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CANINE PAPILLOMATOSIS: CLINICAL OUTCOME AFTER ORAL ISOTHERAPY, SUBCUTANEOUS *TARANTULA CUBENSIS* VENOM AND ORAL LEVAMISOLE-AZITHROMYCIN TREATMENT

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Summary

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The aim was to investigate the clinical outcome of isotherapy with homeopathy and medicated treatment on canine papillomatosis. Total of 30 dogs in Istanbul and Adana from 0.6 to 16 years of age were studied. Samples from warts 0.2–0.5 cm were taken without bleeding and stirred in 5 mL of ethyl alcohol (96%) for 4 min and kept +4 ^{o}C by intermittent shaking every 2 h for a day and left in the +4 ^{o}C for a night. Isotherapy and *Tarantula cubensis* venom each was used at groups of 10 dogs, levamisole + azithromycin was used in 6 dogs. The 4 dogs did not take medication. Diagnosis was based on clinical examination, confirmation was performed by histo-pathological examination and PCR analyses of warts. Nine out of 10 dogs (90%) after isotherapy with homeopathy were recovered, 3 of 10 (30 %) dogs with *Tarantula cubensis venom,* 2 of 6 dogs (33 %) with levamisole + azithromycin were recovered but none of 4 control dogs (0 %). Results indicated oral isotherapy supported by *Thuya occidentalis and silicea* was safe, very effective, easy to use and patient friendly for canine papillamatosis treatment. Azithromycin wasfound to be good to use on infected warts.

Key words: azithromycin, canine papillomatosis, homeopathy, isotherapy, levamisole, *Tarantula cubensis* venom

INTRODUCTION

Papilloma viruses in the *Papovaviridae* family are small, non-enveloped, double stranded DNA viruses. Benign skin tumours caused by the papillomaviruses in dogs are named canine papillomavirus (CPV). Characteristically they are small, cauliflower or solid shaped and rough growths termed warts. Warts contain large amounts of infectious virus which is relatively stable. Transmission between ani-

mals is common via, for example, fence posts or halters. Contaminated tattooing or tagging equipment is another common source of infection (Morter & Horstman, 1999). CPV warts generally occur on the lips, muzzle and skin of dogs and less commonly, on the eyelids and even the surface of the eye or between the toes. Warts usually occur in groups rather than solitary growth. Capsid formation known as a viral protein L1 of canine papilloma viruses. Since the viruses are non-enveloped they are stable in the environment. The duration of infection is very variable from one month to over a year and recurrence is possible in animals (Ogawa *et al.,* 2004; Lange *et al.,* 2011; Salib *et al.,* 2011).

In the absence of the effective treatment for canine papillomatosis, this study was performed to evaluate different treatment regimes in dogs.

MATERIAL AND METHODS

Study population and sampling

This study was performed after taking the client's consent. A total of 30 dogs were consulted in 2014 and 2016. Out of them, 27 were brought to private practitioners clinics, 1 dog to Cukurova University and 1 dog to Istanbul University Veterinary Medicine clinics and 1 dog to Beykoz shelter Istanbul. The age of dogs was from 0.6 to 16 years. Dogs were clinically examined and findings were recorded. Biopsy samples were taken from warts and examined by histopathology as well as PCR analysis in order to make definitive diagnosis as described previously (Ogawa *et al.,* 2004; Lange *et al.,* 2011; Salib *et al.,* 2011; Tan *et al.,* 2012).

Study groups for therapeutic protocols

- Group 1: Isotherapy combination with Thuya occidentalis 9DH and Silicea 7DH.

Preparation of warts for isotherapy: Samples from warts about 0.2 to 0.5 cm were taken without bleeding and put into 5mL of ethyl alcohol (96%). It was then stirred in a glass container for 4 min and kept in +4 ^{o}C by intermittent shaking every 2 hours for a day and stayed +4 ^{o}C for a night. The liquid was taken out for preparation of remedy for isotherapy. For this, 1 mL liquid was transferred into a glass tube containing 9 mL ethyl alcohol (20%) and submitted to vigorous stirring for 2 min to dynamise the composure in a 1DH approach. Same procedure was repeated 8 times and 9DH was obtained. One mL of composure was taken from the last preparation and 9 mL ethyl alcohol (96%) was added and stirred for 2 min. From this preparation, 7.5 mL was taken into brown coloured spray bottle and 42.5 mL drinking water was added. The total of 50 mL final solution was then stirred for 4 min and kept at room temperature until used for isotherapy.

Administration of isotherapy 9DH solution: The preparation was firstly shaken for 20 s before administration then sprayed twice into the mouth directly for the first 3 weeks which is the equivalent of 0.4 mL of mixture. Alternatively the preparation can be given with food which dogs eat eagerly. The administration protocol is given in Table 1.

Preparation of Thuya occidentalis 9DH: These remedies were bought in granulated form from by Boiron Laboratoires (Boiron, Lyon, France). Ten granules from *Thuya occidentalis* 9DH were put into 50 mL brown coloured spray bottle and 7.5 mL of ethyl alcohol (96%) and 42.5 mL water were added. The mixture

was shaken until the granules dissolved and kept at room temperature until use.

Group 1 (isotherapy with *Thuya occidentalis* 9DH and Silicea 7DH) consisted of 10 dogs. The isotherapeutic remedy of 9 DH and *Thuya occidentalis* 9DH solutions were firstly shaken for 20 s. before administration and the protocol is given in Table 1. Additionally at the start of isotherapy and *Thuya occidentalis* 9DH, 5 granules of the *Silicea* 7DH (Boiron, Lyon, France) was also given orally once a week for 4 weeks. The medication completely stopped at the end of the 15th week.

- *Group 2: Tarantula cubensis venom D6:* This group consisted of 10 dogs and all were injected subcutaneously with 2 mL/ *Tarantula cubensis* venom D6 per 10 kg body weight at 5-day intervals, 3 times. After the last injection, same dose was given after a week and after 3 weeks as prescribed by the producer.
- Group 3: *Oral levamisole and azithromycin combination.* This group consisted of 6 dogs. Combination of levamisole 2 mg/kg and azithromycin 10 mg/kg body weight was administered orally every 24 h for 10 days.
- Group 4: Control group. This group consisted of 4 dogs and did not receive any medication.

Histopathology

Biopsy samples from the warts were immediately fixed in 10% neutral buffered formalin for histo-pathological examination. After 24 h fixation the samples were embedded in paraffin blocks and 4–5 µm sections cut, stained with haematoxylin and eosin (HE) and examined under light microscopy.

PCR analyses of the CPV

A biopsy sample was taken from the warts of each dog. For the definitive diagnosis of CPV, PCR was performed as described by others (Lange *et al.,* 2011). Biopsy sampling for PCR was also planned in cases after healing in order to assess the affect of treatment on the presence/consistency of virus in affected tissue. Post sampling was entirely depended on the willingness of owner. Viral DNA was extracted using QIAamp DNA Mini Kit (Qiagen) as recommended by the manufacturer from 20 mg papilloma samples. The amount of DNA in the extracted material was measured using a NanoDrop spectrophotometer (NanoDrop 1000c, Thermo Scientific, Waltham, USA). The primers described by Lange *et al.* (2011). were used. For this, a 50 µL reaction mixture containing 6 µL of DNA (about 200 ng), 25 µL GoTaq® Colorless Master Mix (Promega M7133), 2 µL canPV F primer (5'-CCTTCCTGAWCCTAAYMAKT TTGC-3'; 10 pmol/µL), 2 µL FAP64 R primer (5'- CCWATATCWVHCATNT CNCCATC-3'; 10 pmol) and 15 µL nuclease-free water were prepared. The mixture was placed in a thermal cycler (Biorad, Chromo 4), and the polymerase was activated by incubation at 95 °C for 4 min. The mixture was then cycled at 94°C for 1 minute, at 50 °C for 1 min and 72 °C for 1 min for 45 cycles and final incubation of 72 °C for 10 min. The products of positive PCR reactions were visualised by agarose gel (1.5%) electrophoresis and sequenced to confirm the specificity of this assay.

RESULTS

Group 1: Oral isotherapy – 9 of 10 dogs (90 %) with oral isotherapy with home-

opathy were completely recovered and it has been observed that regression started by the 3rd week and was completed at the 8th week except for 1 dog which had diffused papilloma virus warts, and its recovery started at the 6th week and was completed by the 15th week. The oral isotherapeutic 9 DH and *Thuya occidentalis* 9 DH preparation was firstly shaken for 15–20 s before administration. It was sprayed twice into mouth directly. The 15-week administration protocol (Table 1) was followed strictly for fully recovery. No reinfection and new warts were observed in recovered dogs during the study (Fig. 1 and 2).

Table 1. The 15-week isotherapy administration protocol. It was followed strictly for fully recovery despite that the warts resolved quickly and healing occurred (1 puff is equal to 0.2 mL)

Duration	Dosage
3 weeks twice daily (BID)	2 puff (0.4 mL) morning 2 puff (0.4 mL), evening
3 more weeks once daily	2 puff (0.4 mL) morning only
3 more weeks, every other day	2 puff (0.4 mL)
3 more weeks, twice a week/morning	2 puff (0.4 mL)
3 more weeks, once a week/ morning	2 puff (0.4 mL)

Group 2: Subcutaneous injection of Tarantula cubensis venom extract – 3 of 10 (30 %) dogs were recovered. The regression started by the 3rd week and was completed at the 8th week. No reinfection and new warts has been observed at recovered dogs during the study (Fig. 3 and 4).

Group 3: Oral levamisole and azithromycin combination – 2 of 6 dogs (33%) with oral levamisole and azithromycin combination were recovered. The regression started at the 4th week and was completed at the 8th week. No reinfection and new warts has been observed at recovered dogs during the study.

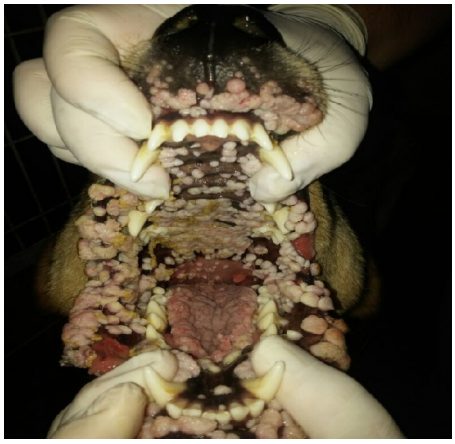

Fig. 1. Day 0, Pre-therapy.

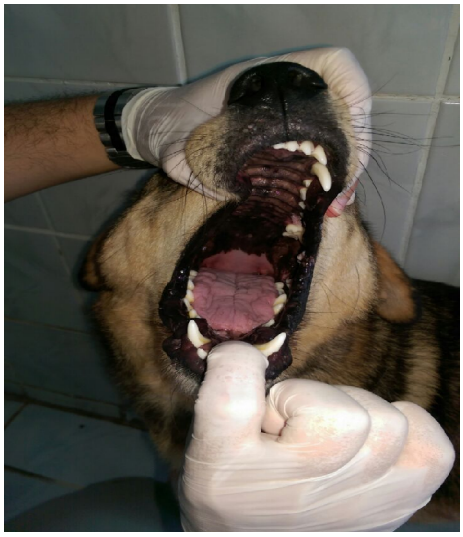

Fig. 2. Post therapy after 15 weeks.

Group 4: Control group – none of 4 untreated dogs (0 %) was recovered.

Results indicated that the oral isotherapy supported by *Thuya occidentalis and*

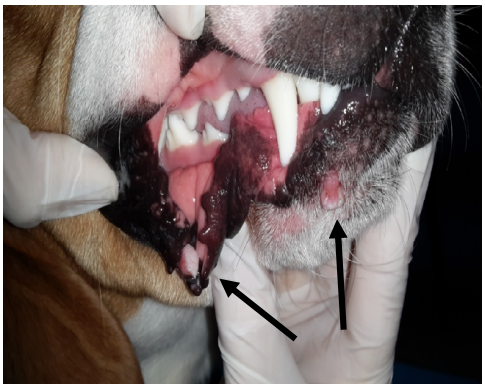

Fig. 3. Day 0, pretherapy.

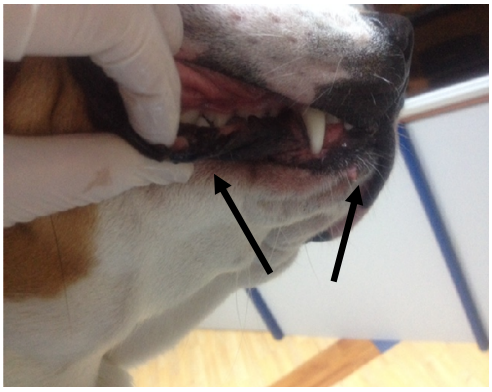

Fig. 4. Post therapy after the 5th week.

Silicea were safe, very effective, easy to use and patient friendly on canine papillomatosis treatment. Azithromycin was found to be good to use on infected warts. The combination of oral levamisole and azithromycin may help the regression of warts and prolong the disease-free status.

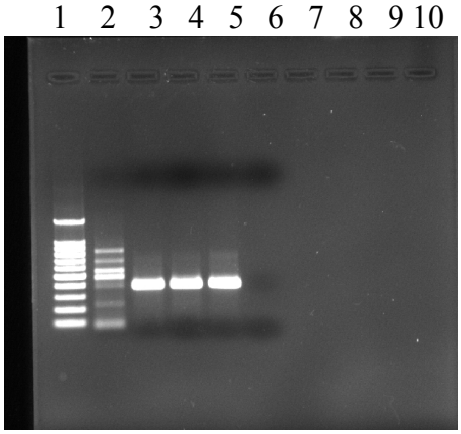

Fig. 5. PCR results of canine papillomavirus performed in samples taken from affected dogs; Lane 1 and 2: DNA ladder; Lane 3: positive control; Lane 4 and 5: positive samples; Lane 6: negative control.

The results of PCR for papillomavirus in samples taken from the affected dogs are shown in Fig. 5. All clinically affected dogs were found to be positive by PCR.

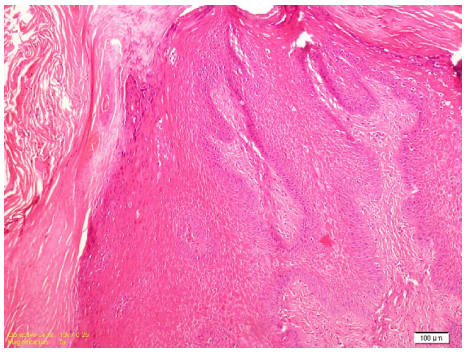

Fig. 6. Acanthosis and hyperkeratosis in canine fibroblastic papilloma.

Histopathology revealed various degrees of acanthosis and hyperkeratosis has been observed on sampled neoplasms. Fibroblastic papillomas have been observed on squamous papillomas of canine papillomas (Fig. 6).

DISCUSSION

In most cases, treatment may not be necessary since the papillomas will usually heal depending on the immune status of the dogs. Fibropapillomas can be troublesome when diffused, present in the oral cavity through pharynx and esophagus and

genital area, causing pain, loss of appetite and interfere with the food intake. Chronically immune suppressed animals may develop extensive papillomatosis in the upper gastrointestinal tract, which can cause difficulties with eating and breathing (Icen *et al.,* 2011). Occasionally papillomas can become infected with bacteria and antibiotic therapy will be needed in these cases to control the secondary infection. Levamisole was reported to be safe and immuno-stimulant both in animals and men (Andrieu, 1977). Canine papillomas may be seen large numbers of warts with the pain, swelling, bad breath and also making it difficult to eat and cause poor life quality (Etinger *et al.,* 2005, Nelson *et al.,* 2009). Warts are also not esthetical. There are other medical choices – surgical removal or cryogenical freezing (Ettinger *et al.,* 2005; Bridger *et al.,* 2007; Nelson *et al.,* 2009) to fight the virus directly. Also at *Tarantula cubensis venom* group, recovery has been observed in dogs when the warts were not diffused and smaller sized. Our results indicated that isotherapy was beneficial for patients. No adverse effects were seen in either groups and no recurrence has been observed during the following 3 months. It is inexpensive, easy to use and patient friendly.

CONCLUSION

Isotherapy with homeopathic protocols, *Tarantula cubensis* venom extract, levamisole and azithromycin appears to be a safe and effective treatment for canine papillomatosis.

ACKNOWLEDGEMENTS

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