (anti-Ki-67, antivimentin, anti-αSMA, antidesmin) and rabbit polyclonal (S-100) primary antibodies were used with Genemed Acu-Stain mouse + rabbit HRP kits according to the manufacturer's instructions. Immunolabeling was done using 3,3-diaminobenzidine (DAB) or 3-amino-9-ethylcarbazole (AEC) as the chromogen. Mayer's hematoxylin was used as the counterstain. Primary antibodies were omitted from negative control sections. Immunopositivity was evaluated using a semiquantitative grading scheme based on the number of cells exhibiting specific labeling for the markers in 3 representative fields (40× objective): (+) low labeling of 1%–10% of cells; (++) moderate labeling of 11%–59% of cells; and (+++) marked labeling of >60% of cells.

3. Results

3.1. Gross findings

Warts of various sizes covered by a hairless skin in eight cattle were seen on the skin of the neck, shoulder, ventral abdomen, preputium, and periorbita, in three of which warts were also seen to growth on teats. In cross-section, the masses displayed a cauliflower-like appearance elevated above the skin, with ulcerations and hemorrhages. In one case, lobulated large masses with irregular surfaces were seen to elongate on the linea alba. The extirpated lobulated masses showed irregular proliferations distributed to all surfaces (Figure 1). In a cow, multiple large masses with whitish appearance were seen to be distributed on the udder skin (Figure 2). Ten cows revealed papillomas on one or more teats without preferential location. Teat masses were often of flattened-round type and measured approximately 0.5–2 cm in diameter. Other proliferative lesions on teat skin consisted of rice grain-like growths, delicate fronds, and exophytic proliferations (Figure 3). Filamentous growths were seen on the external orifice of a teat in one cow. Additionally, solid tumor masses with light hemorrhages were found on the external genitals (two on the vulva and one on the preputium) in three cattle.

3.2. Histopathology

All skin warts were well circumscribed and were characterized by a squamous epidermal thickening of multiple cell layers, severe hyperkeratosis (ortho- or parakeratotic), and many rete ridges' downgrowth into the dermis (Figure 4A). In the epidermis, squamous epithelial cells often revealed diffuse spongiosis and ballooning degeneration, with a few basophilic intranuclear inclusion bodies in the granular cells. On the epidermis, multifocal erosions or ulcerations with bacterial colonies were detected in some

Table 1. Details of primary antibodies used for immunohistochemical analysis.

Primary antibodies	Pretreatment	Primary antibody dilution	Incubationconditions	Commercial reference and clone no.
Polyclonal rabbit anti-BPV	Microwave oven	Prediluted, ready to use	Room temperature	Dako (Catalog no. N1547)
Monoclonal mouse antirat PCNA	Microwave oven	1/2000	Overnight, 4 °C	Chemicon (Clone PC10)
Polyclonal rabbit antihuman CD3	Microwave oven	Prediluted, ready to use	Room temperature	Dako (Catalog no. N1580)
Mouse monoclonal anti-Ki-67	Microwave oven	Prediluted, ready to use	Room temperature	Genemed (Clone GM010)
Mouse monoclonal antibody antihuman vimentin	Microwave oven	Prediluted, ready to use	Room temperature	Leica (Catalog no. PA0640)
Mouse monoclonal antibody to alpha smooth muscle actin	Microwave oven	Prediluted, ready to use	Room temperature	Leica (Catalog no. PA0943)
Mouse monoclonal antibody to desmin	Microwave oven	Prediluted, ready to use	Room temperature	Leica (Catalog no. PA0032)
Rabbit polyclonal antibody to S-100 protein	Microwave oven	Prediluted, ready to use	Room temperature	Novocastra (Code: NCL-L-S100p)

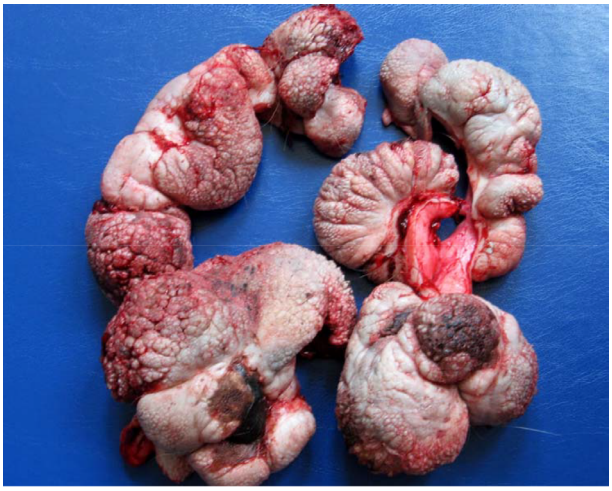

Figure 1. Lobulated large masses with irregular surface extirpated from the linea alba of a cow.

Figure 2. Lobulated whitish tumor mass with exophytic proliferations extirpated from the udder of a cow.

cases. The number and size of keratohyalin granules in the cytoplasm of keratinocytes were evidently increased. The stratum spinosum and granulosa layers showed numerous koilocytes, cells that have large clear cytoplasmic vacuoles and irregular, eccentric, and hyperchromatic nuclei. Dermal connective tissue was severely proliferated and caused the obliteration of normal structures of dermal appendages, covered by hyperplastic epithelium with epithelial pegs extending into the fibrous connective tissue, showing many mitotic figures (Figure 4B). Proliferating fibroblasts were occasionally grouped in bundles that were interwoven, giving the lesion a windblown appearance. The deep dermis of the masses revealed a myxomatous proliferation of fibrocytes and focal hemorrhages. Many capillaries containing few erythrocytes also formed within the fibrotic tissue matrix, indicating a young connective tissue. Multifocal perivascular lymphocytic infiltrations were scattered in the deep dermis. In two cases with fibropapilloma, diffuse necrosis and severe proliferation of connective tissue with numerous blood vessels and bacteria colonies were detected.

Teat proliferations were often diagnosed as cutaneous papilloma where the masses showed severe epidermal hyperplasia, acanthosis, and hyperkeratosis, but not the increase of connective tissue in the dermis. Numerous koilocytes revealed ballooning degeneration and pyknotic

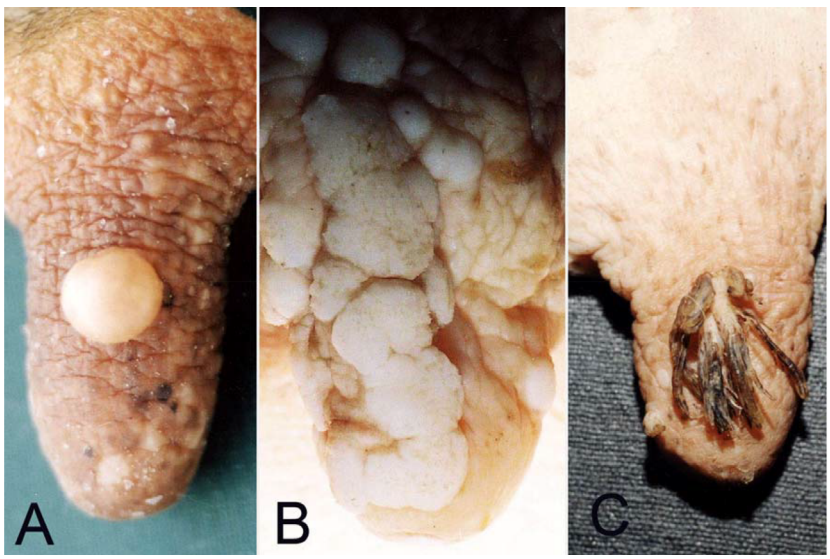

Figure 3. Teat papillomas with different morphological characteristics: flat-and-round type (A), rice grain-like in appearance (B), and filiform proliferations (C).

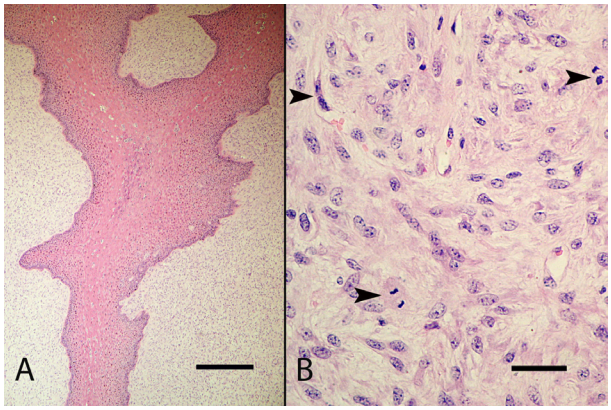

Figure 4. A) Skin fibropapilloma shows rete pegs with hypercellular connective tissue (H&E, bar = 543 µm). B) Numerous mitotic figures in the dermal connective tissue of a fibropapilloma (H&E, bar = 51 µm).

and distorted nuclei with dense chromatin (Figure 5A). Koilocytes were noticed to be larger than those in the skin warts, but the number and size of keratohyalin granules were evidently smaller and fewer than those in the skin warts. Diffuse perivascular cuffing in the upper dermis consisted of mainly mononuclear cells. Fibropapillomas revealed extensive dermal connective tissue with many mitoses. Teat orifice lesions were also characterized by outward digit-like proliferations of the squamous epithelium, an increase of keratohyalin granules, and hyperkeratosis (Figure 5B). Fibromas of external genitals revealed the proliferation of fibrous connective tissue with variably sized fibrocytes and fibroblasts. The nuclei of these cells were pleomorphic and hyperchromatic.

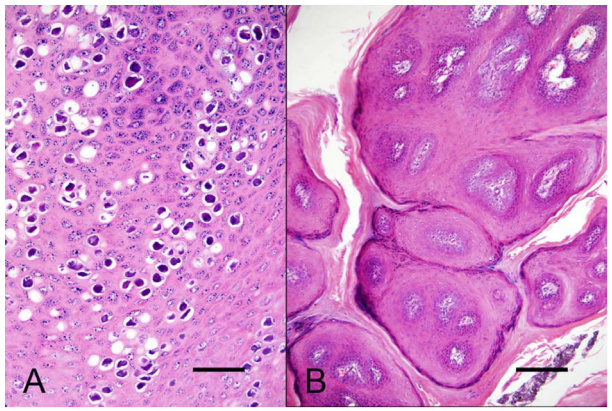

Figure 5. A) Numerous koilocytes with vacuolated cytoplasm with distorted and pyknotic nuclei (H&E, bar = 166 µm). B) The digital-like proliferation of the squamous epithelium at teat orifice (H&E, bar = 543 µm).

3.3. Immunohistochemistry

Samples from both skin and teat warts were studied by immunohistochemistry for BPV, PCNA, Ki-67, and type III intermediate filaments (vimentin, αSMA, desmin, S-100). The nuclei of granular cells in the stratum granulosum layer were stained with the antibody against BPV (Figure 6), and the distorted nuclei of the cells were seen to be strongly positive. However, not all granular layer cells displayed an immunopositive reaction. The connective tissue of the fibropapillomas was immunonegative for BPV antibody. The hyperkeratotic corneal layer also revealed extracellular immunopositive labeling for BPV and it was concluded that the positive labeling was caused by a remnant of the nucleus of granular cells discarded into the stratum corneum. Fibromas of external genitals and normal epidermis in the cattle with warts did not show positive staining for BPV. The highest number of nuclear positive cells for PCNA and Ki-67 was detected in the basal layer cells (Figure 7). The number of PCNA/Ki-67 positive cells was gradually decreased from the basal layer towards the corneum, and few discrete cells in the parabasal and spinous layer showed weak immunostaining with the markers. In fibropapillomas, PCNA-positive reactions in the superficial dermis and perivascular infiltrations were strong, while the number of positively labeled cells was diminished at the bottom of the masses. Some divided cells in the basal layer also showed strong immunopositivity for both markers. PCNA positivity was intense in the cells of both the epidermal and dermal layers, while Ki-67 staining was especially strong in the basal layer cells. $CD3^+$ T lymphocytes were predominantly seen in perivascular cuffings of the dermis (Figure 8). A few $CD3^+$ cells infiltrated into the dermal papilla and epidermal layers. Vimentin immunopositivity occurred predominantly in the fibroblastic proliferations of fibropapillomas (Figure

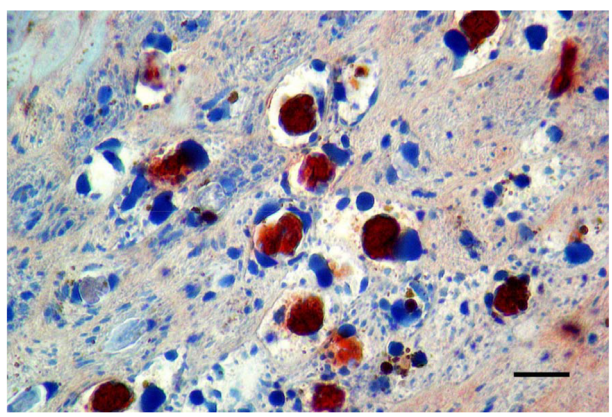

Figure 6. BPV immunopositive reaction in the nuclei of granular layer cells with keratohyalin granules (ABC, bar = 11 µm).

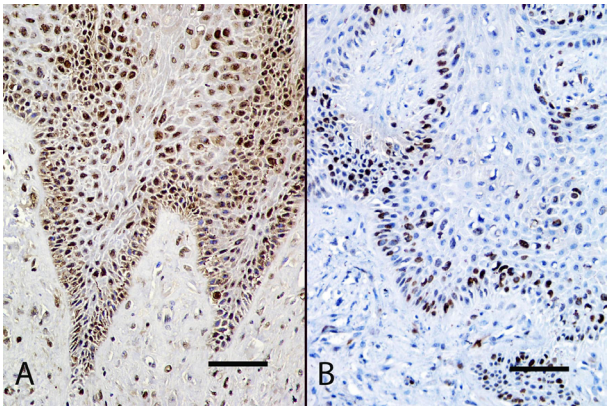

Figure 7. Squamous epithelium and fibroblastic cells show nuclear positivity to PCNA (A) and Ki-67 (B) in teat fibropapilloma (ABC, bar = 51 μm).

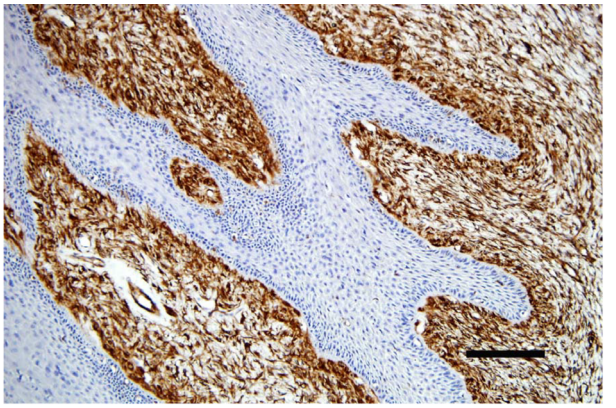

Figure 9. Strong vimentin-positive labeling of dermal connective tissue in a skin fibropapilloma (ABC, bar = 543 μm).

9). Positive immunoreactivity was strong in the superficial dermis, dermal papilla, and islands of connective tissue within the rete pegs. Towards the base of fibropapillomas, where the mass revealed myxomatous proliferation, vimentin positivity gradually decreased. Immunolabeling for vimentin in teat papillomas was weak, and reaction occurred in the dermal papilla, perivascular areas, and normal dermal connective tissue. However, fibromas revealed strong reactivity to vimentin. The tunica media and intima of blood vessels also revealed strong labeling to the marker. αSMA-positive labeling occurred mainly at the smooth muscle cells around the hair follicles and sebaceous glands, and in the media of blood vessels. Mature connective tissue in the upper dermis showed a strong reaction to αSMA (Figure 10), with weak staining in myxomatous proliferations. In teat papillomas, immunopositive staining for αSMA was stronger than that seen in skin warts. All smooth muscle cells in the media of vessels in the dermis and dermal papilla expressed positive reactivity to the desmin. Desmin immunoreactivity within the intima layer was generally weak and preferentially located at the periphery of the media. Desmin positivity was also detected in the muscle tissues around the sebaceous glands, with no staining in normal fibrous tissue or in fibroblastic proliferations of the neoplasm. Immunoreactivity to S-100 was negative in both normal and hyperplastic squamous epithelial cells, and in fibroblastic proliferations with mature or myxomatous connective tissue. However, S-100 immunolabeling was evidently detected at the perivascular neural sheaths, in melanocytes of the basal layer, and in the cytoplasm of vascular endothelial cells, with a strong reaction of neural sheaths in the dermis. Data about animals, tumor locations, and histopathological and immunohistochemical staining results are shown in Table 2.

Figure 8. Predominance of $CD3^+$ immunopositive T lymphocytes around the perivascular spaces in a teat fibropapilloma (ABC, bar = 166 μm).

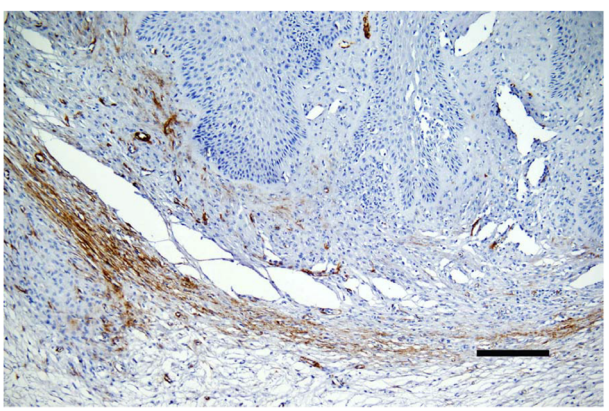

Figure 10. Alpha-smooth muscle-positive reaction in the tunica media of vessels in teat papilloma (ABC, bar = 543 μm).

Table 2: Information on animals, tumor sites, and histopathological and immunohistochemical analysis.

Case no.	Animal	Lesion location	Histopathology	BPV	PCNA/ Ki-67	CD3	Vimentin	αSMA	Desmin	S-100
1	Steer	Neck, shoulder, head, 4 teats	Fibropapilloma	+++	+++	+++	++	++	+	+
2	Cow	Neck	Fibropapilloma	+++	+	++	++	++	+	+
3	Cow	Neck	Fibropapilloma	+++	+++	++	+++	+++	+	+
4	Cow	Linea alba	Fibropapilloma	++	+++	+	+++	++	+	+
5	Cow	Eyelid	Fibropapilloma	+++	+++	++	+++	+++	+	+
6	Cow	Neck, teat	Fibropapilloma	+++	+++	+++	+++	+++	+	+
7	Heifer	Neck, teat	Fibropapilloma	+++	+++	++	+++	+++	+	+
8	Cow	Teat	Fibropapilloma	++	+++	++	++	++	+	+
9	Cow	Teat	Fibropapilloma	++	+++	+++	++	+	+	-
10	Cow	Teat	Fibropapilloma	++	+++	+++	+	+++	+	+
11	Cow	Teat	Papilloma	-	+++	++	+++	++	+	-
12	Cow	Teat	Papilloma	+	+++	+++	+++	+	++	+
13	Cow	Teat	Papilloma	++	++	+++	++	+++	+	+
14	Cow	Teat	Papilloma	-	++	+++	++	+++	+	+
15	Cow	Teat	Papilloma	++	++	++	+	++	+	+
16	Cow	Teat	Papilloma	-	++	++	++	+++	+	+
17	Cow	Teat	Papilloma	+	+++	+++	+++	++	+	+
18	Cow	Teat	Papilloma	+	+	+++	++	++	-	-
19	Cow	Teat orifice	Papilloma	++	++	++	++	+	+	+
20	Cow	External genitals	Fibroma	-	++	+	+++	++	+	-
21	Cow	External genitals	Fibroma	-	+++	+	+++	+++	+	+
22	Steer	Preputium	Fibroma	-	+++	+	+++	++	++	+

+: Weak reaction (0–10), ++: moderate reaction (10–50), +++: strong reaction (50–100).

4. Discussion

Bovine papillomatosis is considered as an experimental model for investigation of human PV infection and viral carcinogenesis (3). Likewise, it would be very helpful to know the oncogenic potentials of the DNA viruses, the relationships between virus and cofactors, and the development of papilloma vaccine (4). The present study was undertaken to evaluate the histopathology and immunohistochemistry of warts from the skin and teats, and diagnosis of the lesions depended on the pathological results with BPV-specific immunolabeling. BPV-induced skin warts were seen on the neck, shoulders, and ventral abdomen, and the masses exhibited circumscribed aspects, pedunculated, cornified, black or grayish in color, as reported by others (5,8,12–18). The reason for the predominant occurrence of warts on the neck might be due to the traumatized skin induced by metal chains used for stabilization of cattle during the winter season in this region. Histopathological examination demonstrated that epidermal lesions of warts mainly consisted of squamous epithelial thickening, hyperkeratosis, and acanthosis with koilocytes having shrunken dark nuclei surrounded by a clear halo, which are hallmarks of the benign papilloma lesions caused by BPV (2,16,18,19). In addition to epithelial hyperplasia, BPV evokes a severe connective tissue proliferation, characteristic of cutaneous papillomatosis. It is reported that stimulation of fibroblastic connective tissue by BPV precedes epidermal hyperplasia (1,20). All fibropapillomas were predominantly composed of severe proliferating mature fibrous tissue in the upper dermis covered by hyperplastic epithelium along with myxomatous proliferation towards the base of the dermis. Connective tissue exhibited a whorled pattern around the small blood vessels and rete ridges of the epithelium extended into the subjacent connective tissue, in accordance with the results reported by others (2,8,12–14,18,20,21).

Teat papillomatosis is very common in cows, and it regresses spontaneously in the field. Outbreaks can reach serious proportions and teats often become secondarily

infected, and sometimes the invasion of tumors can be so severe that milking becomes impossible (3,5,8,22). Teats revealed grossly flat-and-round type, frond-like, and rice grain-like growths, usually multiple in numbers on all teat skin. Similar to our findings, previous researchers (8,22) reported teat warts with similar morphological appearances associated with flower-like and frond-type growths caused by BPV-1 and -10. Squamous papillomas of teats were usually characterized by epidermal thickening, acanthosis, numerous koilocytes, and hyperkeratosis. However, teats with frond and rice grain-like growths did not show dermal fibrosis, in accordance with the results of Maeda et al. (8). It was reported (19) that hyperkeratosis is an effect of the increase in keratohyalin granules in the granular layer because the granules are associated with the keratinization process. Koilocytes with vacuolated cytoplasm and distorted nuclei were detected in all teat papillomas and found as an important factor in the designation of papillomatous proliferation. In accordance with our results, the presence of koilocytosis in warts is considered a prominent sign of the disease and represents a cytopathic effect of PV infection, showing the presence of degenerating, dying cells, highlighted by a prominent halo (15,19).

Although both epithelial and mesenchymal cell lines have been reported to be infected by PVs, viral replication is limited to the basal epithelial cells, which stimulate hyperproliferation and hyperplasia with the formation of warts (4,5,9,14). BPV is first seen in the nucleoli of prickle cells and then spreads throughout the degenerating nuclei of the granular layer cells (23). In the present study, immunohistochemistry for BPV antigen exclusively demonstrated an evident intranuclear positive reaction in the granular cells and in the corneal layer from the skin and teat papillomas, but not in fibroblastic proliferations of the dermis, consistent with results reported by Brobst and Hinsman (23) and Borzacchiello (9). Such a positive reaction evidently confirmed the viral etiology in the occurrence of skin and teat papillomatous growths used in this study, as reported in cattle by others (2,8,12,16,21). BPV immunolabeling in the corneal layer was considered to be related to the nuclear remnants of granular cells, as reported in other studies (2,23).

Proliferation factors are reported to be expressed in all phases of the cell cycle and to be the most important indicators of cellular proliferations (17). The present study found similar expression behavior of PCNA and Ki-67 in the skin and teat papillomas. Immunohistochemistry for the proliferation factors displayed strong positivity of the cells in the basal layer and in the deep zone of the stratum spinosum and in mesenchymal cells of the dermal connective tissue. Similarly, some authors reported proliferative activity in the basal layer and in the spinous layer of the epidermis, and in mesenchymal cells of the superficial dermis using PCNA immunostaining in bovine warts (2,8,16,17). When we compared the reactivity of the proliferating markers, nuclear reactivity to PCNA was strong in both fibroblastic proliferations of the dermis and in the basal layer, while Ki-67 staining was intense in the Malpighian layer. Similar to our results, Özsoy et al. (16) and Kumar et al. (17), in studies on the expression of PCNA and Ki-67 in relation to BPV, found that the expression of PCNA occurred in the dermis and in the stratum basale, while Ki-67 was observed mainly in the basal layer.

Spontaneous papilloma regression in animals and humans is hypothesized to depend on a cell-mediated immune response, the nature of which is still ill-defined and which is accompanied by infiltrations of immune cells (2,24,25). Our study often found perivascular cuffings in dermal connective tissue, which were predominantly immunopositive to $CD3^+$ T lymphocytes, as reported in papillomatosis of cattle (2,12) and dogs (25). As with bovine cutaneous papillomas, Wilson et al. (26) reported a strong labeling of the cluster of $CD3^+$ T lymphocytes in equine sarcoids and concluded that regression of the mass is mediated by cellular immunity. To elucidate the nature and role of the immune cells present in regressing papillomas, tumor specimens from both cattle (24) and dogs (25) were investigated phenotypically and lymphocytes present in these lesions were quantified, and a predominance of CD4 lymphocytes, with only a few CD8 and $\gamma\delta(WC1^+)$ cells, was found.

Immunohistochemical expression of intermediate filaments has not been investigated in bovine warts. Therefore, our study examined the immunohistochemical expression of vimentin, desmin, and αSMA along with S-100. Likewise, fibropapillomas should be distinguished from other spindle cell tumors such as peripheral nerve sheath and smooth muscle cell tumors (27). Positive immunolabeling with vimentin, designated as an important intermediate filament protein of mesenchymal cells (28), was strong in the dermal connective tissue of fibropapillomas and in fibromas. However, vimentin positivity was weak in the squamous proliferations of teats and was found in dermal papilla, perivascular areas, and normal dermal connective tissue. Similarly to our results, in the papillomatous lesions of bovines (29) and deer (30), and in equine sarcoids (26), vimentin positivity confirmed the fibroblastic proliferations. αSMA is the first known marker of differentiated smooth muscle cells that is upregulated during vasculogenesis and expressed predominantly in the media of muscle arteries (31). In this study, skin papillomas revealed positive labeling with αSMA in smooth muscle cells of the tunica media and connective tissue of fibropapillomas, whereas αSMA-positive reaction in teat papillomas was evidently strong in

the vessels and smooth muscles of teats. Positive labeling of connective tissue in the fibropapillomas revealed that smooth muscle actin plays an important role in fibroblast contractility, as reported by others (29,32). Positive staining with desmin was only detected in the smooth muscle cells in the media layer of vessels and smooth muscle cells around the sebaceous glands, but normal dermal connective tissue and fibroblastic proliferations revealed a definitive negativity to the marker, which may confirm benign fibroplasia of warts. Gulbahar et al. (29) stated that even though papilloma was negative to desmin, staining of angiokeratomatous papilloma might be useful in the differentiation of smooth muscle cells and pericytes. S-100 protein expression was not seen in squamous or fibroblastic proliferations, even though numerous melanocytes, endothelial cells, and perivascular neural sheaths were strongly positive for the marker. Thus, S-100 negativity of squamous and fibroblastic proliferations ruled out neoplasms of neural crest origin, as reported by Oryan et al. (27).

In conclusion, the results of the present study demonstrated that epidermal thickening, koilocytes, and hyperkeratosis are very important indicators of bovine warts. However, in papillomatous growths, the presence of BPV needs to be confirmed, which may also be useful in distinguishing the disease from fibromatous growths of skin or external genitalia. Immunohistochemical protocols for the detection of type III intermediate filaments are easily applicable and yield positive results in bovine skin and teat papillomatous lesions. In particular, positive labeling with vimentin may be useful to confirm the fibrotic response to viral replication in fibropapillomas.

References

1. Lancaster WD, Olson C. Animal papillomaviruses. Microbiol Rev 1982; 46: 191-207.
2. Jelinek F, Tachezy R. Cutaneous papillomatosis in cattle. J Comp Pathol 2005; 132: 70-81.
3. Borzacchiello G, Roperto F. Bovine papillomaviruses, papillomas and cancer in cattle. Vet Res 2008; 39: 45.
4. Freitas AC, Silva MAR, Jesus ALS, Mariz FC, Cordeiro MN, Albuquerque BMF, Batista MVA. Recent insights into bovine papillomavirus. Afr J Microbiol Res 2011; 33: 6004-6012.
5. Catroxo MHB, Martins AMCRPF, Petrella S, Souza F, Nastari BDB. Ultrastructural study of bovine papillomavirus during outbreaks in Brazil. Int J Morphol 2013; 2: 777-784.
6. Bocaneti F, Altamura G, Corteggio A, Velescu E, Borzacchiello G. Expression of bcl-2 and p53 in bovine cutaneous fibropapillomas. Infect Agent Cancer 2015; 10: 22.
7. Yaguiu A, de Carvalho C, de Freitas AC, Góes LGB, Dagli MLZ, Birgel EH Jr, Beçak W, dos Santos RCS. Papillomatosis in cattle: in situ detection of bovine papillomavirus DNA sequences in reproductive tissues. Braz J Morphol Sci 2006; 23: 525-529.
8. Maeda Y, Shibahara T, Wada Y, Kadota K, Kanno T, Uchida I, Hatama S. An outbreak of teat papillomatosis in cattle caused by bovine papilloma virus (BPV) type 6 and unclassified BPVs. Vet Microbiol 2007; 121: 242-248.
9. Borzacchiello G. Bovine papillomavirus infections in animals. In: Vilas MA, editor. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Badajoz, Spain: Formatex; 2007. pp. 673-679.
10. Tozato CC, Lunardi M, Alfieri AF, Otonel RAA, Di Santis GW, de Alcântara BK, Headley SA, Alfieri AA. Teat papillomatosis associated with bovine papillomavirus types 6, 7, 9, and 10 in dairy cattle from Brazil. Braz J Microbiol 2013; 44: 905-909.
11. Hsu S, Raine L, Fanger H. Use of avidin–biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981; 29: 577-580.
12. Abdouslam OE, Levkut M, Levkutdva M, Revajova V, Ondrejka R, Benisek Z. Immunohistochemistry of the progressive and regressive stages of bovine papillomatosis. Acta Vet Brno 1997; 66: 245-248.
13. Atasever A, Çam Y, Atalay Ö. Skin papillomatosis in a cattle herd. Ankara Univ Vet Fak 2005; 52: 197-200.
14. Turk N, Župančić Ž, Starešina V, Kovač S, Babić T, Kreszinger M, Ćurić S, Barbić L, Milas Z. Severe bovine papillomatosis: detection of bovine papillomavirus in tumour tissue and efficacy of treatment using autogenous vaccine and parammunity inducer. Vet Arhiv 2005; 75: 391-397.
15. Marins RSQS, Travassos CEPF. Clinicopathologic and epithelial regression study in cutaneous warts of bovines infected by papillomavirus. Afr J Pharm Pharmaco Res 2011; 1: 012-016.
16. Özsoy ŞY, Özyıldız Z, Güzel M. Clinical, pathological and immunohistochemical findings of bovine cutaneous papillomatosis. Ankara Univ Vet Fak 2011; 58: 161-165.
17. Kumar P, Nagarajan N, Kumar D, Bind RB, Somvanshi R. Detection and quantification of bovine papillomaviruses (BPVs) in cutaneous warts of cattle and buffaloes. Adv Anim Vet Sci 2013; 1: 53-58.
18. Hong YJ, Kim JH. Detection of bovine papillomaviruses in skin warts of Korean native cattle from Jeju Island. J Prev Vet Med 2015; 39: 84-88.

19. Araldi RP, Carvalho RF, Melo TC, Diniz NSP, Sant'Ana TA, Mazzuchelli-de-Souza J, Spadacci-Morena DD, Beçak W, Stocco RC. Bovine papillomavirus in beef cattle: first description of BPV-12 and putative type BAPV8 in Brazil. Genet Mol Res 2014; 3: 5644-5653.

20. Cheville NF, Olson C. Epithelial and fibroblastic proliferation in bovine cutaneous papillomatosis. Vet Pathol 1964; 1: 248-257.

21. Elzein EE, Sundberg JP, Housawi FM, Gameel AA, Ramadan RO, Hassanei MM. Genital bovine papillomavirus infection in Saudi Arabia. J Vet Diagn Invest 1991; 3: 36-38.

22. Kumar P, Jangir BL, Saikumar G, Somvanshi R. Co-infection of bovine papillomavirus type-1 and -10 in teat warts of dairy cattle. Vet Med-Czech 2013; 58: 605-608.

23. Brobst D, Hinsman EJ. Electron microscopy of the bovine cutaneous papilloma. Pathol Vet 1966; 3: 196-207.

24. Knowles G, O'Neil BW, Campo MS. Phenotypical characterization of lymphocytes infiltrating regressing papillomas. J Virol 1996; 70: 8451-8458.

25. Nicholls PK, Moore PF, Anderson DM, Moore RA, Parry NR, Gough GW, Stanley MA. Regression of canine oral papillomas is associated with infiltration of $CD4^+$ and $CD8^+$ lymphocytes. Virology 2001; 283: 31-39.

26. Wilson AD, Armstrong ELR, Gofton RG, Mason J, De Toit N, Day MJ. Characterisation of early and late bovine papillomavirus protein expression in equine sarcoids. Vet Microbiol 2013; 162: 369-380.

27. Oryan A, Dehghani S, Yazdi HS, Taravat S, Daneshbod Y. Vaginal fibropapilloma in a heifer. Braz J Vet Pathol 2014; 7: 21-24.

28. Ivaska J, Pallari HM, Nevo J, Eriksson JE. Novel functions of vimentin in cell adhesion, migration, and signaling. Exp Cell Res 2007; 313: 2050-2062.

29. Gulbahar MY, Yuksel H, Aslan L. Angiokeratomatous papilloma associated with papillomavirus in a calf. Vet Pathol 2003; 40: 582-586.

30. Erdélyi K, Gál J, Sugár L, Ursu K, Forgách P, Szeredi L, Steineck T. Papillomavirus-associated fibropapillomas of red deer (*Cervus elaphus*). Acta Vet Hung 2009; 2: 337-344.

31. Shynlova O, Tsui P, Dorogin A, Chow M, Lye SJ. Expression and localization of alpha-smooth muscle and gamma-actins in the pregnant rat myometrium. Biol Reprod 2005; 73: 773-780.

32. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell 2001; 12: 2730-2741.