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# Clinical Efficacy of Bleomycin in Suspected Canine Papillomatosis: Case Report of Two Sibling Puppies

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**Abstract:** The objective of this case report was to assess the clinical efficacy of anti-tumoral bleomycin on canine papillomatosis (CP) in two male Pug breed sibling puppies. Two 8-month-old, male sibling puppies were presented with multiple warts in the lip and chin. A suspected diagnosis of CP was established through only clinical examination with appearance of typical cauliflower-like warts. Bleomycin was subcutaneously administered to both siblings on a weekly by 0.5 IU/kg dosage. At week 6, one of the siblings exhibited complete regression of oral lesions, whereas the other sibling achieved complete healing by week 9. The warts healed completely, permanently, without new lesions. No adverse effects were observed, verified through weekly blood count and physical examination during and post-treatment. Subcutaneous administration of bleomycin at weekly dose of 0.5 IU/kg contributed to the regression of oral lesions and improved clinical outcomes in dogs, suggesting potential efficiency in the treatment of CP.

**Keywords:** Bleomycin, Oral lesion, Papilloma, Warts.

# Şüpheli Canine Papillamatoziste Bleomisinin Klinik Etkinliği: İki Yavru Kardeş Köpeğin Olgu Sunumu

**Özet:** Bu olgu sunumu ile anti-tümoral bleomisinin iki erkek, Pug ırkı yavru kardeş köpekte canine papillamatozis (CP) üzerine klinik etkinliğinin değerlendirilmesi amaçlandı. İki 8 aylık yaşta, erkek yavru kardeş köpek ağız ve çene bölgesinde birden fazla siğil lezyonu ile başvuruda bulundu. Şüpheli canine papillamatozis tanısı yalnızca tipik karnıbahar benzeri siğil görüntüsünü içeren klinik bulgular ile konuldu. Her iki kardeşte bleomisin haftalık 0.5 IU/kg dozda deri altı yolla uygulandı. 6. haftada kardeşlerden birinde oral lezyonlar tamamen kaybolurken diğer kardeşte tam iyileşme 9. haftada sağlandı. Siğiller yeni lezyon olmaksızın kalıcı olarak tamamen iyileşti. Haftalık kan sayımı ve fiziksel muayene bulgularıyla takip yapıldı ve tedavi süresince ve sonrasında hiçbir yan etki görülmedi. Deri altı yolla 0.5 IU/kg dozda haftalık uygulanan bleomisinin köpeklerde oral lezyonların gerilemesine ve klinik sonuçların iyileşmesine katkıda bulunması, CP tedavisinde potansiyel etkinliğini desteklemektedir.

**Anahtar Kelimeler:** Bleomisin, Oral lezyon, Papilloma, Siğiller

## 1. Introduction

Papilloma is a benign growth of squamous epithelial tissue that occurs due to an infection with papillomavirus. This virus, a double-stranded DNA virus lacking an envelope, has a predilection for mucous membranes and skin in both humans and animals (1). Currently, there are 24 known types of canine papillomaviruses (CPVs), most of which are linked to both mucosal and skin lesions (2). These papillomaviruses exhibit a preference for various organs, with the majority affecting the skin (3). Over time, CPVs have traditionally related to oral-skin-inverted or pigmented plaque papillomatosis in dogs (2). On rare occasions, these viruses have been associated with the development of oral and skin squamous cell carcinomas, occurring in cases of immune suppression (4). Puppies, elderly dogs, and dogs with impaired immune systems are particularly vulnerable to infection (5).

Oral papillomatosis is frequently not requiring treatment and tends to resolve on its own within a period of 3 to 12 months. However, the rapid development and spread of lesions can be occurred in the vulnerable dogs. In these dogs the risk of infection may occur when these growths become massive or appear in challenging regions with picking, chewing and swallowing (6).

There are numerous therapy modalities for the treatment of canine papillomatosis (CP), but the majority have not undergone adequate evaluation (7). The treatment often involves a comprehensive approach that combines various

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methods including surgery, vaccines, and immunotherapy. Surgical interventions may encompass the use of electrocautery, scalpels, lasers, or cryosurgery (8,9). Immunomodulatory therapy might be explored as an option when the patient's immune system is compromised (10-14). In cases of persistent CP that require additional treatment, either due to medical concerns or the pet owner's aesthetic preferences, excisional biopsy or electrocautery is frequently the preferred course of action.

Intralesional bleomycin has been used to treatment of human papillomatosis with great effectiveness, even at low concentrations. Bleomycin has an anti-neoplastic effect and cause acute necrosis in warts with impairing DNA synthesis (12,15,16). Therefore, the objective of the current study was to assess the clinical efficacy of anti-tumoral bleomycin on CP in two sibling puppies.

## 2. Case Report

Two sibling puppies, an 8-month-old, male and 4-year-old Mother Pug were presented to the private Veterinarian Clinics with history of multiple warts in the lip and chin. The owner stated that the mother had long-standing these warts which was being tried to cure with different administrations (autologous vaccine, azithromycin and surgical removing) however the lesions never regressed and progressively exacerbated (Figure 1). Similarly, the lesions were first noticed approximately 3–4 weeks prior in sibling puppies and they were firstly represented to the veterinarian clinic. The owner did not accept the histopathological evaluation and treatment, so applied to another private clinic. One month later, the owner returned to the clinic with the puppies and was reported that the mother had been euthanized elsewhere.

**Figure 1:** The unhealed and severe papillomatosis observed in euthanized mother.

Clinical examinations of both puppies were performed before treatment. One puppy had only three small, separated warts present along the lateral aspect of the right lower lip and the middle line of mandible (Figure 2a), other puppy had more severe and bigger multiple warts on the same location with sibling (Figure 3a). A suspected diagnosis of CP was established through only clinical examination. It is important to mention that, apart from oral lesions, no other dermatological abnormalities were observed in siblings.

Upon obtaining consent from the owner, both siblings were initiated on a weekly subcutaneous administration of 0.5 IU/kg bleomycin (®Bleocin-S - 15mg, Onko Koçsel, Istanbul, Türkiye) diluted with 0.9% NaCl. By the week 4 of treatment, despite a reduction in the size of the lesions, an increase in their number was observed (Figure 2b). However, complete resolution was recorded in the following week (Figure 2c). Subsequently, treatment was discontinued after the sixth application for this sibling.

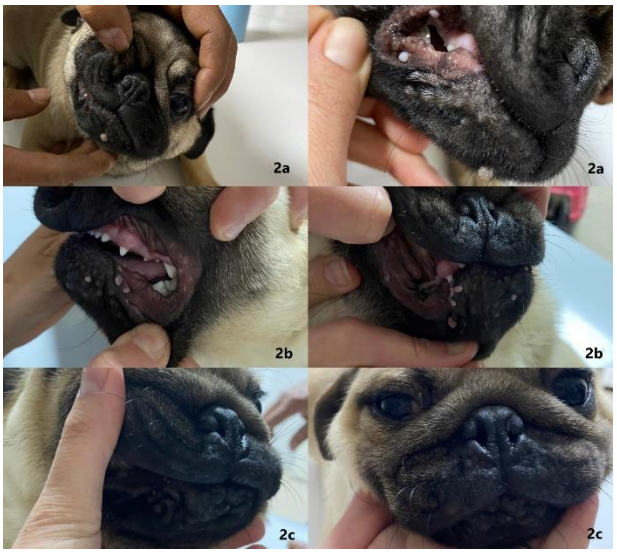


**Figure 2: 2a)** Varied size of papilloma, small smooth papules present along the lateral aspect of the right lower lip and one cauliflower-like papilloma in the middle line of mandible before treatment, **2b)** Multiple small sized papilloma by the week 4 bleomycin application, **2c)** Complete resolution of lesions by the week 5 of treatment

The other sibling had multiple lesions that persisted for eight weeks without improvement and suddenly complete resolution of the lesions without the reduced size of one wart located in the middle line of the mandible was recorded by the week 9 of bleomycin application (Figure 3b).

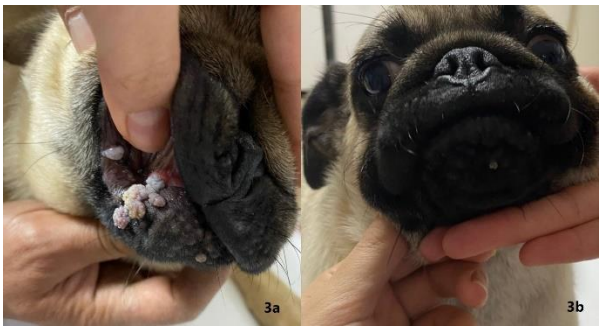


**Figure 3:** Cauliflower like appearance in the pre-administration of bleomycin (3a) and clinical improvement of

lesions without one more smaller size growth by the week 9 of treatment (3b) in the other sibling

As of 1-year post-treatment the warts continue to exhibit complete resolution, with no evidence of new lesions. No adverse effects were observed throughout the treatment and subsequent follow-up, as confirmed by weekly blood count (Tablo 1) and physical examinations.

**Tablo 1**: Pre-post treatment blood count of sibling

| Parameters | Pre-treatment | | Post-treatment |
|---|---|---|---|
| | Case 1 | Case 2 | Case 1 |
| **LYM ($10^9$/L)** | 2,8 | 0,2 | 1,6 |
| **MID ($10^9$/L)** | 1,3 | 0,5 | 0,8 |
| **GRAN ($10^9$/L)** | 10,1 | 9,6 | 8 |
| **RBC ($10^{12}$/L)** | 6,49 | 7,19 | 6,91 |
| **HGB (g/L)** | 158 | 171 | 164 |
| **HCT (%)** | 47,3 | 51,9 | 50 |
| **MCV (fL)** | 73 | 72,2 | 72,4 |
| **MCH (pg)** | 24,3 | 23,7 | 23,7 |
| **MCHC (g/L)** | 334 | 329 | 328 |
| **RDW-CV (%)** | 12,5 | 13,3 | 12 |
| **PLT ($10^9$/L)** | 342 | 474 | 456 |
| **MPV (fL)** | 8,2 | 6,8 | 7,3 |
| **PDW (fL)** | 10,5 | 8 | 8,6 |
| **PCT (%)** | 0,28 | 0,32 | 0,33 |

## 3. Discussion and Conclusion

In this case presentation, both siblings exhibited multiple warts in their lip and chin, clinically consistent with CP. Although spontaneous recovery was observed in these and similar cases, the reason why treatment was applied to the these puppies was the condition of their mothers, to be euthanized due to the severe and chronic process observed despite being exposed to multiple treatments, and the young age of the puppies. However, the owner's reluctance at the referral clinic to undergo histopathological evaluation resulted in a provisional diagnosis of suspected canine oral papillomatosis in these two sibling puppies. Therefore, to assess the clinical efficacy of bleomycin, known for its anti-tumoral activity in human papillomatosis, these two cases were investigated.

Canine oral papillomatosis often resolves spontaneously within 3 to 12 months without requiring treatment, yet susceptible dogs might experience rapid lesion development and spread (6,17). Several surgical and medical approaches are available for treating papillomatosis in dogs, and treatment options may vary depending on the frequency of recurrence, the immunity, and the owner's acceptance of surgical intervention due to aesthetic concerns (8,9). The ideal treatment option aims to eliminate or minimize lesions, preserve skin tissue and integrity, enhance immunity to better combat the disease, and provide lifelong immunity. Numerous therapeutic regimens are considered including the topical application of apple cider vinegar, broad bean wart, vaseline (18), *Thuja occidentalis* (19), imiquimod and 5-fluorouracil (20); oral application of levamisole (21), simethicone (22), alpha interferon (20), human recombinant interferon-alpha 2a (23), *T. occidentalis* (24), acyclovir (25), etretinate (20), azithromycin (10) and homeopathic combination (13); subcutaneous injection of *Tarantula cubensis* extract (26), feline recombinant interferon-omega (27), autogenous vaccine (28) and *T. occidentalis* (29); intramuscular injection of lithium antimony thiomalate (18), *Propionibacterium acnes* (30); intralesional application of alpha interferon (20); intravenous administration of vincristine sulfate, immunoregulin combination (28) and taurolidine (21).

Bleomycin, a cytotoxic agent belonging to the anti-tumor antibiotic subclass and produced from *Streptomyces verticillus* (31), is used in many cancer treatments due to its preferential binding to squamous cells, non-toxic DNA strand breakage/damage (12,33,34). Similarly, it can be administered intralesionally in the treatment of human papillomatosis (12,15,16). Therefore, in the present study, bleomycin was administered subcutaneously once a week in both puppies presented to the clinic with multiple warts on the lip and chin. In one puppy, the lesions were completely resolved after the 5th application, while the other puppy showed improvement after the 9th application. In a meta-analysis encompassing 14 studies involving 2657 patients with common warts (human papilloma), intralesional bleomycin was reported to be more effective than saline or

cryotherapy (32). It's reported that bleomycin on warts induces DNA oxidation by forming metallobleomycin complexes, especially with iron, generating reactive oxygen and causing single-strand and double-strand breaks in DNA between 3'-4' linkages. Furthermore, the activity in tissues is related to bleomycin hydrolase enzyme, also known as cysteine proteinase, and iron content (12).

Pneumonitis and associated pulmonary fibrosis are among the most commonly reported complications of subcutaneous administration of bleomycin in humans and animals (34,35). Gastrointestinal, dermatologic, renal and pulmonary side effects due to high doses of bleomycin have been reported in different experimental studies in dogs (35,36). Pain at the injection site, local swelling and tissue rejection are among the other side effects reported (37). Physical examinations and complete blood analyses were performed weekly on both long-term bleomycin-treated puppies. No symptoms or complications were observed during the treatment period and the following year. A predisposition to pulmonary toxicity has been reported in patients with bleomycin hydrolase deficiency, which detoxifies bleomycin in the lungs (38). It is also stated that high and cumulative doses pose a risk in the development of pulmonary fibrosis (34, 39). In this context, pulmonary side effects have been reported to occur due to the use of doses above 450 IU in adult (40). In the present study, the fact that no side effects were encountered after long-term bleomycin administration in both puppies is consistent with the fact that bleomycin, which is used as part of combined chemotherapy protocols, has lower myelotoxicity than other chemotherapeutic agents (40) and is also reported as a safe agent in combined chemotherapy (41).

In conclusion, in these cases report of suspected CP, subcutaneous administration of bleomycin at a dose of 0.5 IU/kg once a week resulted in regression of oral lesions and clinical improvement.

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