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Introduction

Periodontal disease is diagnosed commonly in small animal practice and may be a potentially serious systemic disease of adult cats.¹ Studies have shown that the development of periodontal disease is related to the growth of bacteria in dental plaque and the release of by-products associated with bacterial metabolism and the host's immune response.²⁻⁴ There is limited information documenting the specific bacteria involved in feline periodontal disease.⁵⁻⁷ However, the microbial flora is considered to be similar to that of humans and canines with periodontal disease. The subgingival microbial flora of healthy gingiva is comprised principally of aerobic, gram (+) cocci changing to anaerobic, gram (-) rods and spirochetes with the establishment and progression of periodontal disease.⁸⁻¹⁰

Therapeutic strategies for the treatment of periodontal disease in cats may include the administration of systemic antimicrobials to inhibit or delay plaque formation following a professional teeth cleaning procedure. However, the development of resistant bacteria, altered bacterial antimicrobial sensitivity, and potential systemic side-effects are factors which should restrict continuous antimicrobial administration.¹¹ Local antiseptic treatment may be an effective method to decrease plaque accumulation while avoiding potential complications associated with chronic antimicrobial therapy.

Zinc has excellent antiseptic activity against both gram (+) and gram (-) bacteria, aids wound healing, acts as an astringent, and may provide unfavorable conditions for bacterial growth.¹²⁻¹⁸

Ascorbic acid is synthesized naturally in all species, except primates and guinea pigs. Stress, infection, or inflammation increase the body's requirement for ascorbic acid. Ascorbic acid is essential for normal wound healing following surgery, collagen production, normal capillary function, and detoxification of toxins, chemicals, and free radicals that may accumulate in inflamed gingival tissue.¹⁹⁻²³

The purpose of the study reported here was to assess the clinical and microbiologic effects of zinc ascorbate gel^a applied orally in cats.

Materials And Methods

The study was performed in 36 client-owned domestic shorthair (DSH) breed cats from a population of cats presented to a private practice for dental examinations during National Pet Dental Week in August, 1999. All cats had outdoor access and were fed a variety of commercial dry and canned cat foods. Cats had not been administered commercial oral hygiene products or any other type of home dental care. Owners were provided financial incentives to return their cats for biweekly recheck evaluations. Cats were divided randomly into two equal groups, with the treatment group (18 cats) receiving zinc ascorbate gel and the control group (18 cats) receiving a placebo (0.9 % sterile saline). The zinc ascorbate group consisted of 10 male and 8 female cats with a mean age of 3.6-years (range = 1.5 to 13 years). The control group consisted of 9 male and 9 female cats with a mean age of 4.1-years (range = 3 to 9 years).

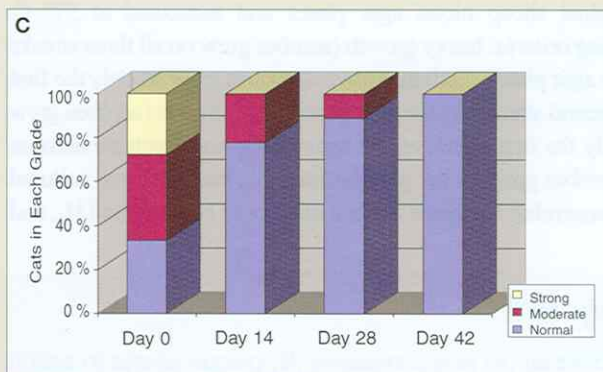
All cats had normal blood hematology and biochemistry parameters, however feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) tests were not performed. Cats had not been administered antimicrobial or anti-inflammatory agents during the 3- months prior to the beginning of the study. Cats were sedated for both the initial (Day 0) and final (Day 42) oral examinations using acepromazine^b (0.05 mg/kg SQ) and buprenorphine^c (0.008 mg/kg SQ). Measurements were performed on all cats without sedation on the buccal or labial tooth surfaces only at Days 14 and 28. All cats had normal periodontal sulcus probing depths and feline odontoclastic resorptive lesions (FORL) were not observed visually or detected during periodontal probing. Intraoral dental radiographs were not performed. A single pea-sized drop of zinc ascorbate gel (0.05 ml) was placed bilaterally on the buccal oral mucosa above the maxillary canine tooth (total dose = 0.1 ml) SID by the owner for 6-weeks (Days 0 to 42). The maxillary canine (104, 204) teeth; maxillary third and fourth premolar (107, 108, 207, 208) teeth;

Figure 1

Individual (A) and cumulative (B) halitosis scores in cats receiving oral zinc ascorbate gel and control group cats. The change in halitosis scores in cats receiving oral zinc ascorbate gel (C) indicated no halitosis at Day 42 of the study, however this result was not statistically significant ($P = 0.14$).

A	Zinc ascorbate group				Control group			
	Day 0	Day 14	Day 28	Day 42	Day 0	Day 14	Day 28	Day 42
1	1	0	0	0	0	0	0	0
2	2	1	1	0	2	2	1	1
3	2	1	0	0	2	2	2	2
4	1	0	0	0	2	2	2	2
5	2	1	1	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	1	1	1	1
9	1	0	0	0	1	1	1	1
10	2	0	0	0	1	1	1	1
11	2	1	0	0	1	1	1	1
12	1	0	0	0	0	0	0	0
13	1	0	0	0	2	2	2	2
14	1	0	0	0	0	0	0	0
15	1	0	0	0	2	2	2	2
16	0	0	0	0	0	0	1	0
17	0	0	0	0	1	1	1	1
18	0	0	0	0	0	0	0	0
Total	17	4	2	0	17	17	17	15

Grading criteria: 0 = normal; 1 = moderate odor, increase in intensity from normal but not offensive; 2 = strong, offensive, unpleasant odor.



mandibular canine (304, 404) teeth; mandibular third and fourth premolar (307, 308, 407, 408) teeth; and, the mandibular first molar (309, 409) teeth were evaluated in each cat.

Assessment Criteria: Halitosis

Halitosis was measured by the examiner's olfactory senses (organoleptic analysis) by smelling the breath of each cat at a distance of approximately 5-cm and grading the odor using three categories.²⁴⁻²⁶ The same examiner graded breath odor throughout the trial period. *Grading criteria:* 0 = normal; 1 = moderate odor, increase in intensity from normal but not offensive; 2 = strong, offensive, unpleasant odor.

Assessment Criteria: Plaque

Plaque scores were determined based on visual assessment of plaque thickness and the surface area of plaque coverage (%) on the buccal or labial crown surface aided by application of a 2.0 % eosin disclosing solution.²⁷ The same examiner graded plaque accumulation throughout the trial period. *Grading criteria:* 0 = no plaque detected; 1 = 1 - 24 %; 2 = 25 - 49 %; 3 = 50 - 74 %; 4 = 75 - 100 %. Plaque thickness was graded as: 0 = no plaque present; 1 = light coverage, tooth enamel still visible through plaque; 2 = medium coverage, no enamel visible; 3 = heavy coverage. The plaque coverage score was multiplied by the

plaque thickness score (if ≥ 1) to obtain an overall plaque accumulation score for each tooth. The sum of all individual tooth plaque scores were averaged to obtain a whole-mouth plaque accumulation score for each cat.

Assessment Criteria: Calculus

Calculus scores were determined based on visual assessment of the surface area of calculus coverage (%) on the buccal or labial crown surface.²⁷ The same examiner graded calculus accumulation throughout the trial period. *Grading criteria:* 0 = no calculus detected; 1 = 1 - 24 %; 2 = 25 - 49 %; 3 = 50 - 74 %; 4 = 75 - 100 %. The sum of all individual tooth calculus scores were averaged to obtain a whole-mouth calculus accumulation score for each cat.

Assessment Criteria: Gingivitis

Gingivitis scores were determined based on visual assessment of inflammation and hemorrhage following periodontal probing of the buccal or labial gingival sulcus.²⁷ *Grading criteria:* 0 = gingiva was coral pink with no bleeding on probing; 1 = slight redness less than 1-mm from the gingival margin and no bleeding on probing; 2 = mean redness greater than 1-mm but less than 2-mm from the gingival margin and/or slight bleeding on probing; 3 = redness covering 2 to 3-mm from

the gingival margin and/or bleeding on probing; 4 = redness > 3-mm from gingival margin and/or bleeding on probing. The sum of all individual tooth gingivitis scores were averaged to obtain a whole-mouth gingivitis score for each cat.

Bacterial cultures were obtained by the examiner from cats in the treatment group at Days 0 and 42 from the buccal or labial periodontal sulcus of the 104, 204, 108, 208, 304, 404, 309 and 409 teeth using sterile # 20 endodontic paper points. Separate paper points were placed into the sulcus to the level of the epithelial attachment (~ 0.5-mm) for 30-seconds. Points were grouped and placed into agar collection vials (aerobes) or sterile meat broth transport media (anaerobes). Care was made to sample only the subgingival flora by avoiding paper point contact with the supragingival tooth surface. The media were transported immediately to a microbiology laboratory^d for analysis. All samples were analyzed in the same laboratory by the same individual. Aerobic cultures were streaked-out three times onto individual sheep blood agar plates and incubated at 37° C. *Grading criteria:* heavy growth (aerobes grew on all three streaks on the agar plate); medium growth (aerobes grew on only the first and second streaks on the agar plate); light growth (aerobes grew on only the first streak on the agar plate); no detectable aerobes (no aerobes grew on the plate). Anaerobic bacteria were cultured in an anaerobic incubator under a mixture of N₂, CO₂ and H₂, and

tested for colony forming units (CFU) and identification of individual species using an APIAN kit^e. Aerobic bacteria were not identified. Antibiotic susceptibility testing was not performed.

Cumulative (total) assessment criteria scores were determined for each group. Differences in clinical and microbiological parameters at the evaluation periods were analyzed using a non-parametric Mann-Whitney test with values of $P < 0.05$ considered significant. Statistical analysis was not performed between individuals within groups.

Results

Clinical Assessment

Halitosis scores on Day 0 were indicative of strong malodor in 5 treatment group cats and 5 control group cats. Halitosis scores were moderate in 7 treatment group cats and 5 control group cats. All other cats had normal breath. On Day 14, no cats in the treatment group and 5 cats in the control group had strong halitosis scores. Halitosis scores were moderate in 4 treatment group cats and 5 control group cats. All other cats had normal breath. Day 28 halitosis scores were normal in 16 treatment group cats while the remaining 2 cats received a moderate halitosis score. Four cats in the control group had strong halitosis scores and 7 control group cats had moderate halitosis. By Day 42, all cats in the treatment group had normal breath, whereas there were

Figure 2

Individual (A) and cumulative (B) plaque scores in cats receiving oral zinc ascorbate gel and control group cats. The change in plaque scores in cats receiving oral zinc ascorbate gel (C) indicated a statistically significant ($P < 0.05$) decrease in plaque accumulation at Day 42 of the study.

Cat #	Zinc ascorbate group				Control group			
	Day 0	Day 14	Day 28	Day 42	Day 0	Day 14	Day 28	Day 42
1	3	2	0	0	4	2	1	2
2	3	4	1	1	4	4	3	4
3	4	2	1	1	4	4	4	4
4	4	2	1	0	1	2	4	4
5	4	2	1	1	4	4	4	4
6	1	0	1	1	1	0	2	1
7	0	0	0	0	3	2	2	2
8	0	0	0	0	2	4	4	3
9	4	4	3	2	3	3	3	3
10	4	2	1	2	2	1	2	3
11	4	1	3	3	2	1	3	3
12	3	2	1	1	1	3	3	2
13	4	2	1	0	4	4	4	4
14	2	1	1	0	3	3	3	2
15	1	1	2	1	2	3	4	4
16	2	2	1	1	2	3	3	3
17	1	0	0	0	3	2	3	3
18	1	0	1	2	3	2	3	3
Total	45	27	19	16	48	49	55	54

Grading criteria: 0 = no plaque detected; 1 = 1 - 24 %; 2 = 25 - 49 %; 3 = 50 - 74 %; 4 = 75 - 100 % of crown surface area. Plaque thickness was graded as: 0 = no plaque present; 1 = light coverage, tooth enamel still visible through plaque; 2 = medium coverage, no enamel visible; 3 = heavy coverage. Overall plaque accumulation score = plaque coverage score x plaque thickness score (if > 1).

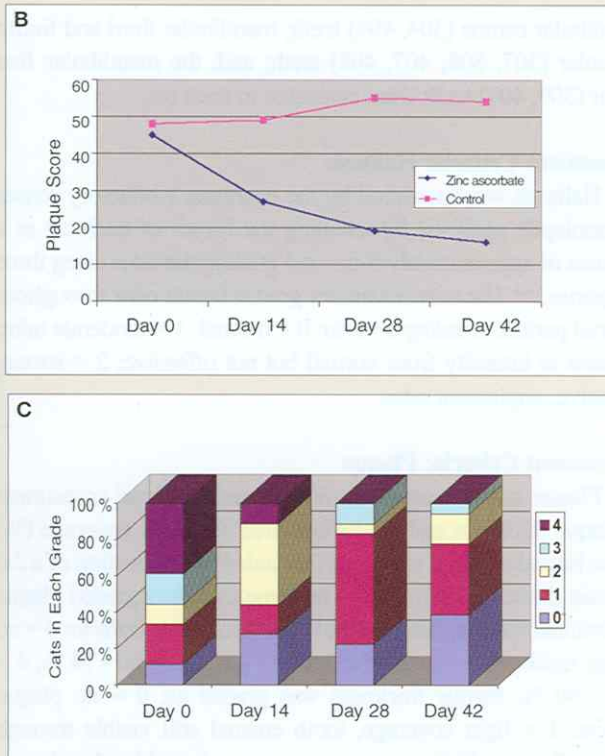
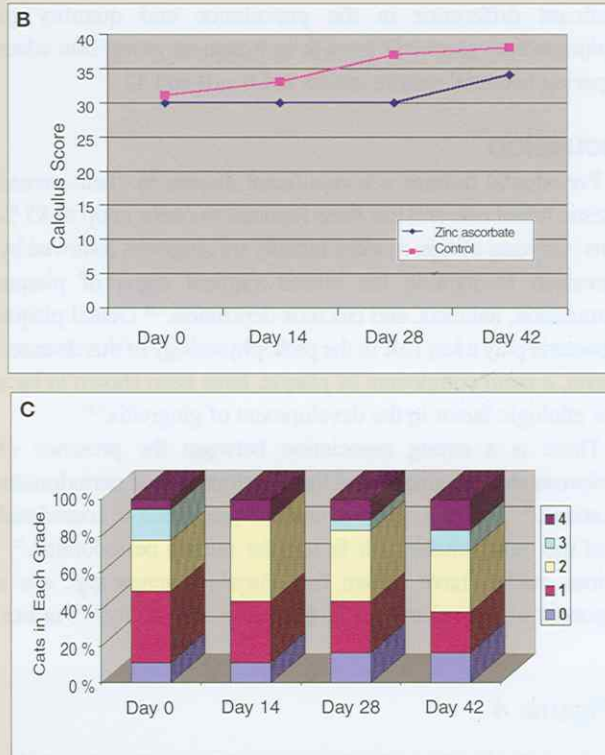


Figure 3

Individual (A) and cumulative (B) calculus scores in cats receiving oral zinc ascorbate gel and control group cats. The change in calculus scores in cats receiving oral zinc ascorbate gel (C) were similar to those for cats in the control group at Day 42 of the study ($P = 0.11$).

Cat #	Zinc ascorbate group				Control group			
	Day 0	Day 14	Day 28	Day 42	Day 0	Day 14	Day 28	Day 42
1	1	1	1	1	1	1	1	1
2	3	1	0	0	1	1	1	1
3	1	1	1	1	4	4	4	4
4	2	1	1	2	2	1	2	2
5	1	2	2	2	3	3	3	3
6	0	0	0	0	1	2	2	2
7	1	1	1	1	1	3	3	3
8	0	0	0	0	1	0	0	2
9	4	4	4	4	2	2	2	3
10	2	2	2	2	2	3	2	2
11	3	4	4	4	1	0	1	0
12	1	1	1	1	2	2	3	3
13	2	2	2	3	3	3	3	3
14	1	2	2	3	1	2	2	2
15	4	4	4	4	1	1	1	1
16	2	2	2	2	2	2	2	2
17	2	2	2	2	1	2	2	2
18	1	2	2	2	2	3	2	2
Total	30	30	30	34	31	33	37	38

Grading criteria: 0 = no calculus detected; 1 = 1 - 24 %; 2 = 25 - 49 %; 3 = 50 - 74 %; 4 = 75 - 100 % of crown surface area.



4, 6, and 8 cats in the control group with strong, moderate, and no halitosis, respectively. Therefore, by Day 42, all of the cats receiving the zinc ascorbate gel had normal breath, whereas there was minimal change in the halitosis scores in control group cats (Fig. 1). Although cumulative halitosis scores decreased from 17 to 0 and 17 to 15 in the treatment and control groups, respectively, these differences were not statistically significant at any of the reevaluation periods.

Day 0 cumulative plaque scores of 45 and 48 were similar for treatment and control groups, respectively. Cumulative plaque scores for treatment group cats had decreased (27) at Day 14, whereas they had increased (49) for control group cats. Day 28 and 42 cumulative plaque scores were 19 and 16, respectively for the treatment group cats and 55 and 54, respectively for control group cats (Fig. 2). During the study period, cumulative plaque scores decreased from 45 to 16 in the treatment group cats and increased from 48 to 54 in the control group cats. Plaque accumulation was significantly less in treatment group compared with control group cats.

Cumulative calculus scores did not change significantly in either group during the treatment period. In the treatment group cats, the cumulative calculus score increased from 30 to 34 with most cats maintaining the same score for calculus at each evaluation period. The cumulative calculus score was similar for the control group cats, increasing from 31 to 38 (Fig. 3). There

was no statistical difference in calculus accumulation when comparing treatment and control group cats during the evaluation period.

Cumulative gingivitis scores were similar for treatment (23) and control (28) group cats at the Day 0 evaluation. Clinical signs of gingivitis were not present in 15 of 18 treatment group cats at the Day 42 evaluation. During the evaluation period, the cumulative gingivitis score for the treatment group cats decreased from 23 to 5, while the cumulative gingivitis score for the control group cats increased from 28 to 40 (Fig. 4). Clinical signs of gingivitis were significantly less in treatment group compared with control group cats at the Day 42 evaluation.

Microbiological Assessment

Aerobic bacteria and *Porphyromonas gingivalis* were isolated from all 18 treatment group cats at Day 0 (Table 5). *Porphyromonas endodontalis*, *Prevotella intermedia*, *Fusobacterium spp.*, or spirochetes were not isolated from any of the cats. There was a heavy or light growth of gram (+) aerobic bacteria in 8 and 10 cats, respectively. Aerobic bacterial culture results were significantly different at Day 42 indicated by 4 cats with light growth of gram (+) aerobic bacteria and no detectable bacterial growth in the remaining 14 cats.

The mean colony count for *Porphyromonas gingivalis* growth in the treatment group cats was 2.7×10^7 CFU at Day 0

and 1.0×10^6 CFU at Day 42 (Fig. 5). At Day 0, 15 cats had *Porphyromonas gingivalis* growth in colony counts $> 1.0 \times 10^5$ CFU. However, at Day 42, 6 treatment group cats had *Porphyromonas gingivalis* colony counts of $< 1.0 \times 10^5$ CFU and 8 cats had no detectable growth of anaerobes. There was a significant difference in the prevalence and quantity of *Porphyromonas gingivalis* growth in treatment group cats when comparing bacterial culture results at Days 0 and 42.

Discussion

Periodontal disease is a significant disease in client-owned domestic breed cats and has been reported to occur in up to 85 % of cats > 6 years of age, marked initially by gingivitis followed by progression to include the classic clinical signs of plaque accumulation, halitosis, and calculus deposition.^{1,28} Dental plaque and bacteria play a key role in the pathophysiology of this disease.¹ Bacteria, a main component of plaque, have been shown to be a major etiologic factor in the development of gingivitis.^{6,29}

There is a strong association between the presence of *Porphyromonas gingivalis* and the development of periodontitis in humans.^{30,31} In fact, *Porphyromonas gingivalis* is considered one of the most reliable risk factors for human periodontitis.^{32,33} Previous studies have shown that *Porphyromonas spp.* are a component of the microflora in the feline oral cavity.^{6,7} Further,

Porphyromonas gingivalis and other *Porphyromonas spp.* have been shown to be highly significant predictors of the grade of periodontal disease in cats.⁷ Evaluation of the changes in the subgingival microflora as reported here indicated a reduction in aerobes as well as *Porphyromonas gingivalis* in treatment group cats. One cat (cat 15) showed an increase in anaerobe colony count, which could have been explained by resistant bacteria, failure of gel distribution in the oral cavity producing suboptimal minimal inhibitory concentrations (MIC), or an unusually high level of *Porphyromonas gingivalis* in one culture site that did not decrease significantly in this cat.

It is not surprising that significant decreases in colony counts of *Porphyromonas gingivalis* were concurrent with significant decreases in plaque and gingivitis scores.³⁰⁻³³ In the treatment group, the majority of individual cats showed reductions in plaque formation, or maintained little or no plaque on all teeth measured. There were 4 cats that did not show a response to treatment (cats 6, 11, 15, 18) possibly due to: lack of owner compliance; tenacious, or virulent plaque; an unusually high level of *Porphyromonas gingivalis* in one culture site; decreased dispersion of the gel in the oral cavity; or, dietary factors which may have enhanced plaque deposition. While there were fluctuations in plaque formation in individual cats, plaque scores were unchanged in the majority of cats within the control group

Figure 4

Individual (A) and cumulative (B) gingivitis scores in cats receiving oral zinc ascorbate gel and control group cats. The change in gingivitis scores in cats receiving oral zinc ascorbate gel (C) indicated a statistically significant ($P < 0.05$) decrease in gingivitis at Day 42 of the study.

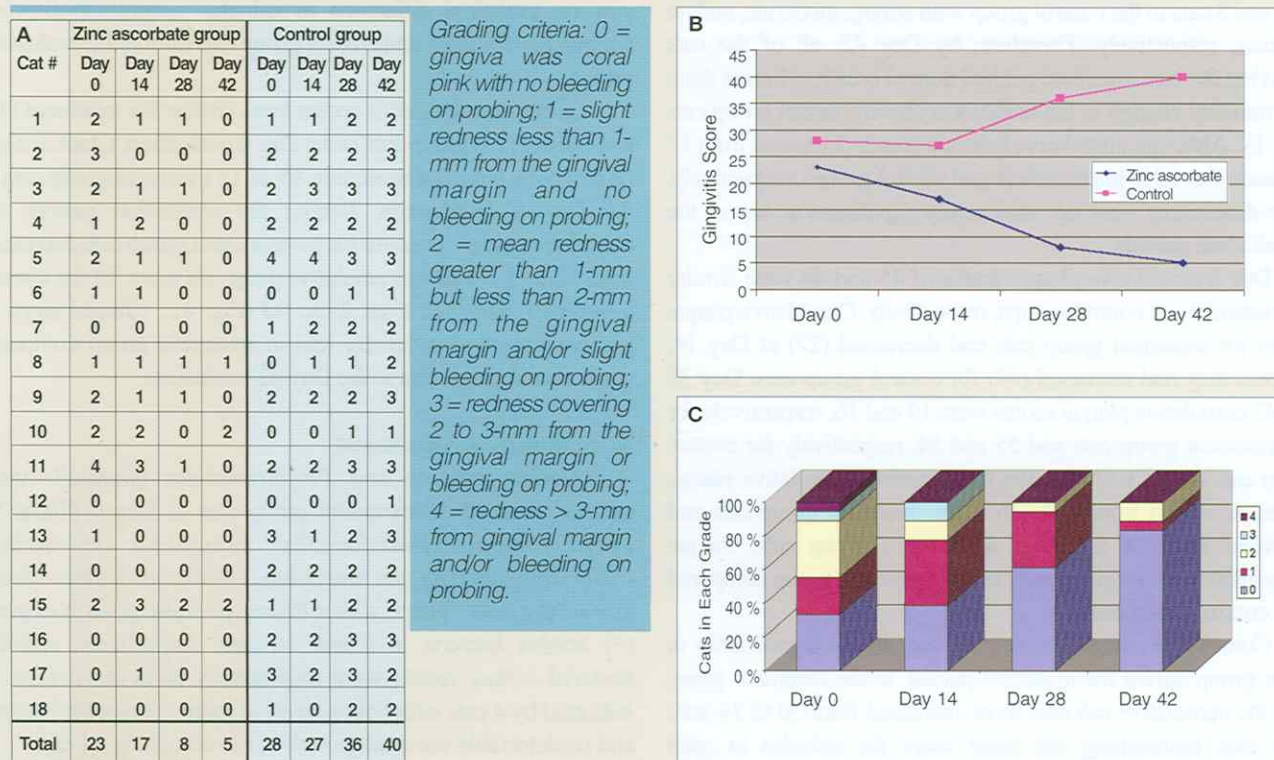
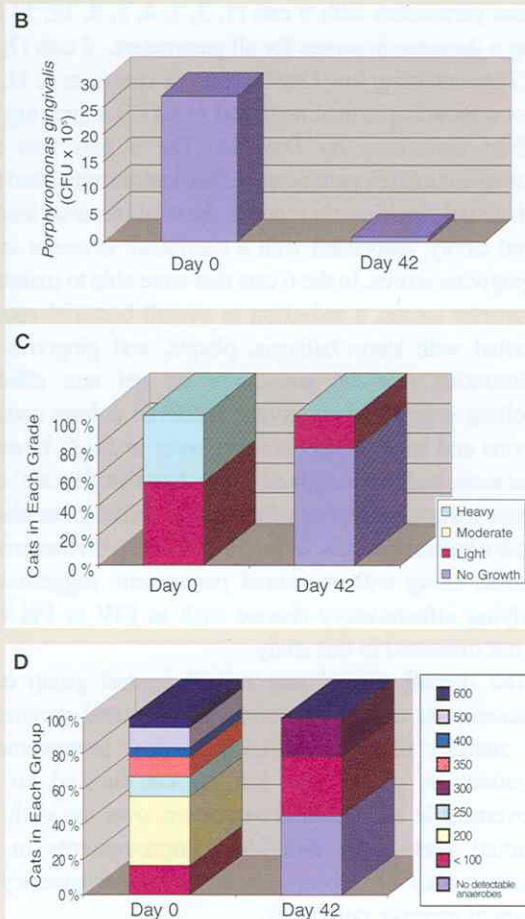


Figure 5

Individual (A) aerobic and anerobic bacterial culture results and cumulative (B) anerobic bacterial culture results in cats receiving oral zinc ascorbate gel. The changes in aerobic (C) and anaerobic [CFU x 10⁵] (D) bacterial culture results in cats receiving oral zinc ascorbate gel at Day 0 compared with Day 42 were statistically significant (P < 0.05).

A	Aerobic bacterial growth		Porphyromonas gingivalis (CFU x 10 ⁵)	
	Day 0	Day 42	Day 0	Day 42
Cat #				
1	Heavy	None detectable	250	<100
2	Heavy	None detectable	400	<100
3	Heavy	None detectable	350	<100
4	Heavy	None detectable	250	None detectable
5	Heavy	None detectable	350	<100
6	Light	None detectable	<100	<100
7	Light	None detectable	<100	None detectable
8	Light	None detectable	<100	<100
9	Heavy	Light	500	300
10	Heavy	Light	500	300
11	Heavy	Light	600	300
12	Light	None detectable	200	None detectable
13	Light	None detectable	200	None detectable
14	Light	None detectable	200	None detectable
15	Light	Light	200	300
16	Light	None detectable	200	None detectable
17	Light	None detectable	200	None detectable
18	Light	None detectable	200	None detectable
Control sample	None detectable	None detectable	None detectable	None detectable
Total			270	10

Grading criteria: heavy growth (aerobes grew on all three streaks on the agar plate); medium growth (aerobes grew on only the first and second streaks on the agar plate); light growth (aerobes grew on only the first streak on the agar plate); no detectable aerobes (no aerobes grew on the plate).



during the evaluation period. Three cats in the control group had a decrease in plaque scores (cats 1, 7 and 14), possibly related to diet abrasion or other environmental factors which were not controlled in this study.

The majority of treatment group cats had a reduction in gingivitis scores, whereas the remainder had little or no gingivitis during the treatment period. The majority of cats in the control group had an increase in gingivitis scores. Two of these cats maintained the same degree of gingivitis (cats 4 and 14) while 2 other cats showed a fluctuation in the severity of gingivitis scores (cats 13 and 17). These 4 cats also had a consistent halitosis score.

The assessment of halitosis was subjective in this study, albeit performed by the same observer. Studies in the veterinary literature have used both portable sulfide monitors as well as examiner's organoleptic analysis.²⁴⁻²⁶ In one study, there was a significant, positive correlation between volatile sulfur concentrations (VSC) concentrations and the human perception of bad breath.²⁶ Halitosis and gingivitis are often associated positively since breakdown products of oral bacteria such as hydrogen sulfide and methyl mercaptan contribute to halitosis.

Bacteria, bacterial by-products, and the host's immune response cause gingivitis.^{24,34} Therefore, when pathogenic oral bacteria are reduced quantitatively, there may be a concurrent decrease in the clinical signs of halitosis and gingivitis. Oral application of zinc ascorbate gel as performed in this study led to quantifiable reductions in aerobic and anaerobic bacteria, and significant reductions in plaque and gingivitis scores. Although there was not a statistically significant difference in cumulative halitosis scores when comparing the treatment and control group cats, there was a trend for halitosis to diminish in cats receiving the zinc ascorbate gel. Decreasing halitosis scores coincided with significant reductions in plaque and gingivitis scores suggesting that there may be minimal threshold levels of plaque and gingivitis to detect subjectively offensive halitosis.

There was not a significant change in the cumulative calculus score when comparing treatment and control group cats during the treatment period. Generally, cats in both groups had increasing calculus scores during the study period. This finding was expected since it is generally believed that calculus removal requires mechanical methods such as those performed during a

professional teeth cleaning procedure.

Overall, the treatment group cats had cumulative decreases in the measured parameters that were clinically apparent in individual cats. Although statistical analysis was not performed on individual cats, there appeared to be a positive relationship between parameters with 9 cats (1, 2, 3, 4, 5, 9, 12, 13 and 14) having a decrease in scores for all parameters, 5 cats (7, 8, 9, 16 and 17) maintaining low Day 0 scores, 4 cats (cats 6, 11, 15 and 18) not responding to treatment, and 1 cat (10) improving initially but then worsening by Day 42. The 9 cats that showed improvement in their parameter scores demonstrated that the zinc ascorbate gel significantly reduced the total bacterial load within the oral cavity, associated with a significant decrease in plaque and gingivitis scores. In the 6 cats that were able to maintain Day 0 parameter scores, a reduction in overall bacterial counts was associated with static halitosis, plaque, and gingivitis scores, demonstrating that the zinc ascorbate gel was effective in controlling established gingivitis. Bacterial colony counts, and gingivitis and halitosis scores were lower in 3 cats however the plaque score had not improved suggesting that the cats' immune response was producing an inflammatory effect in the absence of excessive bacterial levels. In the other cat (cat 15) bacterial levels increased, along with measured parameters, suggestive of an underlying inflammatory disease such as FIV or FeLV which were not measured in this study.

The overall clinical response in control group cats was maintenance or slight worsening of measured parameters. A small number of cats showed individual improvements, or fluctuations in parameters, but no cat showed an overall improvement in all measured parameters over the study period. As noted previously, individual improvements in certain parameters may have been related to diet consistency or the addition of abrasive raw meats.

Previous studies have indicated that antimicrobial and antiseptic preparations, either in liquid or tablet form, have a negative effect on the development of gingivitis in dogs.³⁵⁻³⁷ However, there is limited data showing similar effectiveness in the oral cavity of cats. The zinc ascorbate gel used in this study as an oral antiseptic significantly decreased pathologic factors associated with the development and progression of gingivitis. The results of this study suggest that zinc ascorbate gel used as an oral antiseptic improves feline oral health, and may be most effective in decreasing bacterial growth, plaque formation, and gingivitis when applied as described here following a professional teeth cleaning procedure.

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