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Molecular Characterization of the Presence of Bovine Papillomavirus in Cattle Teat Warts and a Different Treatment Approach

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ABSTRACT

Background: Papillomatosis is a skin condition that is distinguished by benign proliferative tumors with a complicated etiology and epithelial proliferation. There is a need to perform a more thorough study on bovine papillomavirus and identify the virological properties of the agent in detail since a definitive treatment approach against this infection has not yet been completely identified and the suggested treatment outcomes differ.

Methods: Samples were collected from warts developing on the teats of 500 cattle raised for milk production in Burdur Center and its districts. The samples were defrosted in order to extract DNA and the Dneasy Blood and Tissue Kit was used for the extraction procedure. By using a PCR assay, wart sample extracts were examined for 13 different BPV types (excluding BPV Type 7). For BPV type-specific primers, a protocol was utilized. Amplification products were demonstrated using agarose gel electrophoresis.

Result: In the PCR test of 500 wart samples, BPV types (type 1-type 13) were detected in 378 (75.6%). In the general distribution of BPV types (single or mixed types) in teat warts, BPV-2 (n=85; 22.49%), BPV-8 (n=45; 11.90%), BPV-9 (n=48; 12.70%) and BPV-10 (n=52; 13.76%) were determined to be more common. In the study; it was determined that three different combined treatments provided 100% regression or complete recovery in warts developing on the teat of cattle. In conclusion; one of these three treatment combinations may be preferred for teat wart lesions in cattle.

Key words: Bovine papillomavirus, Molecular virology, Teat wart, Treatment.

INTRODUCTION

Papillomatosis; Papovaviridae, found in all mammals and birds It is a disease caused by members of the PV family. Cattle are the most affected domestic animals (Campo, 2006). BPVs have been classified into five genera and one unclassified genus. These are: BPV-1, BPV-2, BPV-13 and BPV-14 in the Deltapapillomavirus genus; BPV-5 and BPV-8 in the Epsilonpapillomavirus genus; BPV-7 in the Dyoxipapillomavirus genus; BPV-16, BPV-18, BPV-22 and Xipapillomavirus in the Yokappapillomavirus genus. BPV-3, BPV-4, BPV-6, BPV-9, BPV-10, BPV-11, BPV-12, BPV-15 and BPV-23 in the genus and BPV-17, BPV-19 and BPV-20 in the unclassified group. BPV-21 and BPV-24 types (Araldi *et al*., 2017; Daudt *et al*., 2016; Knipe and Howley, 2013).

Anthropogenic activities, changes in farming systems and environmental changes all contribute to the development, reemergence and geographical spread of infectious illnesses (Kumar *et al.,* 2023). Among many diseases that cause loss of productivity in meat and dairy farming, papillomatosis cases have an important place. Papillomatosis is a disease characterized by epithelial proliferation in the skin and benign proliferative tumors with a complex pathogenesis and etiology. Papillomas are benign tumors, but sometimes they can transform into malignant epithelial tumors and cause significant disorders (Araldi *et al*., 2017). Bovine papillomavirus (BPV); Mastitis reduces milk output and quality, which results in significant financial losses for milk producers (Safak *et al*., 2023). Mastitis causes economic losses due to decreased milk yield, blunting of the teats, inability of the teats to enter the milking machines due to deformities and inability to milk due to pain (Atasever *et al*., 2005, Yıldırım *et al*., 2022; Özmen and Kale, 2023).

In research, BPV has been detected in blood as well as in papilloma tissues. It has also been stated that it can be transmitted through milk, urine, semen and uterine lavages and vertical transmission has been observed. The presence of viral genomes was also found in the offspring's waste and blood (Bocaneti *et al*., 2016; Lindsey *et al*., 2009). BPV can be transmitted from animal to animal through direct skin contact; Contaminated feed and materials,

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milking machines, ear tagging, procedures, castration devices, tuberculin injections, heredity, malnutrition, hormonal imbalance and mutation and arthropod vectors also indirectly play an important factor in the spread of the disease (Atasever *et al*., 2005). In addition to negatively affecting animal health, BPV has also been reported in recent years to show that it can be transmitted from animals to humans due to its zoonotic nature (Gallina *et al*., 2020). The polymerase chain reaction (PCR) test in the diagnosis of BPV infection is the most widely used method for detecting viral DNA in blood and tissue samples taken from BPV-infected animals (Ataseven *et al*., 2016; Tomita *et al*., 2008; Wobeser *et al*., 2012). Therefore, the conventional PCR method is preferred (Guo *et al*., 2012; Melo *et al*., 2014). Since no definitive treatment method has been defined against BPV infections, different treatment methods are applied for BPV infections.

In this study, BPV types of warts developing on the teats of cattle were determined by PCR tests without classifying macroscopic appearance, virus type, or papilloma/fibropapilloma and the results were evaluated by applying three different combined treatment methods to these animals.

Materials and Methods

Animals

All samples were collected from warts developing on the teats of 500 cattle (Holstein, Simmental and Native Breed) raised for milk production in Burdur-Center and its districts between June 2022 and September 2023. Following the application of a surgical ligature using 0/2 silk thread to the areas where the wart samples meet the normal mucosa, the broken tissue piece was taken and sent to the Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Virology Laboratory for diagnostic purposes. Wart samples were collected and kept at -20°C until extraction.

Extraction and PCR

The samples were frozen for DNA extraction, which was done using the Dneasy Blood and Tissue Kit (Qiagen). Extracts of wart samples were evaluated for 13 types of BPV (except BPV Type-7) by PCR. The procedure of Silva *et al*. (2016) was applied to BPV, specific primers (Table 1). PCR reaction conditions were initial denaturation at 94°C for 3 min, (denaturation at 94°C for 60 s, 35 cycles of 60-68°C annealing, extension at 72°C for 60 s) and final extension at 72°C for 3 min (Thermo). Using ethidium bromide dye and Tris-acetate buffer (2%) agarose gel electrophoresis, amplification products were demonstrated.

Treatment

Three trial groups were established without classification of macroscopic appearance, virus type, or papilloma or fibropapilloma of warts. These trial groups were selected from animals that were positive as a result of the PCR test. The randomization of the treatment groups, consisting of 85 animals each, was determined by lot. Animals with positive PCR test results who were not selected for treatment groups were also determined as the control group. Among the treatment methods compared, PAPILEND™,® cream (Almer Kimya İlaç San.Tic.Ltd.Şti., Ankara, Turkey), ivermectin once daily for 10 days (Zolimectin®, Zolenat İlaç A.Ş., İstanbul, Turkey), levamizol (Levatek® %10, Teknovet İlaç San. Tic., İstanbul, Turkey) and autohemotherapy (right-left scapula bone area; total 20 mL) subcutaneously total of 2 times with an interval of 15 days and Alquermold™ premix powder (Biovet, S.A., 25 Poligono Industrial, Tarragona, Spain) was determined as 150 grams once a day for 10 days. The treatment protocol is given in Table 2. No treatment was applied to the animals in the control group.

Results and Discussion

As a result of the PCR test of 500 animals included in the study, 378 (75.6%) were found to be positive and 122 (24.4%) were negative. 122 (24.4%) animals that were found to be negative were considered the control group in the study (Table 3).

In the general distribution of BPV types (single or mixed types) in teat warts; BPV-2 (n=85; 22.49%), BPV-8 (n=45; 11.90%), BPV-9 (n=48; 12.70%) and BPV-10 (n=52; 13.76%) were determined to be more common (Table 4).

In the study; it was determined that three different combined treatments provided 100% regression or complete recovery in warts developing on the teat of cattle (without classification of macroscopic appearance, virus type, or papilloma/fibropapilloma) (Fig 1, Fig 2, Fig 3). No recurrence of warts was detected in all treatment groups during the six-month period.

The most important function of the teat is to ensure the discharge of produced milk. Its meaning for the calf is that it is the place where milk is absorbed. Mammary papillomatosis cases are one of the most important diseases affecting cattle health and lesions that occur, especially on the udder and teat, cause significant financial losses in the dairy sector.

In this study, BPV types (type 1-type 13) were detected in 378 (75.6%) of 500 wart samples in the PCR test. In the general distribution of BPV types (single or mixed types) in teat warts; BPV-2 (n=85; 22.49%), BPV-8 (n=45; 11.90%), BPV-9 (n=48; 12.70%) and BPV-10 (n=52; 13.76%) were determined to be more common. Jana (2015) emphasized that BPV 1-2 are the viral agents in udder and cutaneous papillomas. As a result of the studies, it has been stated that BPV-6 and BPV-9 are common and responsible for udder papillomatosis (Hatama *et al*., 2009). Rai *et al*. (2011) reported that other types were not detected in the udders of cows in a dairy farm in India and only the presence of BPV-10 was identified. Dağalp *et al*. (2017) explained that BPV-6-7-9-10 are frequently encountered in udder papillomas. Özmen and Kale (2023) determined the highest levels of

Table 1: Spesific primers for BPV.

Primers	Sequences	Fragment size (bp)	Amplified region (gene)
BPV-1	F-5′ GGA GCG CCT GCT AAC TAT AGG 3′	301	L1
	R-5′ ATC TGT TGT TTG GGT GGT GAC 3′		
BPV-2	F-5′ GTT ATA CCA CCC AAA GAA GAC CCT 3′	164	L1
	R-5′ CTG GTT GCA ACA GCT CTC TTT CTC 3′		
BPV-3	F-5′ CAG TCA ATT GCA ACT AGA TGC C 3′	216	L1
	R-5′ GGC TGC TAC TTT CAA AAG TGA 3′		
BPV-4	F-5′ GCT GAC CTT CCA GTC TTA AT 3′	170	E7
	R-5′ CAG TTT CAA TCT CCT CTT CA 3′		
BPV-5	F-5′ GGC ATG TAG AGG AAT ATA AGC 3′	262	L1
	R-5′ TTC TCT GAG ATC AAT ATT CC 3′		
BPV-6	F-5′ TTA GAG ACC TGG AAC TTG GG 3′	294	L1
	R-5′ TAC GCT TTG GCG CTT TTT TGC 3′		
BPV-8	F-5′ TAG AGG ACA CAT ACC GCT TCC AAA GC 3′	196	L1
	R-5′ TTT GCG AGC ACT GCA GGT GAT CCC 3′		
BPV-9	F-5′ AAA GAG CAA ATC GGG AGC ACC 3′	264	L1
	R-5′ AAC TAA TGA CCC ACT AGG GCT CC 3′		
BPV-10	F-5′ AAG GCA TTT GTG GTC TCG AGG 3′	148	L1
	R-5′ CTA AAG AAC CAC TTG GAG TGC C3′		
BPV-11	F-5′ TGC AGA CAC TCA ACC AGG AG 3′	197	L1
	R-5′ CCA TAA GGG TCG TTG CTC AT 3′		
BPV-12	F-5′ AAA GCT GAA CCA TGC AAA CC 3′	159	L1
	R-5′ TAA CAA TGT CAA GGG GCA CA 3′		
BPV-13	F-5′ CCA ACC CCA GTA AGC AAG GT 3′	288	L1
	R-5′ AAG AGG TTG ACC TCG GGA GA 3′		
BPV-13	F-5′ CAC TGC CAT TTG GTG TTC TT 3′	153	E5
	R-5′ AGC AGT CAA AAT GAT CCC AA 3′		

Table 2: Applied treatment methods.

Treatments	Cream	Immunostimulants	Vitamin-premix powder
Treatment I	PAPILEND™,® Cream	Ivermectin (1 cc/50 kg body weight)	Alquermold™
Treatment II	PAPILEND™,® Cream	Levamizol (1 cc/50 kg body weight)	Alquermold™
Treatment III	PAPILEND™,® Cream	Autohemotherapy	Alquermold™

Table 3: PCR (Type-spesific primers) test results.

Papilloma tissue	Positive (%)	Negative (%)	Total
BPV PCR	378 (75.6%)	122 (24.4%)	500

BPV-2, BPV-9 and BPV-10 in single types of udder papillomas. When Özmen and Kale (2023) determining the general distribution, single and mixed types are seen together in the mammary warts of Holstein breed dairy cattle; BPV-2 (37.7%), BPV-6 (26.4%), BPV-8 (28.3%), BPV-9 (34%), they determined that BPV-10 (30.3%) and BPV-12 (20.8%) were more common.

In teat papillomas where mixed types are seen; Lindholm *et al*. (1984) detected 28% BPV-1, 89% BPV-5 and 92% BPV-6. Another group of researchers (Borzacchiello and Roperto, 2008; Tozato *et al*., 2013) identified BPV-1, BPV-5, BPV-6, BPV-9 and BPV-10 in the udder and teats. Ataseven *et al*. (2016) detected BPV-1, BPV-4, BPV-8, BPV-6 and BPV-9. Lunardi *et al*. (2016) found BPV-6 45%, BPV-7 5%, BPV-8 2.5%, BPV-9 5% and BPV-10 32.5%. Ogawa *et al*. (2004) BPV-1, BPV-3, BPV-5. Savini *et al*. (2016) found 52.3% BPV-7, 38.6% BPV-9 and 59.1% BPV-10. Jana (2015) BPV-1, BPV-2, BPV-5, BPV-9 and BPV-10. Bianchi *et al*. (2020) detected BPV-4, BPV-6, BPV-7, BPV-8, BPV-9, BPV-10, BPV-11, BPV-12.

In the study; PAPILEND™,® cream, used in three different combined treatment applications for warts developing on the teat of cattle (without classification of macroscopic appearance, virus type and papilloma/fibropapilloma) is a skin care product consisting of glacial acetic acid, salicylic acid and herbal oils, developed to softenthe wart-like formations seen in cattle and reduce their disturbing effects by hardening (Yigitarslan *et al*., 2023).

The product composition; It comprises salicylic acid, glacial acetic acid, garlic oil, glyceryl monostearate, tea tree oil, podophyllum, cetyl stearyl alcohol, stearic acid, water and hydrogenated castor oil. As main ingredients; they stated that tea tree oil (Melaleuca alternifolia) has antiviral, antifungal and antiseptic properties (monoterpenes terpinen-4-ol, γ-terpinene and linalool) (Alsanad and Alkhamees, 2016). They explained that garlic oil (Allium sativum) has antiviral, anti-inflammatory effects, increases natural killer cells and cellular immunity and that its high allicin substance has anti-DNA activity (Mousavi *et al*., 2018). Apart from these, podophyllum has an antimitotic effect (Rivera and Tyring, 2004), glacial acetic acid has a chemical coagulation of 80-90% of proteins in wart tissues, salicylic acid cleans infected tissues without damaging the epidermis layer and glyceryl monostearate has a stabilizing effect. It has been stated that stearic acid acts as an emulsifier and cetyl stearyl alcohol and hydrogenated castor oil have viscosity increasing properties (Pekcan, 2014; Tırnaksız *et al*., 2015).

BPV is not a good immunogen for the mammalian organism and since it does not cause inflammation, it cannot create a signal to stimulate the immune system (except for the formation of local cellular immunity) regarding the presence of the virus. For this reason, the main goals are the development of neutralizing antibodies against the virus, stimulation of cellular immunity, destruction of infected cells that produce early proteins and introducing the virus to keratinocytes (Campo and Roden, 2010; Bocaneti *et al*., 2016). For this purpose, to stimulate the immune system, Ivermectin, Levamisole and autohemotherapy applications that stimulate the immune system were carried out. Ivermectin has a positive effect on the cellular immune response (T-cell cytotoxicity, natural killer cell cytotoxicity, macrophage activation) of animals with papilloma lesions (Uhlir and Volf, 1992; Nicholls and Stanley, 2000; Campo, 2006). As a matter of fact, ivermectin, used in the treatment of bovine papillomas, increased serum TNF-α levels synthesized by macrophages, endothelial cell and T lymphocytes and IL-6 levels secreted by monocytes, macrophages and T lymphocytes on the 7th day, indicating that ivermectin has an activating role on the immune system, especially T lymphocytes (Bekdur *et al*., 2018). Ivermectin has a cytotoxic effect on papilloma carcinogenic cells (Kim *et al*., 1992). Levamisole is the tetramisole isomer and has been used for viral diseases, tumor treatments and as an immunomodulator in humans and animals for more than 20 years (Miwa and Orita, 1978; Brunner and Muscoplat, 1980; Scheinfeld *et al*., 2004). It has been explained that it is used as a non-specific immune stimulant in many diseases (Ganguly, 2014). It causes an increase in macrophages after blood is taken from the jugular vein and injected directly into the muscle (Lopes *et al*., 2021). While the standard macrophage rate in the blood is 5%, it has been reported that this rate can increase to 20-22% about 8 hours after application (Teixeira, 1940; Ottobelli *et al*., 2016). It is explained that autohemotherapy stimulates RES and increases macrophages in circulating blood (Namgyel *et al*., 2021). It has been reported that autohemotherapy is a complementary treatment method that provides 90% success in the treatment of papillomas in cattle (Jana and Jana, 2009; Jana, 2015). According to Orta *et al.* (2023); Alquermold™ premix powder, which contains

Table 4: General view of single or mixed types.

BPV types	Quantity (n)	Percentage (%)
BPV-1	12	3.17
BPV-2	85	22.49
BPV-3	8	2.12
BPV-4	11	2.91
BPV-5	13	3.44
BPV-6	35	9.26
BPV-8	45	11.90
BPV-9	48	12.70
BPV-10	52	13.76
BPV-11	28	7.40
BPV-12	33	8.73
BPV-13	8	2.12

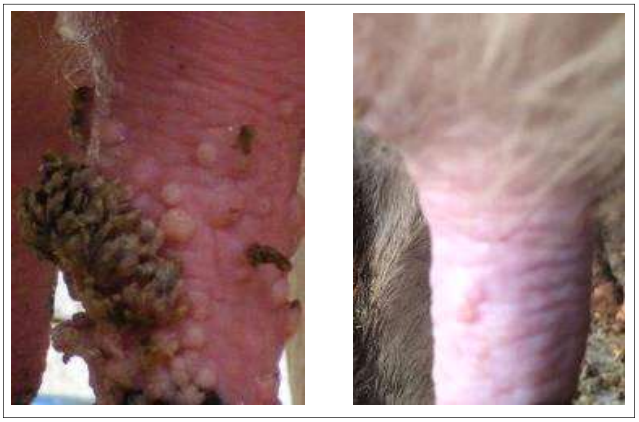

Fig 1: Results of treatment I (A: Before; B: After).

Fig 2: Results of Treatment II (A: Before; B: After).

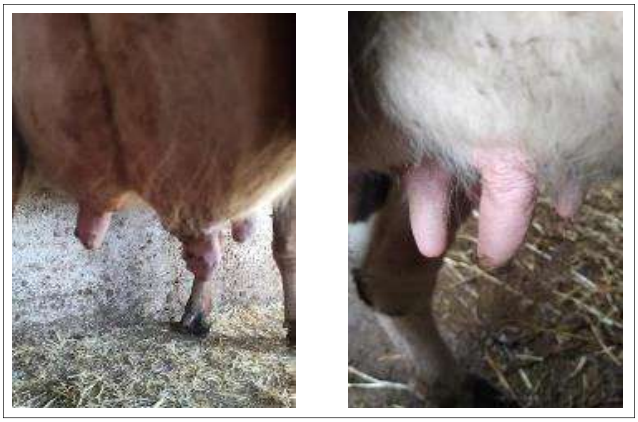

Fig 3: Results of treatment III (A: Before; B: After).

vitamin E, selenium, copper and zinc, was given to sick animals as part of the study in order to boost their resistance to illness (Spears and Weiss, 2008). The main source of vitamin E is food (Baldi *et al*., 2000). The amount of vitamin E diminishes when prepared feed is processed or kept for an extended period of time. Furthermore, grazing may prevent them from getting enough vitamin E from plants (Baldi, 2005). Selenium levels in the soil or in plants may not be adequate (Sharma *et al*., 1983). Selenium is a vital component of the antioxidant defense mechanism of the organism (Kieliszek and Błażejak, 2016). Selenium and vitamin E have a synergistic impact (Willshire and Payne, 2011). Zinc is essential for keratin synthesis (Tomlinson *et al*., 2004). Copper and zinc are crucial for fighting microbes and boosting the immune system (Spears, 2000). Regardless of the macroscopic appearance, virus type and papilloma/fibropapilloma classification of warts developing on the teat of cattle, successful results were obtained as a result of the combined use of PAPILEND cream + immune stimulating drugs + vitamin/premix powders containing vitamin E, zinc, selenium and copper.

Conclusion

Regardless of the macroscopic appearance, virus type and papilloma/fibropapilloma classification of warts developing on the teat of cattle, successful results were obtained as a result of the combined use of PAPILEND™,® cream + immune stimulating drugs or immune system stimulating applications (autohematherapy) + vitamin/premix powders containing vitamin E, zinc, selenium and copper.

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Conflict of interest

All authors declared that they have no conflict of interest.

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