Letters in
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# The Effect of Dental Products and Natural Chews on Canine Oral Bacteria

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## Abstract

Oral hygiene is an important factor in dental and overall health for dogs. Various options are available to help owners reduce the amount of plaque and tartar forming bacteria in their dogs' mouths. These options vary from specifically designed canine toothpaste and chews to natural chews or raw bones. In order to determine which, if any, of these options can help reduce the bacterial load on a dog's teeth, this study examines the change in bacteria after treatment with either CET toothpaste and brushing, VeggieDent chews, bully stick chews, and raw bones. All of these treatments were successful in reducing the amount of bacteria on a dog's teeth with reductions in bacteria by 79.0%, 70.3%, 60.2% and 54.6% respectively for raw bones, brushing, bully sticks and VeggieDent chews. None of the treatments were statistically different from each other ($p=0.330$). This experiment shows that owners have a variety of effective ways at keeping their dogs' teeth and gums healthy and that further research is required to determine the most effective method of reducing bacteria on a dog's teeth.

## Introduction

Many bacteria species normally colonize human and animal oral cavities. Plaque forms when these bacteria are allowed to accumulate on the teeth. Steptoccci are the first bacteria species to accumulate (Ritz 1967). Bacteria continue to reproduce and accumulate on the pellicle of the teeth, forming a biofilm and later, plaque and tartar (Rosan and Lamont 2000). Dental plaque in canines consists mainly of anaerobic gram-negative bacteria. *Bacteroides asaccharolyticus* represents the most common bacteria in the supragingival plaque and *Fusobacterium nucleatum represents* the most common in the subgingival plaque (Syed, Svanberg et al. 1981).

Periodontal disease resulting from plaque buildup is a common problem in both humans and animals. About 20% of one year old dogs and over 80% of three year old dogs suffer from periodontal disease (Kortegaard, Eriksen et al. 2008). Periodontal disease can result in pocketing along the gum line, cavies, and necessary tooth extractions. This disease causes problems not just in the mouth, but throughout the body as well. One study showed a correlation between increased periodontal disease and increased changes in the atrio-ventricular valves as well as increased kidney and liver disease in poodles (Pavlica, Petelin et al. 2008).

Brushing the teeth every day is the best way to prevent tartar (Gorrel and Rawlings 1996). One study showed that beagle dogs did not develop periodontitis or gingivitis on the side of their mouths that had been brushed daily (Lindhe 1973). Dental chews also help reduce tarter accumulation, especially when used in conjunction with brushing (Gorrel and Bierer 1999). Veterinarians often recommend brushing the teeth everyday as the ideal option for promoting oral health, but offer other products, such as dental chews, when brushing every day is not feasible for owners. Little research has been done to examine the effect of natural chews, such as bully sticks and raw bones, on canine oral bacteria. It is unclear how these various dental products compare in their abilities to reduce oral bacteria. If chews reduce bacteria to the same or a similar level as toothpaste does, these would be an easier method of dental cleaning for the owners and a potentially less stressful method for the dogs. In addition, some owners might be more inclined to try a natural chew, so understanding the effect of these options would also help owners and their dogs. This study aims to determine how effective canine toothpaste, dental chews, and natural chews are at reducing canine oral bacteria found on the teeth.

## Materials & Methods

### *Study subjects*

This study examines the effect of dental products on 6 volunteer dogs. These dogs vary in breed, size, diet, and routine dental care. Two of the dogs, a male Australian shepherd and a female jack russell terrier, belong to the researcher. The other four dogs belong to employees at the Animal and Bird Health Care Center and Hospital. Verbal consent to include the dogs in the study was obtained from each owner. The remaining four dogs

include a cocker spaniel, a pit bull, a chow/golden retriever mix and a husky/border collie mix.

### *Treatment and collection methods*

The dental products examined include C.E.T. enzymatic toothpaste applied with a plastic finger brush, regular sized C.E.T. VeggieDent canine chews, bully sticks and raw marrow bones. A pretreatment sample was collected from one side of each dog's mouth using a sterile gauze swab and sterile forceps. The lips were pulled back and each tooth from the molars to the canines was swabbed with the gauze. The sample was then transferred back to a sterile container. After the pretreatment sample was collected, the teeth were treated with one of the experimental products. To test the effect of the canine toothpaste, a half inch strip of toothpaste was applied to the toothbrush. The teeth on one side of the mouth were then brushed for 15 seconds. A post-treatment swab was obtained in the same manner as the pretreatment swab. The swabs were taken from opposite sides of the mouth to avoid any decrease in bacteria resulting from the action of the first swab. The plastic finger toothbrush was cleaned under running water and sterilized with 70% ethanol between treatments.

At least 24 hours between treatments were given to allow the bacteria levels to return to a normal level for each dog. The second treatment examines the effect of C.E.T. VeggieDent canine chews. A pretreatment swab was collected in the same manner as described. Each dog was given one regular sized chew and was allowed to finish the chew at its own pace, which lasted between 5 and 10 minutes. After finishing the chew, a post treatment sample was collected from the opposite side of the mouth as the pretreatment sample, again, to avoid any effect of the first swab.

The third treatment examines the effect of a natural chew treat not specifically designed for dental health – a "bully stick". A bully stick is a dried bull penis cut into various lengths and sometimes braided or twisted. Corkscrew bully sticks of about 6 inches in length were used for this treatment. The dogs were allowed to chew the treat at their own pace.

The fourth treatment examines the effect of feeding raw bones to dogs. Raw beef marrow bones were used in this experiment. The dogs were allowed to chew on the bone for 30 minutes. Only the researcher's two dogs were used for the chew portions of the experiment. Treatments were performed on different days in order to increase sample size while maintaining independence of samples.

### *Bacteria growth*

After sample collection, swabs were transferred to sterile tubes containing 15ml of sterile water and mixed using a vortex mixer to remove bacteria from the swab. From this, 1:100 and 1:1,000 dilutions were created and mixed using the same method. Bacteria were grown using 100 microliters from each dilution spread on LB agar plates and incubated at 37 degrees Celsius for 48 hours. After that time period, bacteria colonies from each plate were counted and used to calculate the amount of bacteria collected.

### *Statistics*

Due to the potential differences in pretreatment bacteria levels, the change in bacteria between pre and post-treatment samples were calculated for each dog, and these values were used in the statistical analysis. An ANOVA test was used to determine whether significant differences existed between the change in bacteria levels after using toothpaste, dental chews, bully sticks or raw bones. All statistical analysis were performed with 95% confidence level, $\alpha = 0.05$.

## Results

Plates created from 1:100 dilutions were preferentially used for data analysis – this concentration allowed enough bacteria colonies to grow that could be accurately counted but were numerous enough to closely examine differences between samples. Data were eliminated from the study when less than 10 colonies grew on either the pre or post treatment plates. This occurred in 29 out of 51 potential replicates. Data were also rendered unusable when the colonies were too numerous to accurately count or estimate. This occurred in 3 of the pretreatment replicates. Overall, 19 out of the total 54 replicates were used for the analysis.

Raw bones reduced bacteria by 79.0% (SD=11.2%, N=4), brushing by 70.3% (SD=15.8%, N=7), bully sticks by 60.2% (SD=29.8%, N=4) and VeggieDent chews by 54.6% (SD=20.9%, N=4). None of these treatments were statistically different (p=0.330).

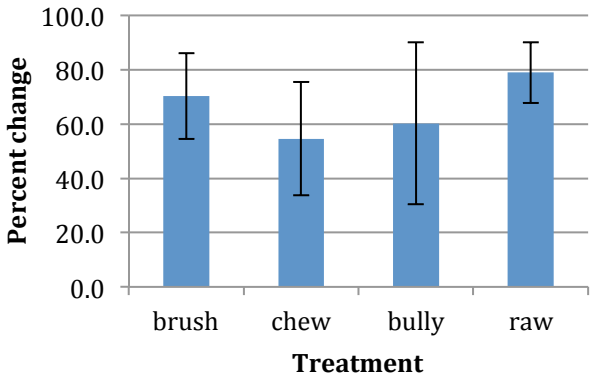


Figure 1. The percent decrease in bacteria after treating with toothpaste/brushing, VeggieDent chews, bully stick chews and raw bones. Results were not statistically different between treatments (p=0.330).

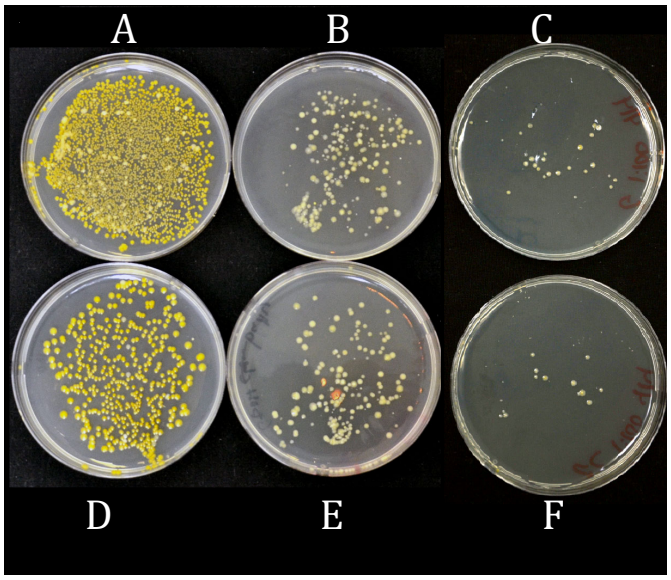


Figure 2. LB agar plates grown at 37 degrees Cecius for 48 hours, all representing 1:100 dilution of original sample. A. Brushed pretreatment B. Bully stick pretreatment C. VeggieDent pretreatment D. Brushed post treatment E. Bully stick post treatment F. VeggieDent chew post treatment.

## Discussion

This study was designed to determine the effectiveness of various canine dental products at reducing the amount of oral bacteria. Good dental hygiene is important to maintain healthy teeth and gums, but also to keep internal organs such as the heart, kidney and liver healthy (Pavlica 2008). Pet owners have many options when it comes to dental health for their pets, including canine toothpaste, dental chews, and other natural chew products. Products designed specifically for dental benefits have antibacterial ingredients, but some also claim that the act of chewing also helps to remove some of the tartar from the teeth. This study examines these claims, seeking to determine the relative effectiveness of brushing the teeth, giving a dental chew and giving a natural chew treat.

The results show that brushing the teeth and giving a VeggieDent chew reduce the amount of oral bacteria by an average of 70.3% (SD = 15.8%) and 54.6% (SD=20.9%) respectively (Fig. 1). The effect of brushing and chewing a VeggieDent were not significantly different (p=0.330). Canine enzymatic toothpaste contains two active ingredients, glucose oxidase and lactoperoxidase (Department 2009). These ingredients produce hydrogen peroxide, which is bactericidal (Bankar 2009). The active ingredient in VeggieDent chews is Chlorhexidine digluconate (Department 2009). This ingredient is both bactericidal upon initial contact, but it also gets absorbed into the pellicle of the teeth, providing a longer lasting bacteriostatic effect (Jenkins 1988). These antibacterial effects could account for the decrease in oral bacteria observed after treating with toothpaste or a dental chew.

In addition to antibacterial ingredients in dental products, the act of chewing might also have an effect on the amount of bacteria on the teeth. Chewing a bully stick (a natural chew treat) reduced the amount of bacteria by 60.92% (SD = 28.9%). Raw bones also successfully reduced bacteria by 79.0% (SD=11.2%) (Fig. 1). Again, this could be due in part to the chewing action physically removing bacteria from the teeth. Anecdotal evidence suggests that feeding a raw diet or at least recreational raw bones keeps the teeth and gums free of tartar. Dogs fed this kind of diet have teeth with a visibly lower amount of tartar and better smelling breath than those fed commercial diets (Lee 2009), but these claims have not yet been verified by scientific analysis. Canine saliva has bacteriocidal properties, and is specifically effective against *Escherichia coli* and *Streptococcus canis* (Hart 1990). Saliva also serves to buffer the oral cavity and physically cleanse the teeth (Dowd 1999). The reduction of bacteria due to natural chews, especially raw bones, could be an antibacterial result, perhaps relating to an antibacterial nature of saliva, or a physical effect of scraping tartar off the teeth. Further research should seek to elucidate the exact mechanism by which natural chews are able to reduce tartar. Research should focus specifically on the raw diet, as this diet has anecdotally been shown to keep teeth and gums healthy. Scientific analysis could take these accounts from sheer experience and word of mouth to scientific fact.

This study was effective at showing that various dental products and natural chew treats are able to reduce the amount of bacteria on a dog's teeth. Many of the samples taken showed a significant reduction in the amount of bacteria after treatment. However, many samples were also inconclusive and inconsistent with results supporting the effectiveness of these products. In some cases, plates derived from the same sample would differ greatly. For example, two plates could grow too many bacteria to count, and yet when re-plated from the same sample at the same concentration would only grow a few bacteria. This result calls into question the validity of any of the plates that did not grow many bacteria. It brings in the question of whether the reduction in bacteria was due to the treatment, or whether that plate simply did not grow a representative number of bacteria. Despite this great variation in some plates, others did grow a large enough amount of bacteria to suggest not an error or randomness in plating, but rather a true effect. In an attempt to isolate the most representative data, only plates that grew at least 10 colonies were used. This number should be increased in studies that have more data available. A low number was used as the cutoff because some of the dogs appeared to have fewer bacteria in their mouths to start, perhaps due to differences in home dental care. In the interest of keeping as much data in the analysis as

possible, a low limit of 10 colonies was used for this report.

The differences in bacteria seen between dogs could have increased the variances, leading to nonsignificant results. Future studies should increase the number of dogs used in order to lessen the effect of variances. This study is unique among animal experiments in the that treatments have little to no aversive effects on the animals and are already being used by many dog owners. A future study could use this fact by incorporating owners that already regularly use these various dental treatments. If experimental treatment data cannot be obtained, surveys regarding dental health practices coupled with mouth swabs could also potentially indicate the most effective but convenient dental health practices.

Overall, this experiment suggests there is an observable effect of various dental products and natural chews on the amount of bacteria in a dog's mouth. However, many more replicates, additional study subjects, and refined methods would increase the confidence in the results. More replicates would allow the researcher to better determine when the plates were representative of the amount of bacteria present. Further research should also better examine mechanism of the effect of natural products. Whatever the method, keeping a pet's teeth and gums healthy is an important part of overall health that owners should not ignore. Researchers should continue to examine the effect of dental products, both commercial and natural, to determine the best and most convenient method to reduce bacteria, plaque and tartar on animals' teeth.

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