Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.192

RESEARCH ARTICLE

Molecular Detection of Bovine Papillomaviruses in Cattle in Konya Province: The Emergence of BPV-12

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ARTICLE HISTORY (26-617)

Received: May 22, 2026
Revised: June 23, 2026
Accepted: June 24, 2026
Published online: July 10, 2026

Key words:
BPV
Co-infection
Molecular virology
Papilloma

ABSTRACT

Bovine papillomaviruses cause a contagious viral disease in cattle known as papillomatosis. This study was conducted to determine the molecular presence of papillomavirus in cattle raised in Konya Province. For this purpose, a total of 25 papilloma lesion samples developing in the head, neck, and teat regions of 25 cattle raised for milk and meat production in the Ahirli, Bozkir and Yalihuyuk districts of Konya Province were collected. These lesions were analyzed at the molecular level using the conventional PCR method with the FAP59/64 and MY11/09 consensus primers. While no positivity was detected in any of the samples analyzed using the FAP59/64 primer pair, positive results were obtained in 2 of the 25 samples (8%) examined with the MY11/09 primer pair. In the second-stage analysis performed using type-specific primers in all samples, Bovine Papillomavirus (BPV) types were detected in 24 of the 25 samples (96%). BPV-3, BPV-5 and BPV-11 types were not detected in any of the samples. The BPV types identified using type-specific primers were determined to be BPV-1, BPV2, BPV-4, BPV-6, BPV-8, BPV-9, BPV-10 and BPV-12. In the overall distribution, BPV-12 (95.83%) was identified as the most prevalent type for the first time. Macroscopic examination revealed that 52% of the lesions were classified as having a typical morphological form. No statistically significant association was found between the macroscopic appearance, anatomical localization of the lesions, and the detected BPV types (P>0.05). In conclusion, various BPV types responsible for papillomatosis were determined to be present in cattle raised in Konya Province.

To Cite This Article: Kaya Y, Bulut O and Kale M, 2026. Molecular detection of bovine papillomaviruses in cattle in konya province: the emergence of BPV-12. Pak Vet J, 46(8): 2025-2031. http://dx.doi.org/10.29261/pakvetj/2026.192

INTRODUCTION

Papillomaviruses are viruses that infect many vertebrate species, including cattle, horses, dogs, cats and humans (Van Doorslaer*et al.,* 2018). *Bovine Papillomavirus* (BPV) is a viral agent that causes highly contagious hyperproliferative lesions, known as papillomas, in the skin and mucosal tissues of cattle. Papillomas manifest as benign tumors that can occur in almost all parts of the bovine body, as well as malignant lesions affecting the upper alimentary tract organs and the urinary bladder (Nasir and Campo, 2008).

Papillomavirus (PV) genomes consist of a non-segmented, covalently closed circular double-stranded DNA molecule approximately 8,000 base pairs in length (Van Doorslaer*et al.,* 2018). Papillomavirus genomes contain ORFs designated as early and late regions, which encode early and late proteins that vary according to the PV type (McBride, 2008). Since the L1 protein is the most highly conserved gene among PVs, it is still widely used in the classification of papillomaviruses (Van Doorslaer*et al*., 2018).

The disease is caused by multiple BPV types. To date, 29 BPV types have been identified, which are classified within the genera *Deltapapillomavirus, Dyokappapapillomavirus, Dyoxipapillomavirus, Epsilonpapillomavirus*, and *Xipapillomavirus*, with three additional types remaining unassigned to any genus (Van Doorslaer*et al.,* 2018; Matias*et al.,* 2024). In addition, there are also newly identified types reported in recent studies that are still awaiting official classification (Sauthier*et al.,* 2021; Gilio Gasparotto*et al.,* 2024; Chang*et al.,* 2025).

Although the disease can affect cattle of all age groups, it is more commonly encountered in young cattle

under 2 years of age (Jelínek and Tachezy, 2005). Bovine papillomaviruses are thought to be transmitted primarily through direct contact (Nasir and Campo, 2008). It has also been reported that animals raised in confined environments are more susceptible to the disease and that indirect transmission may occur via fomites. Therefore, there is a risk of disease transmission through fomites contaminated indirectly by contact with infected animals, such as fences, halters, milking equipment, grooming tools, and ear-tagging forceps (Nasir and Campo, 2008; Araldi*et al.,* 2015). Although direct contact is considered the most widely accepted route of transmission, there is evidence suggesting that transplacental transmission may also occur (Freitas*et al.,* 2003; Yaguiu*et al.,* 2008).

In addition to cutaneous papillomas and fibropapillomas that can occur almost anywhere on the body, BPVs also cause vulvovaginal, penile, gastrointestinal, udder, and teat papillomas, as well as malignant lesions in the urinary bladder and gastrointestinal tract organs (de Alcântara*et al.,* 2021; Medeiros-Fonseca*et al.,* 2021; Sauthier*et al.,* 2021). Lesions developing in the skin exhibit various morphological patterns. Monteiro*et al.* (2008) morphologically classified the lesions caused by BPVs in cattle. Accordingly, the lesions were classified as typical, pedunculated, atypical or flat papillomas, filiform, and rice-grain forms (Fig. 3).

Bovine papillomatosis can be diagnosed based on clinical findings, lesion histology, immunohistochemistry, or molecular techniques. Polymerase chain reaction (PCR) assays are among the most widely used molecular methods for the detection of BPVs in blood and tissue samples (Silva*et al.,* 2013; Yıldırım*et al.,* 2022).

In this study, the relationship between the macroscopic appearance of papilloma lesions developing at different anatomical sites in infected cattle in the Konya region and the animals age, sex, and viral types detected by PCR was evaluated.

MATERIALS AND METHODS

All sampling procedures were conducted in accordance with animal welfare and ethical guidelines. This study was approved by the Local Ethics Committee for Animal Experiments of Selcuk University (Ethical approval number 2023/05, dated 27.04.2023).

Animals: Following the screening of 3,270 bovine animals from 298 herds located in the Bozkır, Yalihüyük, and Ahırlı districts of Konya Province, wart samples were collected by random sampling from 25 cattle of different breeds, ages, and sexes that clinically exhibited pointed and rounded lesions. Only one infected animal from each herd was sampled. The sampled animals were classified according to sex, age, anatomical location, and breed. All samples were obtained from infected animals between May 2023 and January 2024, either following the application of surgical silk ligation or from tissue fragments detached during palpation, and were transported under sterile cold-chain conditions to the Virology Laboratory of the Faculty of Veterinary Medicine, Selcuk University. The samples were stored at -80ºC until molecular analysis.

DNA extraction and PCR: DNA extraction was performed from 25 tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Germany). The extraction procedure was performed according to the manufacturer's instructions. The obtained extracts were stored at -20°C until PCR analysis. The consensus primers FAP59/64 and MY11/09, which amplify the highly conserved L1 gene region, were previously described and were used for the screening of potential novel BPV types (Table 1). Subsequently, the samples were evaluated for 11 previously identified BPV types using the procedure developed by Silva*et al*. (2016) (Table 3). The PCR reaction conditions are presented in Tables 2 and 4.

Table 1: Consensus oligonucleotide primer designs

Primer		Sequence	Fragment Size (bp)	Gen
FAP59/64	F	5′- TAACWGTIGGICAYCCWTATT-3′	478	L1
	R	5′-CCWATATCWVHCCATITCICCATC-3′		L1
MY11/09	F	5′- GCMCAGGGWCATAAYAATGG -3′	450	L1
	R	5′- CGTCCMARRGGAWACTGATC-3		L1

Table 2: PCR reaction conditions using consensus oligonucleotide primers

Primer	Denaturation	Annealing	Extension	Number of cycles
FAP59/64	1 dk. 94℃	1 dk. 50℃	1 dk. 72℃	45
MY11/09	30 sn. 95℃	30 sn. 48℃	1 dk. 72℃	35

Table 3: Type-specific oligonucleotide primer designs

Type		Sequence	Fragment Size (bp)	Gen
BPV1	F	5′GGAGCGCCTGCTAACTATAGG3′	301	L1
	R	5′ATCTGTTGTTTGGGTGGTGAC3′		
BPV2	F	5′GTTATACCACCCAAAGAAGACCCT3′	164	L1
	R	5′CTGGTTGCAACAGCTCTCTTTCTC3′		
BPV3	F	5′CAGTCAATTGCAACTAGATGCC3′	216	L1
	R	5′GGCTGCTACTTTCAAAAGTGA3′		
BPV4	F	5′GCTGACCTTCCAGTCTTAAT3′	170	L1
	R	5′CAGTTTCAATCTCCTCTTCA3′		
BPV5	F	5′GGCATGTAGAGGAATATAAGC3′	262	L1
	R	5′TTCTCTGAGATCAATATTCC3′		
BPV6	F	5′TTAGAGACCTGGAACTTGGG3′	294	L1
	R	5′TACGCTTTGGCGCTTTTTTGC3′		
BPV8	F	5′TAGAGGACACATACCGCTTCCAAAGC3′	196	L1
	R	5′TTTGCGAGCACTGCAGGTGATCCC3′		
BPV9	F	5′AAAGAGCAAATCGGGAGCACC3′	264	L1
	R	5′AACTAATGACCCACTAGGGCTCC3′		
BPV10	F	5′AAGGCATTTGTGGTCTCGAGG3′	148	L1
	R	5′CTAAAGAACCACTTGGAGTGCC3′		
BPV11	F	5′TGCAGACACTCAACCAGGAG3′	197	L1
	R	5′CCATAAGGGTCGTTGCTCAT3′		
BPV12	F	5′AAAGCTGAACCATGCAAACC3′	159	L1
	R	5′TAACAATGTCAAGGGGCACA3′		

Table 4: PCR reaction conditions using type-specific oligonucleotide primers

Type	Denaturation	Annealing	Extention	Number of cycles
BPV-1	40 sn. 94˚C	40 sn. 68˚C	60 sn. 72˚C	35
BPV-2	40 sn. 94˚C	40 sn. 55˚C	60 sn. 72˚C	35
BPV-3	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-4	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-5	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-6	40 sn. 94˚C	40 sn. 65˚C	60 sn. 72˚C	35
BPV-8	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-9	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-10	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-11	40 sn. 94˚C	40 sn. 60˚C	60 sn. 72˚C	35
BPV-12	40 sn. 94˚C	40 sn. 54˚C	60 sn. 72˚C	35

The obtained amplicons were visualized using UView™ 6x Loading Dye (BioRad, USA) staining with 1.5% Tris Acetate Buffer agarose gel electrophoresis.

Statistical Analysis: The types and co-infections detected as a result of macroscopic and molecular analyses in papilloma samples found in the bodies of cattle were

statistically analyzed. The association of the macroscopic forms of papillomas with anotomical location and BPV types was analyzed using Chi-square and Fisher's exact tests.

Numerical and categorical data were analyzed using percentages (%) and mean values. SPSS 15.0 for Windows® software was used in the analysis.

RESULTS

In the present study, samples obtained from 25 animals with macroscopically evident lesions were used. Macroscopically, the overall prevalence of pointed and rounded papillomas was determined to be 7.38% among the screened herds. Among the screened cattle, the overall prevalence of pointed and rounded papillomas was determined to be 1%. The obtained lesions were anatomically classified as follows: 2 in the head region, 13 in the neck region, and 10 in the udder region (Table 5). The mean age of the sampled animals was determined to be 27.64 months (Table 5). In the molecular analysis, positivity was detected in 2 of the 25 samples using the MY11/09 consensus primer, whereas no positivity was detected in any sample with the FAP59/64 consensus primer. In the type-specific PCR analysis performed to determine the BPV types present in the lesions, positivity was detected in 24 of the 25 samples (Table 6). BPV-3, BPV-4, BPV-5, and BPV-11 types were not detected in any of the samples. In the analysis performed using type-specific primers, BPV-12 was identified as the most prevalent type in the 24 positive samples, with a prevalence rate of 92%, followed by BPV-2 (56%), BPV-6 (20%), and BPV-1 (16%). BPV-12 was the most frequently detected type in all age groups. Only in animals aged 24-35 months, BPV-12 and BPV-2 were detected concurrently in 5 of the 6 animals. The distribution of single and multiple BPV type co-infections in the samples showed that dual-type infections were the most common, with a rate of 54.17% (Table 6).

Table 5: Age, sex, and breed of the sampled animals, macroscopic forms, and anatomical locations of the lesions.

Animal	Age (Month)	Sex	Breed	Macroscopic Form	Anatomical Location
1	18	Female	Brown Swiss	Pedunculated	Head
2	42	Female	Simental	Filiform	Teat
3	30	Female	Brown Swiss	Atypical	Teat
4	30	Female	Simental	Typical	Neck
5	30	Female	Holstein	Typical	Neck
6	16	Female	Holstein	Typical	Neck
7	30	Female	Simental	Typical	Neck
8	16	Male	Simental	Typical	Neck
9	16	Female	Holstein	Filiform	Teat
10	15	Female	Simental	Typical	Neck
11	17	Female	Simental	Atypical	Neck
12	16	Female	Holstein	Typical	Neck
13	56	Female	Holstein	Filiform	Neck
14	37	Female	Simental	Typical	Teat
15	41	Female	Holstein	Typical	Teat
16	43	Female	Brown Swiss	Pedunculated	Teat
17	50	Female	Holstein	Filiform	Teat
18	57	Female	Holstein	Pedunculated	Teat
19	6	Female	Simental	Typical	Head
20	30	Female	Simental	Atypical	Teat
21	17	Female	Simental	Filiform	Teat
22	13	Female	Simental	Pedunculated	Neck
23	25	Female	Holstein	Typical	Neck
24	19	Female	Holstein	Typical	Neck
25	21	Female	Simental	Typical	Neck

Table 6: Results of the molecular analysis of the samples

Animal	FAP59/64	MY09/11	Type Spesific	Viral Types
1	-	-	+	BPV-12
2	-	-	+	BPV-6,12
3	-	-	+	BPV-2,12
4	-	-	+	BPV-2,12
5	-	-	+	BPV-2,12
6	-	+	+	BPV-1,2,12
7	-	-	+	BPV-1
8	-	-	+	BPV-2,12
9	-	-	+	BPV-2,6,12
10	-	-	+	BPV-12
11	-	+	+	BPV-12
12	-	-	+	BPV-1,12
13	-	-	+	BPV-12
14	-	-	+	BPV-6,8,12
15	-	-	+	BPV-2,12
16	-	-	+	BPV-8,12
17	-	-	+	BPV-2,8,10,12
18	-	-	+	BPV-2,9,12
19	-	-	+	BPV-2,12
20	-	-	+	BPV-2,6,9,12
21	-	-	+	BPV-2,6,12
22	-	-	-	
23	-	-	+	BPV-2,12
24	-	-	+	BPV-1,2,12
25	-	-	+	BPV-2,12

Macroscopically, the lesions were characterized as typical, atypical, pedunculated, and filiform forms. The rice-grain form was not observed in any of the lesions. Of the lesions, 52% were determined as the typical form (Fig. 1; Fig. 2).

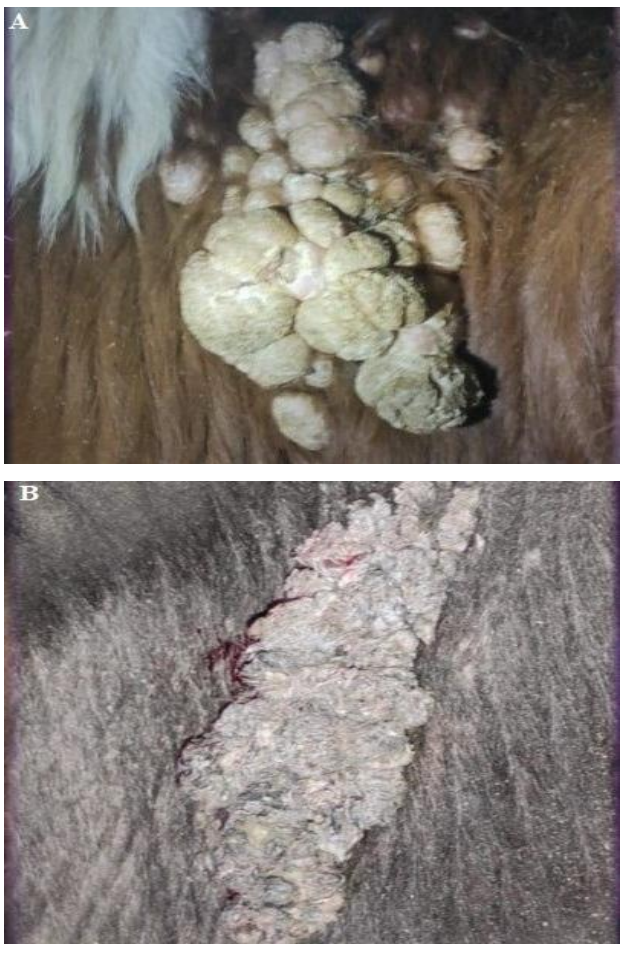

Fig. 1: A) Typical form, Neck, Types detected in the lesion: BPV-1 and BPV-2, Yenikoy neighborhood, Bozkir. B) Atypical form, Neck, Types detected in the lesion: BPV-2, BPV-12, Yenikoy neighborhood, Bozkir.

Fig. 2: A) Pedunculated form, Eye, Types detected in the lesion: BPV-12, Karabayir neighborhood, Bozkir. B) Filiform, Teat, Types detected in the lesion: BPV-2, BPV-6, BPV-9, BPV12, Sorkun neighborhood, Bozkir.

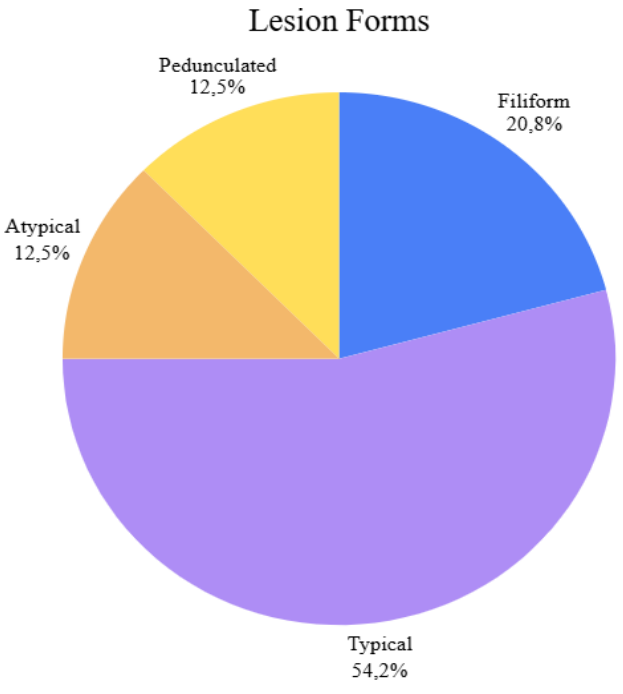

Fig. 3: Macroscopic distribution of the lesions observed in the samples.

Statistical results: The warts with macroscopically defined forms were classified according to their anatomical location as head, neck, and teat regions. The analyses were performed based on this classification. Accordingly, no statistically significant association was found between the macroscopic appearance of the lesions and their anatomical location or the detected BPV types.

DISCUSSION

Although bovine papillomavirus-associated lesions can be observed in cattle of all ages, they have been reported to occur more frequently in young animals (Jelínek and Tachezy, 2005). Studies conducted in Türkiye and worldwide have reported that animals with papilloma lesions are generally young animals (Araldi*et al.,* 2015; Mathewos*et al.,* 2021; Taşatan and Kale, 2024). In the present study, similar to previous reports, the animals clinically presenting with papilloma lesions were predominantly young, with a mean age of 27.64 months. Of the 24 animals that tested positive molecularly, 10 (41.66%) were found to be between 12 and 23 months of age. Young cattle are thought to be more susceptible to infection due to factors such as the alkaline range of skin pH at an early age, the time required for the development of specific immune responses, the loss of maternal antibodies rendering animals more susceptible to other infections, parasitic infestations, and stress factors such as parturition (Salib and Farghali, 2011).

On the other hand, there are studies reporting a significant association between BPV prevalence and sex, indicating that the disease occurs more frequently in female animals (Salib and Farghali, 2011; Hamad*et al.,* 2017; Mathewos*et al.,* 2021). Similarly, in the present study, 24 (96%) of the sampled animals were female cattle. It has been suggested that male animals are generally slaughtered for meat production after a certain period, whereas female animals remain in the production cycle and live for many years (Hamad*et al.,* 2017). Furthermore, female animals have been reported to be more frequently exposed to physiologically immunosuppressive conditions associated with pregnancy, lactation, and aging, whereas male animals are generally slaughtered for meat production before being subjected to such immunosuppressive physiological events (Salib and Farghali, 2011). In the present study, the finding that the only infected male animal identified in the screened herds was 15 months old supports previous reports suggesting that male animals are generally slaughtered after reaching slaughter weight rather than being maintained for prolonged rearing.

Lesions associated with Bovine Papillomaviruses can be observed in cattle in the head, neck, udder and teat regions, legs, urinary bladder, genital and paragenital regions, as well as in the upper gastrointestinal tract organs (Somvanshi, 2011; Jana*et al.,* 2024). However, lesions are generally reported to occur in friction-prone areas of the skin that are more susceptible to physical abrasion and trauma, such as the head and neck regions (Araldi *et al.,* 2015). In the present study, lesions were detected in the head region in 2 animals, in the neck region in 13 animals, and in the udder region in 10 animals. The localization of 15 (60%) of the sampled

lesions in the head and neck regions was found to be consistent with the findings of previous studies. On the other hand, the sampled farms were observed to be small-scale enterprises employing traditional husbandry practices, in which animals were restrained using individual tethering equipment that continuously rubbed against and abraded the areas where lesions were observed. Moreover, overcrowding observed in some farms was considered highly important in terms of transmission, as infected animals were frequently in close contact with one another.

Several studies have been conducted regarding the macroscopic appearance of the lesions (Kumar*et al.,* 2013; Jangir and Somvanshi, 2021; Özmen and Kale, 2023). However, no association has been reported between the diversity of macroscopic appearances and the molecularly identified BPV types (Bertagnolli*et al.,* 2020; Alfaro-Mora*et al.,* 2023). Furthermore, there is also a study reporting that different BPV types can cause lesions with similar macroscopic appearances (Claus*et al.,* 2009). Although there are claims that the diversity in macroscopic appearance is due to co-infections with BPV types (Carvalho*et al.,* 2012; *Batista et al.,* 2013), and diversity may vary depending on tissue specificity, and this diversity is driven by viral genotypes (de Villiers*et al.,* 2004; Hatama*et al.,* 2008; Ozmen and Kale, 2023), no consensus has yet been reached on this issue. In the present study, 13 lesions were identified as typical, 5 as filiform, 4 as pedunculated, and 3 as atypical forms. The detection of BPV-12 in 95.83% of the molecularly positive lesions, together with the presence of multiple macroscopic appearances, indicated that lesion morphology is not determined solely by viral types and that no association was found between lesion appearance and the co-infections detected in 19 of the 24 positive samples.

MY11/09 and FAP59/64 are among the primer sets most frequently used in the molecular diagnosis of papillomaviruses (Dagalp*et al.,* 2017; Özmen and Kale, 2023. In the present study, none of the samples obtained from 25 animals with papilloma lesions located in different body regions tested positive with the FAP59/64 primer set, whereas 2 samples were identified as positive using the MY11/09 primer set. In the analysis of the positive samples using type-specific BPV primers, BPV-1 and BPV-12 were detected in one sample, whereas only BPV-12 was identified in the other positive sample. According to the type-specific PCR analysis, positivity was detected in 95.6% of the 23 samples that had tested negative with the consensus primers. Previous studies have similarly reported that type-specific primers are more sensitive than consensus primers (Grindatto*et al.,* 2015; Özmen and Kale, 2023). However, it is evident that the sensitivity of primer designs should be reconsidered and further optimized.

In this study, BPV positivity was detected in 24 (96%) of the 25 papilloma lesion samples using seven type-specific primer sets (excluding BPV-7). In addition, BPV-3, BPV-4, BPV-5, and BPV-11 types were not detected in any of the samples. The positively identified types were BPV-1, BPV-2, BPV-6, BPV-8, BPV-9, BPV-10, and BPV-12. In the overall distribution, including both single and co-infected lesions, the prevalence rates were determined as follows: BPV-12, 95.83%; BPV-2, 58.33%; BPV6, 20.83%; BPV-1, 16.67%; BPV-8, 12.50%; BPV-9, 8.33%; and BPV-10, 4.17%. The detection of BPV-1 and BPV-2 in the present study is particularly noteworthy, as these genotypes have previously been identified in urinary bladder carcinomas (Ropert*et al.,* 2010; de Alcântara*et al.,* 2021; de Falco*et al.,* 2024). Furthermore, it is noteworthy that the immunity developed following infection in animals is type specific(Jarrett *et al.,* 1990), while the level of viral diversity remains remarkably high. Viral typing studies may contribute epidemiologically to the identification of circulating types among cattle, the detection of potential novel types, and the development of viral vaccines and therapeutic strategies.

There are no definitive data identifying the most frequently detected BPV genotype across cattle populations worldwide. However, BPV-1 and BPV-2 are generally the most frequently detected genotypes in cattle. (Dagalp*et al.,* 2017; Bertagnolli*et al.,* 2020; Kale*et al.,* 2023; Atlı*et al.,* 2025). Among the samples analyzed in the present study, the most frequently detected genotypes were BPV-12 (95.83%) and BPV-2 (58.33%). Compared with previous studies conducted worldwide and in Türkiye, this study is the first to identify BPV-12 as the most frequently detected genotype, including in co-infected lesions..Previous studies have reported BPV-12 in cutaneous papillomas and teat papillomas (das Chagas*et al.,* 2021; Özmen and Kale, 2023). At the same time, while only BPV-1, BPV-2, and BPV-13 had previously been reported to cross the species barrier and infect horses (Nasir and Campo, 2008; Lunardi*et al.,* 2013),a recent study identified BPV-12 in a papilloma lesion obtained from the perianal region of a horse (Orta*et al.,* 2023). This finding suggests that BPV-12 may also possess the potential to infect different host species. These findings suggest that the geographical distribution, host range, and prevalence of BPV-12 may be considerably greater than currently recognized.

In the present study, BPV-1, BPV-2, and BPV-12 were more frequently identified in lesions located in the head and neck regions, including co-infected lesions, whereas BPV-2, BPV-6, BPV-8, BPV-9, BPV-10, and BPV-12 were more commonly detected in udder and teat lesions, including co-infected cases. The lower viral diversity observed in papilloma lesions of the head and neck regions compared with those of the udder and teat regions may be associated with the fact that the majority of the examined cattle were animals used for milk production. The greater exposure of the udder and teat tissues to mechanical irritation during milking, together with environmental factors such as milking hygiene, may have contributed to this difference. Furthermore, the identification of BPV-1 and BPV-2 in the present study, which have been associated in the literature with urinary bladder carcinomas and upper gastrointestinal tract neoplasms, suggests that the presence of these types may be of considerable importance in terms of disease-related economic losses and mortality.

Previous studies on the disease have reported co-infections in which multiple BPV types were detected simultaneously within a single papilloma lesion (Yaguiu*et al.,* 2006; Santos*et al.,* 2016; Sauthier*et al.,* 2021; Taşatan and Kale, 2024). In the present study, co-infection was detected in 24 samples that tested positive, with two types

in 50%, three types in 20.83%, one type in 20.83%, and four types in 8.33%. Among all lesions, co-infected lesions constituted 79.16% of the sampled cases. Previous studies have reported that it remains unclear whether the detected BPV types are transcriptionally active; however, these types were found to be present in the lesions at low copy numbers (Freitas*et al.,* 2011). Although there are suggestions that co-infections may be a consequence of immunosuppression (Batista*et al.,* 2013; das Chagas*et al.,* 2021) and that viral diversity and co-infections may contribute to lesion persistence, as proposed by Grindatto*et al.,* (2015), these hypotheses have not yet been conclusively demonstrated. Santos*et al.* (2016) reported that BPV types or co-infections were not associated with the clinical status, age, sex, or immunological condition of the animals. Similarly, in the present study, no statistically significant association was observed between lesion morphology, anatomical location, and the detected BPV genotypes ($P>0.05$). However, considering the predefined sampling strategy and the limited number of molecularly analyzed lesions, these findings should be interpreted within the scope of the present study. Furthermore, it should be noted that the absence of sequencing confirmation constitutes a limitation of the present study.

Conclusions: In conclusion, our study provides general epidemiological information regarding BPV infection in Türkiye, which may be highly valuable for conducting broader epidemiological investigations in the future. The genotypes detected in this study demonstrate the presence of these BPV types within the investigated cattle population. Moreover, the identification of BPV-12 as the most frequently detected genotype across herds for the first time highlights the need for further studies investigating BPV infection–associated risk factors and the epidemiology of viral genotypes. Nevertheless, sequencing and phylogenetic analyses are planned as part of our ongoing research to further confirm the molecular identification of the detected BPV genotypes and to investigate their genetic diversity. Furthermore, considering the involvement of certain BPV types in carcinogenesis, the identification of additional internal and external cofactors influencing this process may contribute to the development of novel therapeutic approaches.

Conflict of interests statement: Authors declares that there are no conflicts of interest related to the publication of this article.

Financial disclosure statement: This study was financed by the project number 23212019 supported by the Selcuk University Scientific Research Projects Unit.

Animal rights statement: Selcuk University Faculty of Veterinary Medicine, Animal Experiments Ethics Committee 27.04.2023, 2023/05 Number Ethics Committee Decision

Authors contribution: YK: research, sampling, data analysis, writing – original draft preparation and editing; OB and MK: writing- review and editing, project design and consulting.

Acknowledgement: Part of this study was derived from a doctoral dissertation titled “Molecular Diagnosis of Bovine Papillomavirus-Associated Warts in the Konya Region,” completed in 2025.

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