Oral-Fluid Thiol-Detection Test Identifies Underlying Active Periodontal Disease Not Detected by the Visual Awake Examination

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ABSTRACT

Periodontal disease in dogs is highly prevalent but can only be accurately diagnosed by performing an anesthetized oral examination with periodontal probing and dental radiography. In this study, 114 dogs had a visual awake examination of the oral cavity and were administered an oral-fluid thiol-detection test prior to undergoing a a full-mouth anesthetized oral examination and digital dental radiographs. The results show the visual awake examination underestimated the presence and severity of active periodontal disease. The thiol-detection test was superior to the visual awake examination at detecting the presence and severity of active periodontal disease and was an indicator of progression toward alveolar bone loss. The thiol-detection test detected active periodontal disease at early stages of development, before any visual cues were present, indicating the need for intervention to prevent periodontal bone loss. Early detection is important because without intervention, dogs with gingivitis (active periodontal disease) progress to irreversible periodontal bone loss (stage 2+). As suggested in the current AAHA guidelines, a thiol-detection test administered in conjunction with the visual awake examination during routine wellness examinations facilitates veterinarian-client communication and mitigates under-diagnosis of periodontal disease and underutilization of dental services. The thiol-detection test can be used to monitor the periodontal health status of the conscious patient during follow-up examinations based on disease severity.
(*J Am Anim Hosp Assoc* 2018; 54:■■■–■■■. DOI 10.5326/JAAHA-MS-6607)

Introduction

Periodontal disease involves an infection of the gingiva that, if left untreated, leads to alveolar bone loss and loss of the periodontal ligaments that attach to the tooth.[1–4] The structures destroyed by periodontal infection cannot be fully evaluated in the awake animal because most of them lie beneath the gingiva, hidden from view.[5–8] Only a full-mouth examination performed under general anesthesia, including full-mouth periodontal probing with full-mouth dental radiographs, can determine the extent of underlying soft and calcified tissue damage and permit diagnosis of periodontal disease and disease staging.[9]

In the examination room, veterinarians must rely upon a thorough but relatively rapid visual inspection of the oral cavity to make an initial assessment of the patient's oral health.[9] Because periodontal disease involves an infection that begins in the gingival sulcus and therefore is not visible upon the visual awake examination, and because pet owners tend to underestimate the disease burden patients bear, it is challenging for veterinarians to communicate to clients the critical importance of routine prophylaxis, treatment, and home care.[10] Routine prophylaxis, treatment, and home care are critically important in order to preserve the integrity of periodontal structures and maintain the overall health of the animal.[11] Periodontal disease has a direct effect on the oral health of the animal and has been linked to many systemic diseases including but not limited to cardiovascular, renal, and hepatic disease.[12–16]

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Accepted for publication: March 23, 2016.

The present study was undertaken to determine the effectiveness of the visual awake examination at detecting underlying periodontal disease, and to compare its effectiveness with that of a point-of-care oral-fluid thiol-detection test developed to aid in detecting the presence and severity of underlying periodontal infection and progression toward bone loss. The present study also documents the prevalence of periodontal disease in a general veterinary and dental referral practice and finds the prevalence to be high, and clinical attachment loss to occur even in young dogs 1 yr of age or less.

Materials and Methods

Subject Recruitment

Subjects were recruited for this prospective study from a veterinary practice consisting of both a general practice hospital and a dental referral clinic (The Carolinas Animal Hospital & Dental Clinic, Charlotte, North Carolina). Informed consent was obtained from each client, and the contemporary standard of care was provided to each subject. Each client was instructed not to provide any form of dental home care to the subject on the day of presentation for examination. Each client was also instructed not to permit the subject to consume any solid food after 10 p.m. on the night before the day of presentation for examination.

Visual Awake Examination of the Oral Cavity

Each subject was fully evaluated in an examination room with the owner present, where subject history was collected. A visual awake oral examination was also performed at that time. During the visual awake oral examination, the veterinarian assessed the gingival health by careful visual inspection of the oral cavity of the awake subject, as described previously.[17] Gingival health was categorized into any one of the following mutually exclusive categories:

- No gingival inflammation
- Mild gingival inflammation
- Moderate-to-severe gingival inflammation

Thiol-Detection Test

While in the examination room and awake, each subject ~~was~~ also had a colorimetric thiol-detection test[a] performed by a veterinary technician. The test strip was glided along the subject's maxillary facial gingival margin to collect a sample of oral fluid. After 10 seconds, the color test result was read by an examiner blinded to the result of the visual awake oral examination. The color test result was recorded and placed into one of the following categories:

- 0 (0 μM thiol)
- 1 (12.5 μM thiol)
- 2 (50 μM thiol)
- 3 (100 μM thiol)
- 4 (200 μM thiol)
- 5 (400 μM thiol)

The veterinarian responsible for performing the visual awake oral examination and the full-mouth anesthetized oral examination was blinded to the results of the thiol-detection test.

Full-Mouth Anesthetized Examination

Each subject received propofol intravenously and an inhalant anesthetic. In those patients in which a pre-anesthetic anticholinergic was indicated, the anticholinergic was administered only after the visual awake examination and the thiol-detection test was conducted to ensure the gingival tissues were not abnormally dry. A full-mouth oral examination using a periodontal probe and full-mouth digital dental radiographs were performed while the subject was under general anesthesia. The Gingival Index as developed by Löe and Silness was recorded as previously described to determine the severity of active periodontal disease.[17,18] Each tooth was assigned one of the following Gingival Index scores:

- 0: Normal (absence of inflammation)
- 1: Mild inflammation (slight change in color, little change in texture, no bleeding on probing)
- 2: Moderate inflammation (moderate redness, edema, and hypertrophy, bleeding on probing)
- 3: Severe inflammation (marked redness and hypertrophy, tendency to spontaneous bleeding or ulceration)

For each subject, the maximum Gingival Index was determined for purposes of classification of the severity of active periodontal disease. A subject with one or more teeth having Gingival Index ≥ 1 was considered to have active periodontal disease. A subject with one or more teeth having Gingival Index ≥ 2 was considered to have moderate-to-severe active periodontal disease. Full-mouth digital dental radiographs were obtained for each subject to determine the presence and extent of alveolar bone loss by staging each tooth. Classification of periodontal disease was according to current American Veterinary Dental College Nomenclature Committee recommendations.[19]

Data Analysis

Summary statistics were prepared from compiled subject history and recorded data. Fisher exact test was employed for comparison of proportions (null hypothesis: no difference).[20] A standard significance level of 0.001 was used for all comparisons (i.e., differences were considered significant if $P < .001$).

Results

Of 118 subjects recruited for enrollment, 114 presented for study examination in conformity with the study protocol. The proportion of subjects who were 25 lbs or less, between 25 and 50 lbs, or greater

than 50 lbs were not statistically different, and no single breed predominated (data not shown). All study subjects who presented ($n = 114$) were determined by the full-mouth anesthetized examination to have active periodontal disease. 101 of those subjects had moderate-to-severe active periodontal disease, and 85 had alveolar bone loss. A breakdown of subject ages and the relationship of age to alveolar bone loss ($P < .001$) is shown in **Table 1**.

All dogs presented with active periodontal disease (Gingival Index ≥1) on the full-mouth anesthetized examination (data not shown). Of the 114 dogs presenting with active periodontal disease on the full-mouth anesthetized examination, only 94 presented with inflammation on the visual awake examination, whereas 113 out of 114 presented with a positive result on the thiol-detection test. Of the 20 subjects that appeared to have no visible inflammation of the gingiva on the visual awake examination, all 20 presented with active periodontal disease, and 19 of the 20 yielded a positive result on the thiol-detection test.

Table 2 shows summary data concerning the relationship of the visual awake examination result or the thiol-detection test result to a definitive clinical finding of moderate-to-severe active periodontal disease (Gingival Index ≥2; i.e., bleeding on probing) on the full-mouth anesthetized examination. Of the 114 subjects, 101 presented with moderate-to-severe active periodontal disease. Of the 101 dogs presenting with moderate-to-severe active periodontal disease on the full-mouth anesthetized examination, only 40 presented with moderate-to-severe inflammation on the visual awake examination, whereas 94 presented with a result of 2–5 on the thiol-detection test. The proportion of dogs presenting with visible inflammation on the visual awake examination (94/114) was statistically significantly different ($P < .001$) from the proportion of dogs actually having active periodontal disease (114/114), whereas the proportion of dogs having a positive thiol-detection test result (113/114) was not statistically different from the proportion having active periodontal disease. Of the 20 subjects who appeared to have no visible inflammation of the gingiva on the visual awake examination, 11 presented with moderate-to-severe active periodontal disease, of whom all 11 yielded a positive result on the thiol-detection test.

TABLE 1

Prevalence of Alveolar Bone Loss in Subjects of Various Ages

Age, yr	No. of Subjects	No. of Subjects with Alveolar Bone Loss	Prevalence of Alveolar Bone Loss
<1	12	2	17%
1–2	17	9	53%
≥3	85	74	87%
Total	114	85	75%

TABLE 2

Visual Awake Examination Observation or Thiol-Detection Test Result as Related to Definitive Clinical Finding of Moderate-to-Severe Active Periodontal Disease (Gingival Index ≥2; i.e., Bleeding on Probing) on Full-Mouth Anesthetized Examination

Clinical Finding	Positive (GI ≥2) on Full-Mouth Anesthetized Examination ($n = 101$)	Negative (GI ≤1) on Full-Mouth Anesthetized Examination ($n = 13$)
Observation on visual awake examination		
No inflammation	11	9
Mild inflammation	50	3
Moderate-to-severe inflammation	40	1
Result of thiol-detection test		
0 (0 μM thiol)	0	1
1 (12.5 μM thiol)	7	5
2 (50 μM thiol)	28	6
3 (100 μM thiol)	36	1
4 (200 μM thiol)	21	0
5 (400 μM thiol)	9	0

GI, Gingival Index.

Table 3 shows summary data concerning the relationship of the visual awake examination result or the thiol-detection test result to a definitive clinical finding of alveolar bone loss (periodontal stage ≥2) on the full-mouth anesthetized examination. Of the 114 subjects, 85 presented with alveolar bone loss. Of the 85 dogs presenting with alveolar bone loss on the full-mouth anesthetized examination, only 41 presented with moderate-to-severe inflammation on the visual awake examination, whereas 81 presented with a result of 2–5 on the thiol-detection test. The proportion of subjects having alveolar bone loss who had a thiol-detection test result of 2–5 (81/85) was significantly different ($P < .001$) from the proportion showing moderate-to-severe inflammation (41/85) on the visual awake examination.

Table 4 shows summary data concerning the thiol levels detected in oral fluid and the maximum Gingival Index determined during the full-mouth anesthetized examination. Maximum Gingival Index increased with increased inflammation noted on the visual awake examination ($P < .001$). Maximum Gingival Index also increased with increased result on the thiol-detection test ($P < .001$).

TABLE 3

Visual Awake Examination Observation or Thiol-Detection Test Result as Related to Definitive Clinical Finding of Alveolar Bone Loss (Periodontal Stage ≥2) on Full-Mouth Anesthetized Examination

Clinical Finding	Positive (Alveolar Bone Loss) on Full-Mouth Anesthetized Examination ($n = 85$)	Negative (No Alveolar Bone Loss) on Full-Mouth Anesthetized Examination ($n = 29$)
Observation on visual awake examination		
No inflammation	4	16
Mild inflammation	40	13
Moderate-to-severe inflammation	41	0
Result of thiol-detection test		
0 (0 μM thiol)	0	1
1 (12.5 μM thiol)	4	8
2 (50 μM thiol)	18	16
3 (100 μM thiol)	34	3
4 (200 μM thiol)	20	1
5 (400 μM thiol)	9	0

Discussion

The results of the present study show how the prevalence and the severity of periodontal disease are underestimated when the visual awake examination is inappropriately relied upon as a substitute for the anesthetized oral examination. Although all 114 subjects presented with active periodontal disease (stage 1 or higher periodontal index), only 101 of these subjects had a maximum Gingival Index of 2 or 3, (showed bleeding on probing during the full-mouth anesthetized examination). 85 subjects presented with alveolar bone loss (stage 2 or higher periodontal index). Two of the subjects with alveolar bone loss were less than 1 year of age, and more than half (53%) of the subjects 1 to 2 years of age already had alveolar bone loss (Table 1).

Because all subjects presented with active periodontal disease on the full-mouth anesthetized examination, the present study did not compare the effectiveness of either test modality at distinguishing between dogs with active periodontal disease and those without. However, the proportion of dogs presenting with visible inflammation on the visual awake examination (94/114) was statistically and significantly different ($P < .001$) from the proportion of dogs actually having active periodontal disease (114/114), whereas the proportion of dogs having a positive thiol-detection test result (113/114) was not statistically different from the proportion having active periodontal disease. Reliance solely on the visual awake examination would therefore result in underestimation of active periodontal disease.

Of the 114 subjects, 101 were found to have moderate-to-severe active periodontal disease on the full-mouth anesthetized examination (Table 2). The proportion of subjects having moderate-to-severe active periodontal disease who had a thiol-detection test result of 2–5 (94/101) was significantly different ($P < .001$) from the proportion showing moderate-to-severe inflammation (40/101) on the visual awake examination.

Of the 114 subjects, 85 were found to have alveolar bone loss on the full-mouth anesthetized examination (Table 3). The proportion of subjects having alveolar bone loss who had a thiol-detection test result of 2–5 (81/85) was significantly different ($P < .001$) from the proportion showing moderate-to-severe inflammation (41/85) on the visual awake examination. Of the 20 subjects showing no inflammation on the visual awake examination, 4 (20%) had alveolar bone loss; of the 53 subjects showing only mild inflammation on the visual awake examination, 40 (75%) had alveolar bone loss.

As shown in Table 4, maximum Gingival Index increased with increased inflammation noted in the visual awake examination ($P < .001$). However, even dogs with no inflammation on the visual awake examination had active periodontal disease (average maximum Gingival Index = 1.6). Maximum Gingival Index also increased with increased thiol-detection test result ($P < .001$). Even dogs with the lowest detectable level of thiol (12.5 μM) had active periodontal disease (average maximum Gingival Index = 1.6).

For veterinary professionals to properly focus on early detection of disease, along with prevention of disease progression once that

TABLE 4

Visual Awake Examination Observation or Thiol-Detection Test Result as Related to Severity of Active Periodontal Disease Detected on Full-Mouth Anesthetized Examination

Clinical Finding	No. of Subjects	Maximum Gingival Index (Average)
Observation on visual awake examination		
No inflammation	20	1.6
Mild inflammation	53	2.1
Moderate-to-severe inflammation	41	2.7
Result of thiol-detection test		
0 (0 μM thiol)	1	1
1 (12.5 μM thiol)	12	1.6
2 (50 μM thiol)	34	2
3 (100 μM thiol)	37	2.4
4 (200 μM thiol)	21	2.5
5 (400 μM thiol)	9	2.7

disease is detected, leading indicators of disease are needed.[21] With respect to periodontal disease, the need is particularly pressing, because dogs with more advanced gingivitis (i.e., active periodontal disease) progress more rapidly to irreversible bone loss.[22] The present study shows that the visual awake examination can disclose the presence of underlying periodontal disease, but only after the disease has progressed. The insufficiency of the visual awake examination is most apparent in subjects who presented with no inflammation or only mild inflammation visually when the awake examination was performed. In contrast, the thiol-detection test disclosed underlying active periodontal disease at its earliest stages of progression. Each tested modality generally gave a higher result when disease was more severe, but with the thiol-detection test result starting to trend upward earlier in the disease process. This is consistent with the natural history of periodontal disease, which develops in the gingival sulcus, an anatomical compartment that cannot be seen during the visual awake examination.[1–8] Rather, visible gingival inflammation detected during the visual awake examination reflects soft tissue damage that has already taken place. In many instances, this visible soft tissue damage is already accompanied by loss of calcified tissue (i.e., alveolar bone loss).

In a landmark survey of 52 private veterinary practices in the United States, veterinarians reported a prevalence of gingivitis among 31,484 dogs of up to 20% but a prevalence of periodontitis of as low as 2%.[23] However, the study expressly excluded referral and specialty practices.[23] In a recent study from England, the prevalence of periodontal disease was reported by general practice clinics as 9.3%.[24] When a full-mouth anesthetized examination has been performed, the reported prevalence of periodontal disease is much higher, ranging from 60% to more than 80%.[25,26] The present study supports the conclusion that the prevalence and the severity of periodontal disease are underestimated, and periodontal disease is therefore underdiagnosed if the visual awake examination is relied upon to detect periodontal disease. A positive result on the visual awake examination typically discloses periodontal disease that may already be in advanced stages.

The present study confirms the results of Manfra et al. in proving the clinical utility of a thiol-detection test at disclosing the severity of underlying periodontal disease.[17] The present study also extends the results of Manfra et al. by validating the clinical utility of the thiol-detection test in disclosing the presence of active periodontal disease, when tissue destruction is occurring.

A limitation of the present study is that all examinations were performed by one examiner within a single veterinary dental clinic. The examiner in this instance was a veterinarian with 30 yr of experience in veterinary dentistry. The concordance of the present results with those of Manfra et al., in which there was an examiner different from the examiner in the present study but also an expert in veterinary dentistry, indicates that this limitation does not endanger the validity of the present results.

There is a compelling reason to detect periodontal disease as early in its development as possible: to prevent its progression. This requires action by the veterinarian and staff members and compliance by the pet owner. It has been shown that treatment of the early stages of periodontal disease is safe and can lead to better quality of life for the patient.[27] As the present study shows, this is important throughout a patient's life, beginning as soon as permanent teeth have erupted, and demands increasing attention as patients age.[28] For the patient to gain maximum benefit, home care is critical, requiring a high degree of consistent compliance to be most effective.[29]

While periodontal infection often goes unseen beneath the gingiva, it can affect the health of the dog from its very earliest stages.[16] When periodontal infection is treated, however, there can be marked improvement in the animal's overall health.[12] A thiol-detection test may be used to demonstrate to the pet owner the presence and severity of the patient's periodontal infection and to highlight the need for a full-mouth anesthetized examination to make a proper diagnosis and develop a treatment plan.[9,30] This is particularly important because pet owners tend to underestimate the disease burden their pets bear.[10]

Periodontal disease has been shown to be closely associated with life-threatening comorbidities including heart, liver, and kidney disease.[10–16] Long-term client compliance with a veterinarian's recommendations, and hence the patient's well-being, may be enhanced when there is a straightforward and efficient way to demonstrate to the client in the examination room that a periodontal infection is present and needs to be acted upon. The present study demonstrates that a thiol-detection test can detect the presence and severity of periodontal disease in the examination room in the awake animal, opening the door for timely dialogue with the pet owner concerning the need for a full-mouth anesthetized examination, dental radiographs, and development of a treatment plan, including implementing daily oral home care. Such an approach holds the promise that there will be fuller utilization of veterinary dental services and consequently the improved long-term health of canine patients.[31]

Conclusion

In conclusion, the present study found that periodontal disease was highly prevalent in study subjects, and that alveolar bone loss had occurred even in dogs less than 1 year of age. In the examination room, a thiol-detection test identified underlying active periodontal disease at early stages of the disease, and earlier than the visual awake

examination. Reliance on the visual awake examination alone would have led to underestimation of the presence and severity of periodontal disease present beneath the gum line. This is consistent with current American Animal Hospital Association guidelines which recommend the use of a thiol-detection test in conjunction with the visual awake examination as the standard of care in routine wellness examinations.[13]

Funding for this study was provided by PDx BioTech (Lexington, Kentucky).

FOOTNOTES

[a] OraStrip QuickCheck Canine; PDx BioTech, Lexington, Kentucky

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