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Quantification of Canine Dental Plaque Using Quantitative Light-Induced Fluorescence

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Abstract

The aim of this work was to evaluate Quantitative Light-induced Fluorescence (QLF™) as an alternative to the established Logan and Boyce method for determining plaque coverage of dogs' teeth. In a series of studies in conscious and anesthetized dogs, QLF showed good intra-photographer repeatability (coefficient of variation [CV] of 7.5% for undisclosed teeth) and inter-photographer reproducibility (CV of 3.2% for undisclosed teeth and 8.5% for disclosed teeth). The QLF software accurately identifies areas of plaque as demonstrated by comparison to the variability of 5 human scorers, manually marking plaque on QLF-acquired images ($P = 0.1$). There was good agreement with the modified Logan and Boyce method in the percentage reduction in plaque accumulation measured when dogs were fed an oral care chew versus no chew. To see a 15% difference in plaque accumulation, which is considered sufficient by the Veterinary Oral Health Council to differentiate between 2 treatments, a retrospective power analysis (90%) of the data established that only 7 dogs would be required, compared to 19 dogs for the modified Logan and Boyce method. QLF is a reliable method for measuring dental plaque in dogs with the added advantage that it is not subjective and requires fewer animals.

Keywords

dental plaque, dog plaque index, planimetry, reproducibility, sensitivity, accuracy, QLF

Introduction

Periodontal disease is the most widespread oral disease in dogs with prevalence estimates ranging from 44% to 64%.[1-4] Dental plaque is an important etiological factor in the development of the disease.[5] If allowed to accumulate and mature, plaque leads to an inflammatory response (gingivitis) that can ultimately give rise to periodontitis and destruction of the periodontal ligament and alveolar bone that supports the tooth. This can be painful and ultimately lead to tooth loss.[6] The earliest stage of the disease can be managed with early identification and intervention, which could be in the form of oral hygiene products.

Evaluating the quantity of plaque on the tooth surface is essential for determining the efficacy of oral hygiene products. Numerous methods for plaque quantification have been used in human dental research including plaque indices and planimetric analysis. Plaque indices generally involve the use of a disclosing solution and then quantification of plaque based on estimates of the area of tooth covered by the dye or the intensity of the color to estimate thickness of plaque.[7-10] There has been much criticism of these methods with respect to their resolution, subjectivity, and need for examiner training.[11] Planimetric analysis generally involves disclosing plaque, with subsequent photography of the tooth surfaces.[12,13] The images are then either traced by hand and the area of plaque calculated or they are digitized and analyzed using computer software. Studies found that computer-based plaque analyses are more reliable,[14] more precise,[15] more objective,[14-16] and more sensitive[15] than classic plaque indices. Furthermore, the calculation of plaque coverage on a continuous scale, as opposed to an ordinal scale as used in index methods, permits greater resolution. One aspect of planimetric techniques frequently mentioned is that they take account of plaque coverage but not plaque thickness.

Quantitative Light-induced Fluorescence (QLF™), a technique initially used for detecting caries lesions in humans, has

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also been employed to detect dental plaque.[11,17-19] This method either relies on the natural fluorescence of plaque under blue light (405 nm) or uses a standard disclosing solution to enhance bacterial fluorescence. The images are captured in real time using a modified version of a standard SLR camera, and image analysis software is then used to quantify the amount of plaque. The advantages and disadvantages of this technique are similar to other planimetric methods, but there is the additional major advantage that the greater contrast between the gingiva and the tooth, which is a feature of this technique, circumvents the need to manually define the tooth area accurately. This difference reduces the analysis time considerably and potentially increases accuracy when determining the plaque coverage of the tooth surface.

Several techniques for the quantification of plaque have been developed for use in cats and dogs. Routine methods include the modified Logan and Boyce plaque index which is used to quantify plaque accumulation on the buccal surface of the whole tooth[20] and the gingival contour plaque index (GCPI), which focuses on plaque that accumulates along the buccal gingival margin.[21,22] Both of these methods have been endorsed by the Veterinary Oral Health Council (VOHC; www.vohc.org) for supporting product claims relating to plaque control. *In vivo* product efficacy trials require a clean mouth model, where cats or dogs are anesthetized at the start of the study and at the end of each test phase so the teeth may be scaled and polished. Alternative methods that reduce the number of anesthetic procedures per animal, decrease the duration of anesthesia, reduce subjectivity, and improve accuracy (thereby reducing the number of animals required) are desirable to reduce the impact of the testing procedure on the animals involved. To our knowledge, the more reliable, objective, and sensitive planimetric methods such as QLF have not been described for use in dogs. Therefore, the aims of these studies were to evaluate the repeatability, reproducibility and accuracy of QLF for quantification of canine dental plaque and to compare this to an established clinical scoring system, namely, the modified Logan and Boyce[20] method.

Materials and Methods

The dogs included in the studies detailed subsequently were pair housed at the WALTHAM Centre for Pet Nutrition in environmentally enriched kennels and provided with a comprehensive dog–dog and dog–human socialization program adjusted to the needs of individual dogs. All dogs received a pre-study veterinary examination to ensure suitability for trial, which included a physical examination and an assessment of the dog's veterinary history. The studies were approved by the WALTHAM Animal Welfare and Ethical Review Body and run under licensed authority in accordance with the UK Animals (Scientific Procedures) Act 1986.

Intra-photographer Repeatability—Undisclosed Teeth

Eleven miniature schnauzer dogs, aged between 2.5 and 6.9 years (6 females and 5 males, weight range 7.0-10.2 kg), which had received a recent scaling and polishing and had little or no visible calculus, were recruited to the study. Dogs' teeth were brushed daily for approximately 1 week prior to the start of the trial using compact medium or soft brushes (TePe Oral Hygiene Products Ltd, Bronsåldersgatan 5213 76 Malmö, Sweden) and water. Dogs received no subsequent tooth brushing for 21 days when images of undisclosed teeth were captured using the commercially available QLF-D Biluminator 2 system (Inspektor Research Systems, Amsterdam, Netherlands; see section on QLF image acquisition and analysis for further details). Three repeated sets of images, 2 in the morning and 1 in the afternoon, were taken of conscious dogs by a single photographer. A set of images comprised 4 views around the mouth, 2 images on both the left and right hand side of the dog's mouth, were taken to visualize the maxillary first premolars (P1; 105, 205), second premolars (P2; 106, 206), third premolars (P3; 107, 207), and fourth premolars (P4; 108, 208).

Inter-photographer Reproducibility: Undisclosed Teeth

Twelve miniature schnauzer dogs, aged 3.1 to 7.5 years (7 females and 5 males, weight range 7.5-10.6 kg), that had their teeth brushed every other day as part of their normal oral care regimen from 1 year of age were allocated to 1 of the 3 groups based on time since their teeth were previously brushed. The purpose of this was to ensure that the reproducibility of QLF was assessed across the whole of the plaque coverage range: Group A had their tooth brushing stopped 21 days prior to examination, group B 10 days before the examination, and group C was tooth brushed the day before their examination. Three dogs, one from each group, were allocated to 1 of 4 consecutive assessment days on which 5 photographers captured images of undisclosed teeth using QLF.

The dogs were trained so that QLF images could be captured without the need for anesthesia and with minimal restraint (see section on QLF image acquisition and analysis for further details). Each photographer took 4 images of each dog capturing left and right maxillary third incisor (I3; 103, 203), canine (C; 104, 204), P1, P2, P3, and P4.

Inter-photographer Reproducibility: Disclosed Teeth

Seven miniature schnauzer dogs, aged 3 to 5.3 years (2 females and 5 males, weight range 7.2-10.8 kg), had their teeth brushed every other day up to the day before the start of the trial. As the dogs had not received a recent scale and polish, there was sufficient natural variation in the amount of plaque present to allow reproducibility across the plaque coverage range to be assessed adequately. The dogs were trained so that the teeth could be disclosed and QLF images captured without the need for anesthesia. For imaging plaque, the teeth on the dogs' right sides were first washed with 3 mL water using a plastic Pasteur pipette, and then 1 mL undiluted disclosing solution (GUM Red Cote liquid, Sunstar, Butler) was applied on the buccal surface of the teeth. The lip was dropped back to spread the disclosing solution, and excess solution was washed off with a further 3

mL of water. The QLF images were immediately taken of the disclosed teeth by 3 photographers in close succession to reduce the effect conferred by loss of stain over time on the observed plaque coverage. This method was then repeated on the left side of the dog. Each photographer took 4 images of the maxilla of each dog capturing the I3, C, P1, P2, P3, and P4 on each side.

Accuracy

The ability of the QLF software to identify plaque correctly was determined by comparing the software results with those from 5 human scorers who had manually marked plaque on QLF-acquired images in an image-processing package as described subsequently. The 5 human scorers (including 2 veterinary dentists) were trained to be able to assess plaque coverage using the modified Logan and Boyce method. A test set of QLF images, anesthetized dogs with disclosed teeth, were selected to contain examples of teeth with a range of plaque coverages. This set contained 54 teeth in 30 QLF images from 9 dogs. Raw images were opened in Adobe Photoshop software (Version CC, Adobe Systems Inc, San Jose, California), and 54 teeth were selected as individual layers using the quick selection tool to outline each tooth. Each scorer independently marked plaque areas using a brush (hardness 100%), scorers were allowed to resize the brush as appropriate. Plaque coverage for each tooth was determined by the percentage of pixels within the tooth area marked as plaque in relation to total tooth area. For visual comparison of the agreement between the 5 scorers and the QLF software, an image projection for each tooth was rendered using Image J (image processing program developed at the National Institutes of Health) by stacking each plaque image from the 5 scorers.

Comparison to Modified Logan and Boyce Method

A randomized cross-over trial, a study design endorsed by the VOHC, was undertaken to determine the agreement between QLF and modified Logan and Boyce in distinguishing the levels of plaque on the teeth of dogs fed a commercially available oral care chew (OC chew) compared to with no chew. Twenty-six miniature schnauzer dogs aged between 1.4 and 8.2 years (11 females and 15 males, weight range 7.1-12.5 kg) were included in the study. They were divided into 2 groups where 1 group was fed a daily OC chew in phase 1 and no chew in phase 2 of the study, and the other group received no chew in phase 1 and a daily OC chew in phase 2. Each test phase lasted for 28 days. For the duration of the study, all dogs received a single batch of a commercially available dry diet (Royal Canin Medium Adult), which conformed to the National Research Council Nutrient Guidelines 2006[23]; dogs were fed according to their individual energy requirement to maintain bodyweight. On chew feeding days, the amount of main meal was reduced to account for the calorie content of the chew. Each day, 30 g of the diet was removed from the main meal and used for the purpose of training the dogs as part of their normal socialization routine.

At the start of the study, each dog received a full mouth scaling and polishing followed by 7 days of tooth brushing to maintain oral health. Dogs also received a full mouth scaling and polishing at the end of each test phase. All examinations and full mouth scale and polishes were performed under general anesthesia. Dogs were fasted overnight and following a premedication of acepromazine (0.05 mg/kg) and buprenorphine (0.02 mg/kg), general anesthesia was induced by an injection of propofol (4 mg/kg) via an intravenous catheter. Gas anesthesia was maintained with oxygen and isoflurane via a cuffed endotracheal tube.

At the end of each test phase, plaque (coverage and thickness) was scored using a modified Logan and Boyce technique.[20] The overall plaque score for each tooth half (gingival and coronal) was calculated by multiplying the coverage and thickness scores. Gingival and coronal scores were then added to give the total tooth score. The mean of all tooth scores provided the mouth score. The following teeth were included in the assessments: Maxillary I3, C, P2, P3, P4 and first molar (M1; 109, 209), and mandibular C, P2, P3, P4, and M1 (309, 409). Five examiners determined plaque coverage and thickness scores, and all received training by a recognized European specialist in veterinary dentistry and were calibrated 2 weeks prior to the start of the trial to ensure consistency between examiners.

During anesthesia, QLF images of undisclosed and disclosed teeth were captured. In addition, undisclosed QLF images were taken from 10 of the dogs consciously at the end of each test phase prior to the dog being placed under general anesthetic. Only images of the maxillary I3, C, P3, and P4 were captured consciously due to difficulties accessing the caudal maxillary and mandibular teeth.

Data were excluded from the analysis where the protocol was not correctly followed. This included occasions where the dog consumed the chew on fewer than 26 of the 28 days offered, where the dog was inappropriately fed the chew or where the dog's teeth were brushed by mistake. This resulted in 5.7% of the data being excluded. In addition, images where all 18 teeth specified by the VOHC were not visible by QLF were also excluded to allow direct comparison with the standard modified Logan and Boyce protocol. This accounted for a further 8.7% of the data. The teeth defined by the VOHC are the maxillary I3, C, P3, P4, M1, and mandibular C, P3, P4, M1 which must be scored for any trials that support VOHC product claims relating to plaque coverage.

QLF Image Acquisition and Analysis

For conscious imaging, dogs were trained to sit on a low table and to have their lips held open, either using fingers or a plastic cheek retractor (Mirahold child's cheek retractor, Henry Schein, 135 Duryea Road, Melville, NY 11747), to allow visualization of the upper jaw. In addition, dogs were trained to accept the presence of the QLF camera.

On average, it took 6 weeks to train dogs for QLF image capture when provided with 30-minute sessions each day (20-25 hours). These dogs had also received mouth handling from about 4 weeks of age and were confident with tooth brushing.

The QLF-D Biluminator 2 system was used for imaging of both undisclosed and disclosed teeth. It is based on a full-sensor

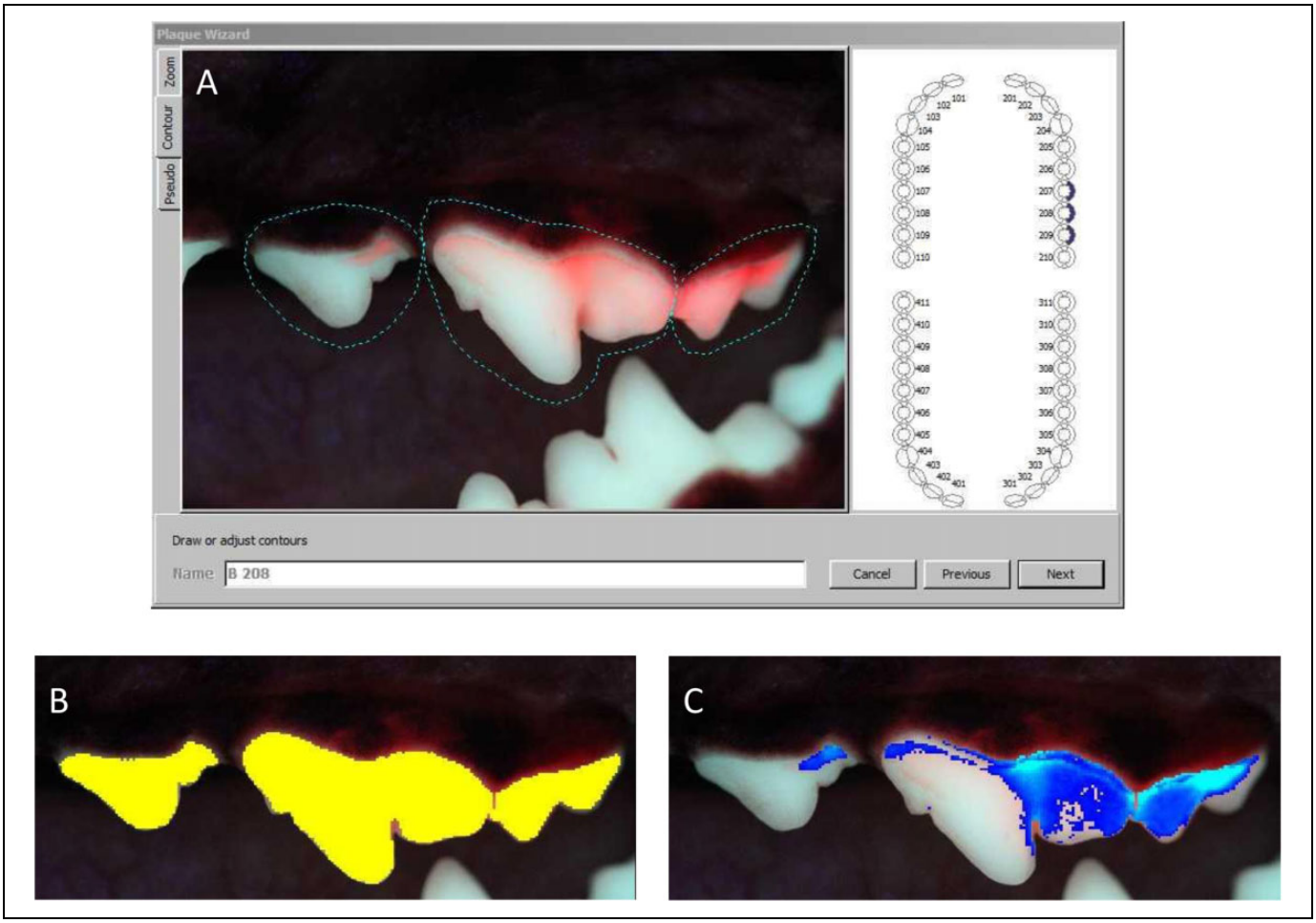

Figure 1. Inspektor Pro Image analysis software. (A) contouring and naming of teeth, (B) software identification of tooth (yellow); and (C) software identification of plaque (blue).

SLR camera Canon 450D. The camera is equipped with an illumination tube with white and blue LEDs placed in a ring around the lens opening (the Biluminator). The lens also comprises differential filtering allowing both normal and fluorescence photography using the same camera. Photograph capture is managed via image capture software on an attached personal computer.

For undisclosed teeth, the QLF system works on the principle that if teeth are illuminated with a blue light (405 nm), the plaque will naturally fluoresce with red light, which is then captured via a band-pass filter and camera. Disclosed plaque also fluoresces red against the white fluorescence of the teeth. The examinations were conducted in a darkened room to maximize the quality of the QLF images captured.[24] The individual image was inspected at the time of taking for quality control and if teeth were missing from the frame, obscured, or blurred, another image was immediately taken.

The red fluorescence of plaque in the undisclosed QLF images was analyzed using a modified version of the proprietary software associated with the unit (Inspektor-Pro QA2 version 1.23). The modifications were co-developed by Inspektor Research Systems BV to enable the more rapid annotation and analysis of imaged teeth. Modifications included a new tooth masking tool and canine dentition-specific annotation of each mask to reduce transcript error when the data were exported. Briefly, a region of interest was defined by drawing roughly around the tooth using an interface within the masking software (Figure 1). The software was then able to identify the tooth area within this outline. Each contoured tooth was named and the software calculated the percentage plaque coverage, which is the percentage of pixels within the tooth surface classified as plaque in relation to total tooth area ($\Delta R\%$).[25] The $\Delta R30$ values were used for all subsequent analyses.

For images of disclosed teeth, when the level of plaque coverage was very high (and there was therefore very little clean tooth for comparison), the algorithm occasionally had difficulty identifying the area of plaque. To combat this, an image pre-processing step was included for all disclosed images prior to analysis. The QLF images were opened in Photoshop CC, and a standardized spot of clean tooth devoid of plaque was added as a reference point to each image in order to baseline the algorithm. Images were analyzed in QA2 software with the additional tooth spot included in the contouring. The additional spot added a negligible increase in pixel counts.

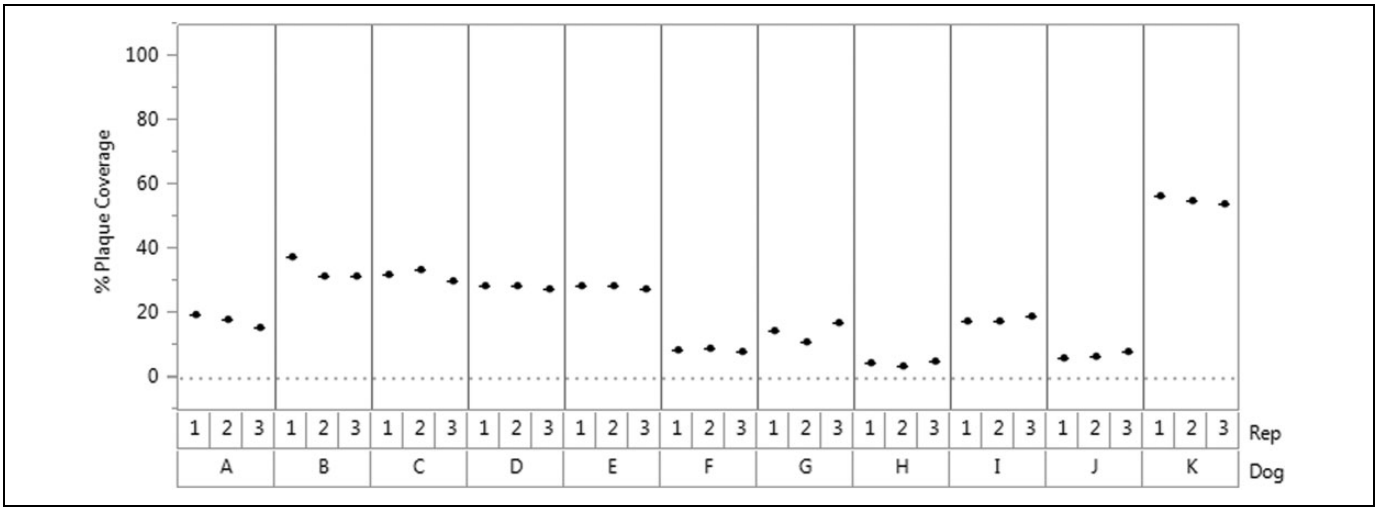

Figure 2. Intra-photographer repeatability of a single photographer taking images of undisclosed teeth of conscious dogs. Variability chart of percentage plaque coverage (whole mouth average: maxillary first, second, third, and fourth premolars) as determined by Quantitative Light-induced Fluorescence (QLF) on undisclosed teeth, by dog (A-K) and repetition (1-3).

Images were scrutinized for quality in terms of focus, parts of teeth obscured, illumination, or any other artifacts that could have affected the analysis. During this process, it was observed that for undisclosed images, in rare instances where there were very high levels of plaque, the algorithm occasionally identified that the whole tooth was covered in plaque but reported plaque coverage as 0%. In this instance, a value of 100% plaque coverage was imputed.

Statistical Analysis

Intra-photographer repeatability. Linear mixed effects models (Restricted maximum likelihood [REML]) were used to estimate variance components of the percentage plaque coverage, using repeat nested within dog as random effects. First, a model for an average mouth (maxillary P1, P2, P3, and P4) was used, followed by assessment of each tooth type. The percentage of variability that was accountable to repeatability and the percentage of coefficient of variability (%CV; repeatability standard deviation relative to the overall mean of the model) were then calculated.

Inter-photographer reproducibility. Linear mixed models (REML) were used to estimate variance components of the percentage plaque coverage, with photographer nested in dog as the random effects. The percentage of variability accountable to the photographer and the %CV (reproducibility standard deviation relative to the overall mean of the model) were then calculated.

Accuracy. The accuracy of the software was determined by comparing its results with those of human scorers. Whole mouth scores from 9 dogs, as assessed by 5 human scorers, were analyzed by a linear mixed model with scorer nested in dog fitted as the random effects. The variance estimates were then used to inform a simulation of 1000 scorers (assuming each scorer assessed 9 dogs) with an average of 46.8% plaque coverage (as was found from the 5 human scorers). The probability of the QLF software results falling within the distribution of the human scorers' results was calculated by the percentage of simulated scorers with an average less than the average QLF software score. A test level of 5% was used.

Table 1. Intra-photographer Repeatability, One Photographer Capturing Images of Undisclosed Teeth of Conscious Dogs: Average Percentage Plaque Coverage and Variability on Premolars; P1 (105, 205), P2 (106, 206), P3 (107, 207), and P4 (108, 208).

Teeth	Average Percentage Plaque Coverage	Standard Deviation	Percentage Variability	Percentage Coefficient of Variation
105	12.9	1.0	0.3	7.4
106	14.9	1.5	1.0	10.1
107	32.8	1.1	0.5	3.4
108	41.9	1.1	0.3	2.5
205	13.0	1.8	1.2	14.1
206	11.0	1.9	3.7	17.5
207	26.1	1.4	0.5	5.4
208	36.4	2.1	1.3	5.8

Comparison to modified Logan and Boyce. The percentage plaque coverage measured by QLF and modified Logan and Boyce, averaged for all teeth, were analyzed by linear mixed models with dog as a random effect and chew type as a fixed effect. This was used to assess the difference in mean plaque scores between chew types, at the 5% significance level. The mean and difference between mean plaque scores for each chew type are reported with 95% confidence intervals. These data, and their associated variances, were then used to inform retrospective sample size analyses for a 2-way crossover trial to detect a 15% reduction (as defined as relevant by the VOHC) in plaque accumulation compared to no chew with at least 90% power.

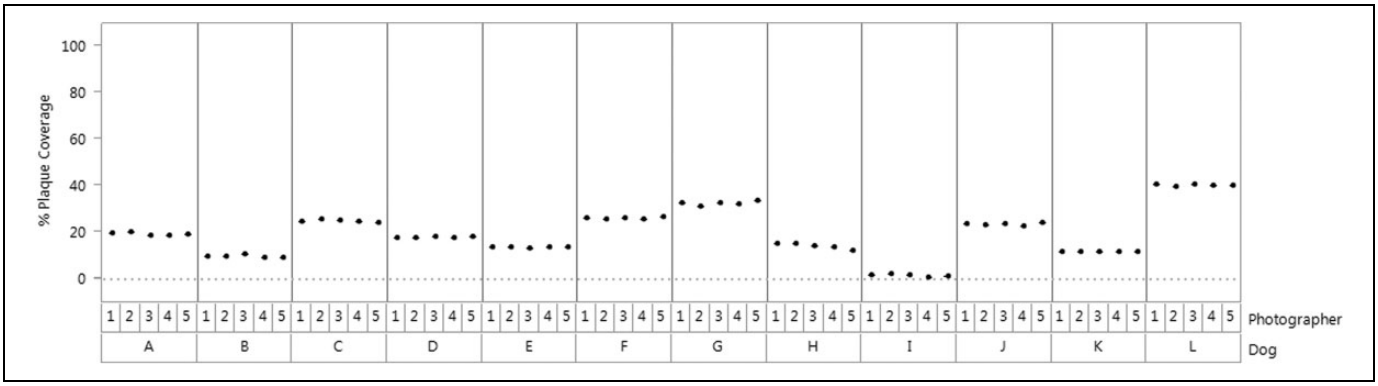

Figure 3. Inter-photographer repeatability of 5 photographers taking images of undisclosed teeth of conscious dogs. Variability chart of percentage plaque coverage (whole mouth average: maxillary third incisors, maxillary and mandibular canines and third and fourth premolars) as determined by Quantitative Light-induced Fluorescence (QLF) by dog (A-L) and photographer (1-5).

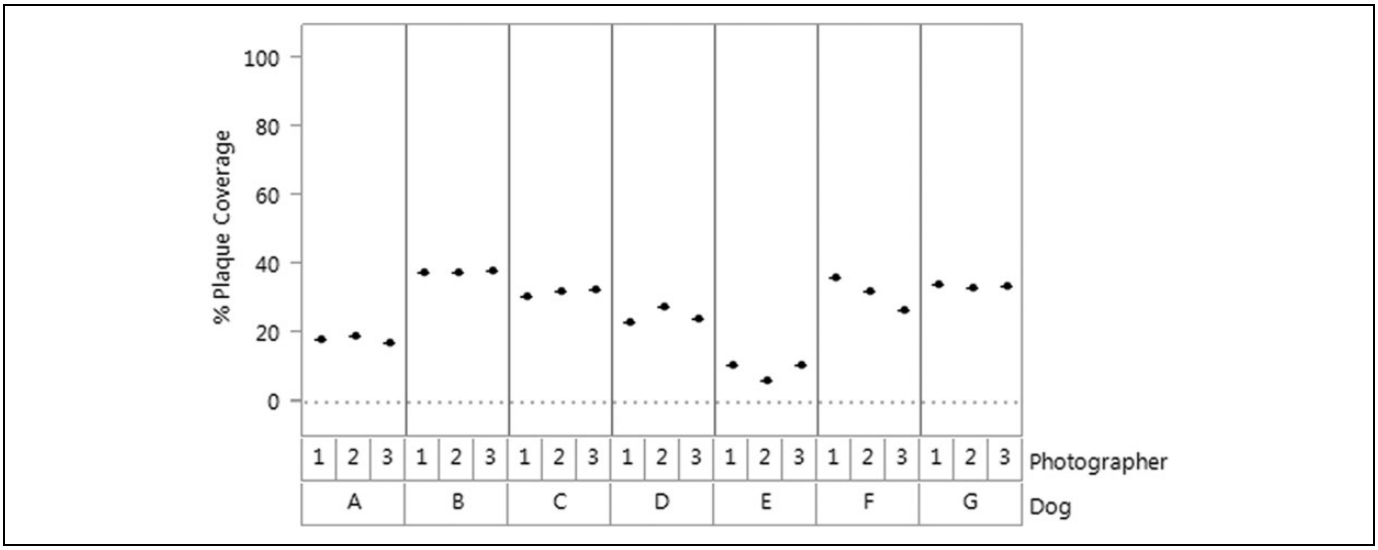

Figure 4. Inter-photographer repeatability of 5 photographers taking images of disclosed teeth of conscious dogs. Variability chart of percentage plaque coverage (whole mouth average: maxillary third incisors, maxillary and mandibular canines, and third and fourth premolars), as determined by Quantitative Light-induced Fluorescence (QLF) by dog (A-G) and photographer (1-3).

Comparison between conscious and unconscious imaging. The percentage plaque coverage as measured by QLF of undisclosed teeth from conscious (average of upper jaw teeth) and anesthetized (average of all teeth) dogs was analyzed using linear mixed models. Dog was included as a random effect and chew type, measure type, and their interactions were included as fixed effects. Contrasts were performed within and between measure types at a family wise controlled error rate of 5% (R v3.02 using libraries nlme and multcomp).

Results

Intra-photographer Repeatability: Undisclosed Images

Variance components analysis of data from 264 images of undisclosed maxillary teeth (P1, P2, P3, and P4) from 11 conscious miniature schnauzers was used to quantify the intra-photographer repeatability of a single photographer and showed that the repeatability coefficient of variability (standard deviation relative to the mean plaque coverage) was 7.5% (Figure 2).

The intra-photographer repeatability component of variability showed that the QLF method was highly repeatable and accounted for <1.4% of the total variability for most teeth. The exception was tooth 206 where it accounted for 3.7% of the variability (Table 1). When the variance components were made relative to the mean plaque coverage for each tooth, this showed that the %CV ranged from 2.5% to 17.5% (Table 1). The P1 and P2 had the highest %CV ranging from 7.4% to 17.5%, and these teeth also had the lowest average percentage plaque coverage ranging from an average of 11.0% to 14.9%. The average percentage plaque coverage for the P3 and P4 ranged from 26.1% to 41.9% with %CVs ranging from 2.5% to 5.8%.

Figure 5. Visual representation of plaque identified by five human scorers marking plaque in Photoshop and plaque identified by the Quantitative Light-induced Fluorescence (QLF) software, on 4 sample disclosed teeth. "Overlay" is an amalgamation of the 5 scorers.

Inter-photographer Reproducibility: Undisclosed Images

The percentage plaque coverage was determined for 480 undisclosed maxillary teeth (I3, C, P3, and P4), 96 per photographer (n = 5), from 12 conscious miniature schnauzers. The teeth selected were based on the teeth scored using the modified Logan and Boyce method as is the case in standard product testing protocols. The mouth averages ranged from 1.2% to 41.2% plaque coverage. The inter-photographer reproducibility coefficient of variability was 3.21% (Figure 3). The variability in percentage plaque coverage scores for individual teeth, dogs, and photographers is shown in supplementary Figure 1.

Inter-photographer Reproducibility: Disclosed Images

The percentage plaque coverage was determined for 228 disclosed maxillary teeth (I3, C, P1, P2, P3 and P4), 76 per photographer (n = 3), from 7 conscious miniature schnauzers. The average mouth plaque values ranged from 6.5% to 38.4%. Again, if the whole mouth plaque score is based on the teeth scored using the modified Logan and Boyce (I3, C, P3 and P4), the variances attributable to the photographer were approximately 5% of the total variation, and the %CV was 8.5% (Figure 4). The variability in percentage plaque coverage scores for individual teeth, dogs, and photographers is shown in supplementary Figure 2.

Accuracy of QLF

The ability of the QLF software to identify plaque correctly was determined by comparison with plaque coverage levels determined by 5 human scorers manually marking plaque on QLF-acquired images (Figure 5). A high agreement in identification of plaque was seen between the 5 scorers ("overlay") and in comparison to the QLF software (Figure 6). Agreement

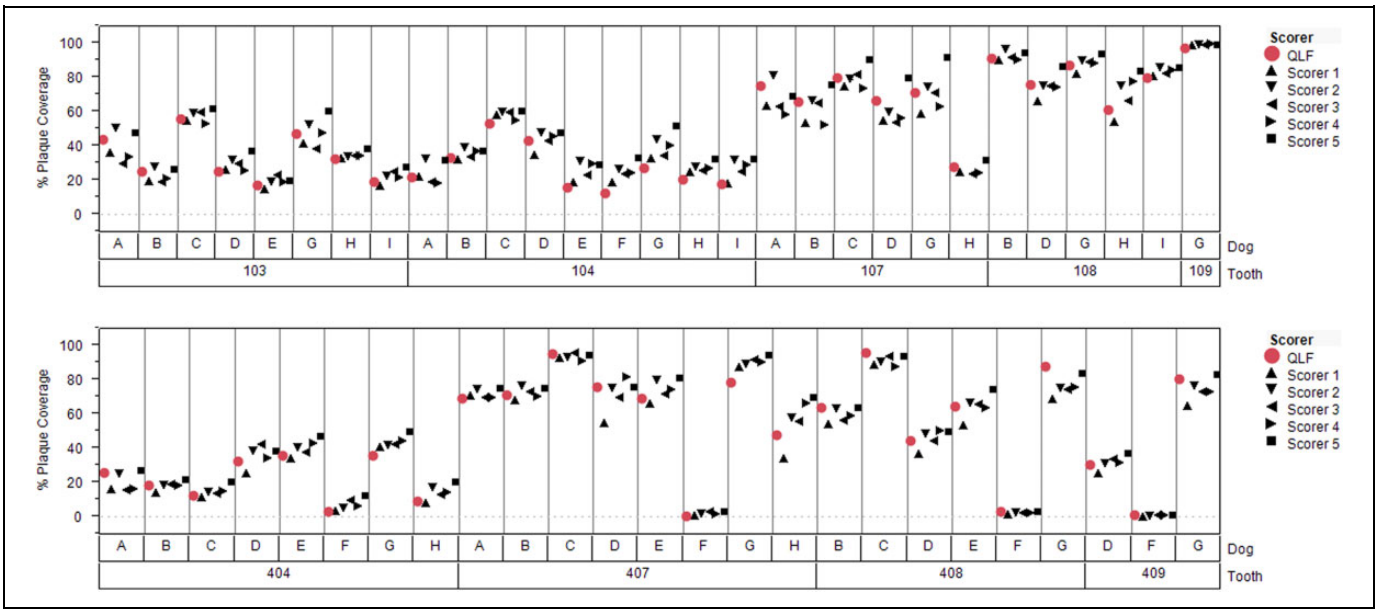

Figure 6. Variability chart of percentage plaque coverage identified by 5 human scorers marking plaque in Photoshop (black data points) and Quantitative Light-induced Fluorescence (QLF) software (red data points): Maxillary third incisors, maxillary and mandibular canines, and third and fourth premolars (disclosed teeth).

Table 2. Comparison of QLF (Undisclosed and Disclosed) to Modified Logan and Boyce for Measuring the Difference in Percentage Plaque Reduction Between Dogs Fed an OC Chew Compared With No Chew.

	Percentage mean plaque coverage (95% confidence intervals)			
Data type	OC Chew	No Chew	% Plaque Reduction	*P* Value
Modified Logan and Boyce	9.79 (8.83, 10.75)	12.57 (11.54, 13.59)	22.13 (12.64, 31.62)	<0.001
QLF disclosed	54.78 (51.72, 57.85)	67.73 (64.48, 70.98)	19.12 (14.09, 24.14)	<0.001
QLF undisclosed	10.35 (7.03, 13.66)	32.97 (29.48, 36.46)	68.62 (58.96, 78.27)	<0.001

Abbreviations: QLF, Quantitative Light-induced Fluorescence; OC, oral care.

was seen across the entire range of plaque coverage from 0.6% to 100% (min, max). Simulations of the variance of the 5 scorers showed the QLF software was not significantly different from the human scorers, with 10% of simulated human scorers having lower average percentage plaque coverage than the QLF software.

Comparison With Modified Logan and Boyce

A product efficacy trial was undertaken to determine the agreement of QLF to the modified Logan and Boyce scoring system. Analysis of QLF images of disclosed teeth showed an average reduction in plaque accumulation of 19.12%, with 95% confidence intervals (14.09%, 24.14%) when dogs received an OC chew compared to no chew (Table 2). This was similar to the results obtained using modified Logan and Boyce which gave an average reduction in plaque accumulation of 22.13% (12.64%, 31.62%). The QLF images were also taken of undisclosed teeth, while dogs were under anesthesia, and this showed a much greater difference, with an average reduction in plaque accumulation of 68.62% (58.96%, 78.27%). The mean percentage plaque coverage for the QLF images of disclosed teeth was 54.8% (51.7%, 57.9%) and 67.7% (64.5%, 71%) for dogs receiving an OC chew compared to no chew, respectively. However, for images of undisclosed teeth, the plaque levels were nearly 20% lower, 10.35% (7%, 13.7%) for dogs on the OC chew, and approximately 50% lower, 32.97% (29.5%, 36.5%), for dogs not receiving a chew.

A retrospective power calculation was performed and showed that for future studies 19 dogs would be required to see a 15% reduction in plaque accumulation for dogs receiving an OC chew compared with no chew using the modified Logan and Boyce method (with at least 90% power). By comparison, the number of dogs required to measure the same difference with QLF with disclosed and undisclosed images was 7 and 14 dogs, respectively (Figure 7).

The QLF images of undisclosed teeth were also taken of 10 dogs consciously at the end of each test phase of the cross-over study prior to being placed under anesthesia. There was a significant difference between OC chew and no chew for both dogs imaged consciously ($P < 0.001$), and the same 10 dogs imaged unconsciously ($P < 0.001$). The average plaque

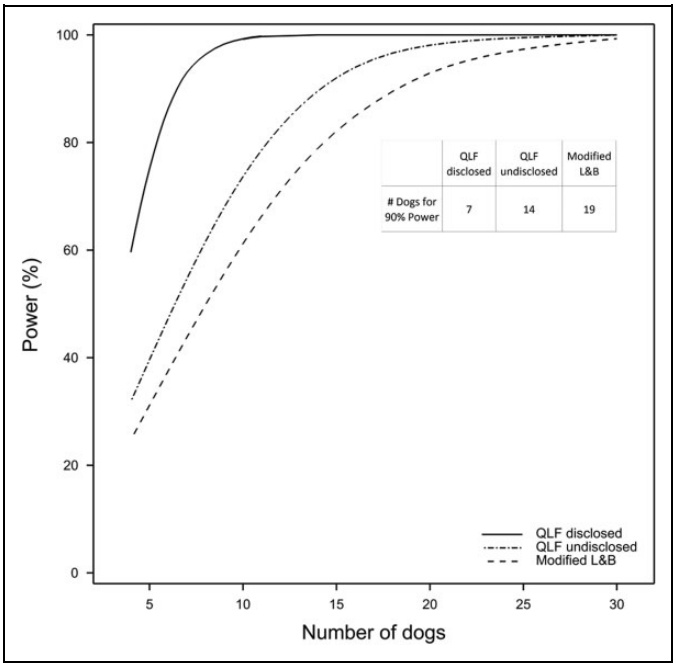

Figure 7. Number of dogs required to detect a 15% reduction in plaque accumulation when fed an oral care (OC) chew compared to no chew in a 2-way crossover trial. Solid line depicts Quantitative Light-induced Fluorescence (QLF; disclosed teeth), dashed line depicts the modified Logan and Boyce method, and the dot dashed line QLF (undisclosed teeth).

coverage for the dogs that were imaged consciously (undisclosed) was 27.7% (22.2%, 33.2%) and 7.6% (2.1%, 13.1%) for no chew and OC chew, respectively, which is a 72.6% (54.0%, 91.2%) reduction in plaque accumulation (Figure 8). When the same 10 dogs were imaged under anesthesia (undisclosed), the average plaque coverage was 30.5% (25.0%, 36.1%) for no chew and 9.5% (4.0%, 15.0%) when fed an OC chew which is a reduction in plaque accumulation of 69.0% (52.1%, 85.8%). No significant difference was found between conscious and unconscious dogs in the percentage of reduction in plaque accumulation between dogs fed the OC chew and no chew ($P = 0.984$; Figure 8), even though the conscious dogs were imaged on the upper jaw only. Examples of QLF images taken of conscious and unconscious dogs (disclosed and undisclosed teeth) are shown in Figure 9.

Discussion

We have shown that QLF is a reliable technique for measuring the plaque coverage on undisclosed and disclosed teeth of both anesthetized and conscious dogs. QLF showed good intra-photographer repeatability with a %CV of 7.5%. In the majority of teeth assessed, QLF accounted for <1.4% of the total variability with %CV ranging from 2.5% to 17.5%. The P1 and P2 had the highest variability (%CV of 7.4% to 17.5%) and the lowest levels of plaque coverage (<16% on average) but are not teeth usually assessed as part of product efficacy trials. The VOHC has defined a number of teeth (maxillary I3, C, P3, P4, M1 and mandibular C, P3, P4, M1) that should be scored for any trials that support product claims relating to plaque coverage. These were selected on the basis of functional importance, likelihood of accumulation of plaque and calculus, likelihood of being present in the mouth in the face of moderate periodontal disease, and size for ease of recording. The P3 and P4 teeth were the only VOHC teeth assessed in the intra-photographer repeatability study, and these teeth had high levels of plaque (average plaque coverage of 26% to 40%) and low %CVs (<6%).

Good Inter-photographer reproducibility for both undisclosed and disclosed dog's teeth has been demonstrated with whole mouth %CVs of 3.2% and 8.5%, respectively. This compares favorably with other plaque scoring methods. For instance, the whole mouth inter-grader variability of GCPI was reported as 18%.[21] In addition, it has been previously reported that experience is a significant factor when scoring plaque for research purposes using plaque index methods such as Logan and Boyce.[20] For our studies, photographers received a half-day training session on how to acquire the QLF images and also how to interact with the dogs, which clearly demonstrates that experienced photographers are not required to obtain precise measurements using QLF.

Although many studies report the precision and discriminating power of indexes for measuring plaque, very few determine the accuracy. We have shown, by comparing the identification of plaque by QLF software to 5 human scorers manually marking plaque on QLF acquired images, that the software is able to accurately identify areas of plaque and is accurate throughout the coverage scale. Visual inspection of the areas of plaque identified by the QLF software in comparison with the human scorers showed a high level of agreement.

This study has shown that it is possible to determine the plaque coverage on disclosed dog's teeth using QLF and that the reduction in plaque accumulation when dogs received an OC chew compared to no chew is comparable to the results obtained using the modified Logan and Boyce Index. A retrospective power analysis showed that fewer dogs are required to measure a reduction in plaque accumulation using QLF compared to the modified Logan and Boyce method. The modified Logan and Boyce method required 19 dogs to statistically show a 15% reduction in plaque accumulation when dogs are being fed an OC chew compared to no chew (with 90% power), whereas the QLF method required only 7 dogs.

It is also possible to visualize plaque using QLF without the need to disclose the teeth. However, the percentage of reduction in plaque accumulation observed for disclosed and undisclosed teeth when dogs were fed an OC chew compared to no chew was very different. This may relate to the bacteria responsible for the fluorescence. In human plaque, the fluorescence is attributed to porphyrins from the human periodontal pathogen *Porphyromonas gingivalis*.[26] Porphyromonad species are even more common in canine plaque than in human plaque, with *Porphyromonas cangingivalis* being the most prevalent of all canine oral species.[27] The fact that the mean plaque coverage for undisclosed teeth is lower than for disclosed teeth suggests

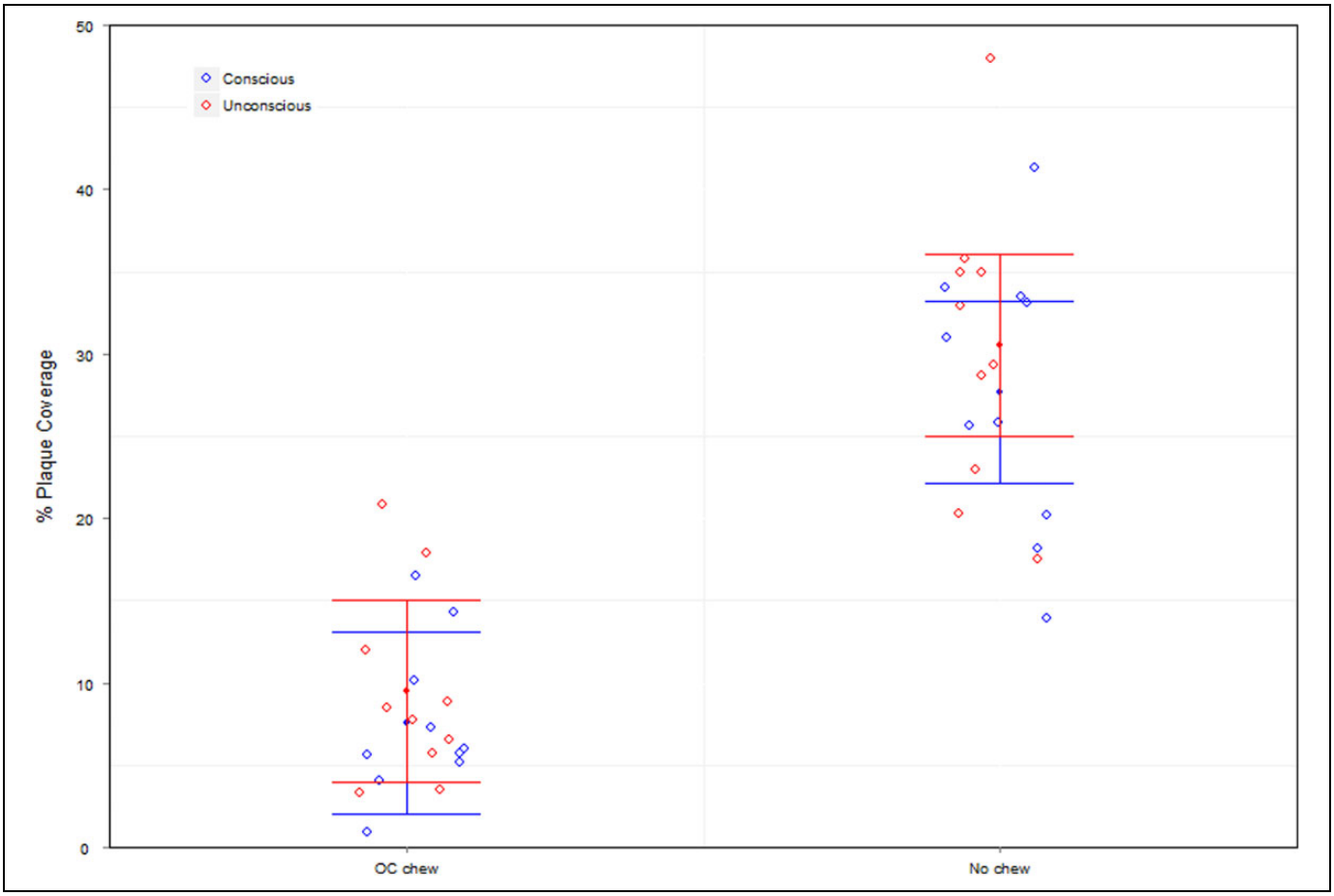

Figure 8. Average plaque coverage of maxillary jaw only (third incisors, canines, third and fourth premolars) of conscious dogs (blue dots) and maxillary jaw (third incisors, canines, third and fourth premolars, and first molars) and mandibular (canines, third and fourth premolars and first molars) of unconscious dogs (red dots) when fed an oral care chew versus no chew. Dots represent average percentage plaque coverage and bars depict 95% confidence intervals.

that not all the bacteria in mature biofilms autofluoresce and therefore QLF underestimates the amount of total plaque on undisclosed teeth. This has also been reported in a study that assessed the potential for using QLF for measuring plaque coverage on human teeth.[11] It is not yet known which canine bacterial species autofluoresce and at what stage of biofilm development undisclosed plaque can be visualized by QLF. Recent work indicates that *Porphyromonads* are not primary colonizers in dog plaque and do not predominate in the first 24 hours of biofilm development.[28] This may explain some of the difference between disclosed (average plaque coverage of 54.8%) and undisclosed (average plaque coverage of 10.1%) images for the dogs fed an OC chew as they had 18 to 20 hours of new plaque accumulation between the feeding of the last OC chew and the QLF assessments. This plaque may have consisted mainly of bacteria that do not naturally fluoresce. Therefore, while QLF analysis of undisclosed teeth is suitable for distinguishing between canine dental products, plaque needs to be disclosed to measure the efficacy of products in reducing total plaque volume.

This study has shown that QLF can be used on conscious dogs that have been appropriately trained. Dogs that have received regular mouth handling from an early age require approximately 6 weeks of training to be confident when having QLF images taken. In conscious dogs, it is currently only possible to capture images of the upper jaw and it is not possible to visualize M1 teeth in every dog and therefore further work is required to capture all the teeth currently required for VOHC approval. It may be possible to train dogs to hold something in their mouths, such as a wedge shaped toy, to enable the lower teeth to be visualized, and with the use of lip retractors, it may also be possible to visualize M1 in some breeds of dog. Nevertheless, this may not be necessary as the results from the conscious dogs were comparable to the whole mouth data obtained from anesthetized animals when teeth are undisclosed (Figure 8).

One potential limitation of QLF, as for other planimetry methods, is that it is currently not possible to measure plaque depth. This should be possible in the future with further modification to the algorithm and validation of how plaque color intensity relates to plaque thickness. There has been uncertainty about clinical relevance of methods that allow equal weighting of the gingival and coronal halves of the tooth.[29]

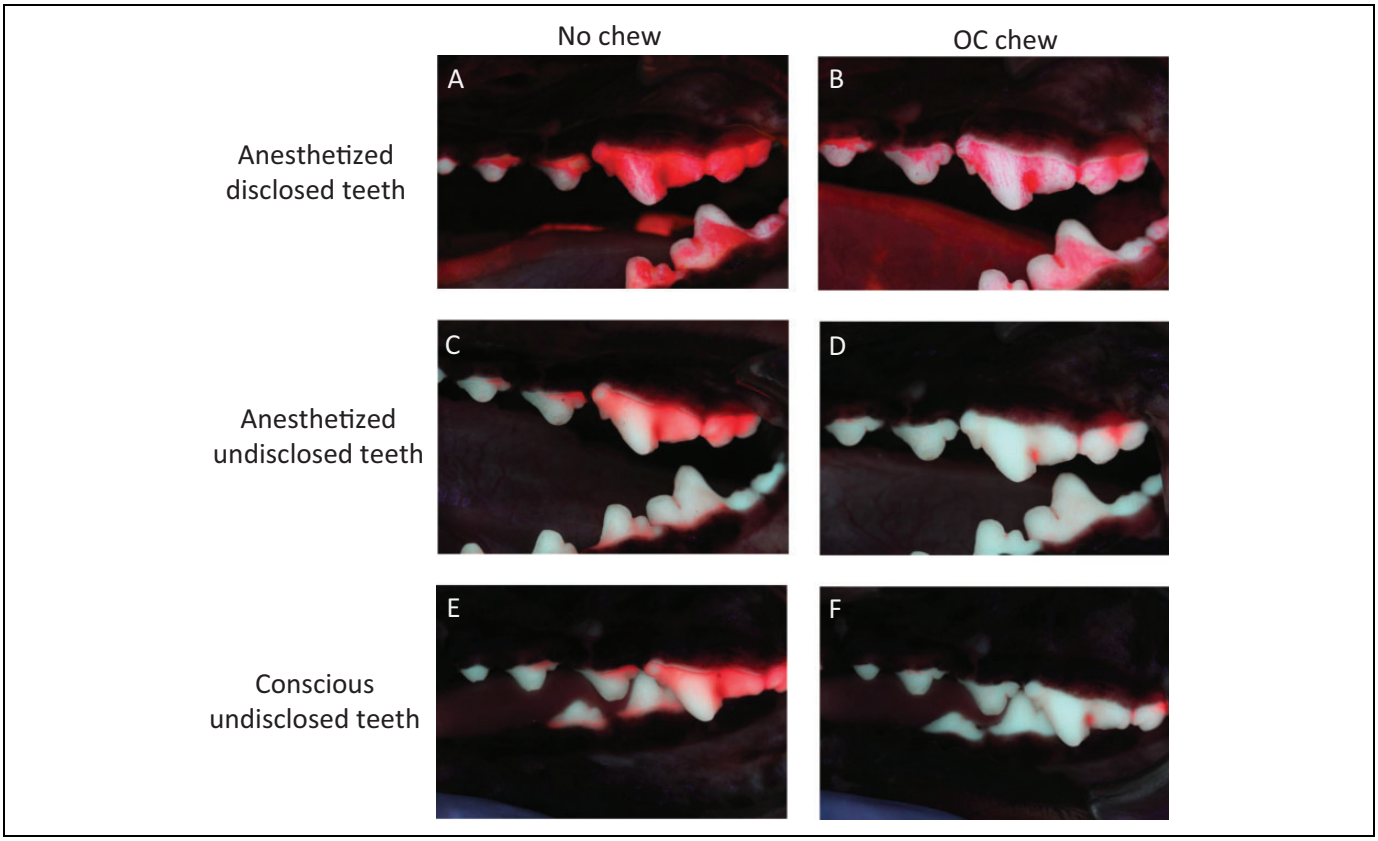

Figure 9. Examples of Quantitative Light-induced Fluorescence (QLF) images of disclosed teeth (second, third, and fourth premolars and first molars) of anesthetized dogs receiving (A) no chew and (B) an oral care (OC) chew, undisclosed teeth of anesthetized dogs (second, third and fourth premolars and first molars) receiving (C) no chew and (D) an OC chew and undisclosed teeth (first, second, third, and fourth premolars) of conscious dogs receiving (E) no chew and (F) an OC chew. The plaque can be seen as red against the white tooth.

Again, with modifications to the masking algorithms, it should be possible to calculate plaque coverage at the gingival margin as for methods such as GCPI. Although GCPI has been shown to be quick and less resource intensive than plaque index methods for quantifying plaque and evaluating the efficacy of canine oral care products, it is still subjective. The ability to automate QLF image analysis means that it is less subjective. Finally, methods for measuring plaque coverage treat all teeth equally in their contribution to the total mouth plaque score (regardless of their size). The use of planimetric methods such as QLF that record the size of every tooth make it possible to calculate a whole mouth plaque score that accurately reflects the contribution of each tooth to the total amount of plaque in the mouth. It is worth considering whether this would more truly reflect a product's ability to reduce plaque, especially since there is evidence that the total amount of plaque in the mouth is a key predictor of oral health.[30] Harvey quantified the similarities and differences among the crown of teeth used to generate plaque and calculus scores in dogs and cats and, due to the buccal surface area variability between teeth, questioned whether equal weighting should be given to each tooth.[31] Harvey et al later proposed a system for more accurately scoring gingivitis and periodontitis on a whole-mouth basis.[32] This system, called the Total Mouth Periodontal Score, uses weighting factors to take into consideration the differences in size of dogs' teeth. The use of QLF for plaque assessment would allow the ideas explored in these papers to be taken to their logical conclusion by calculating the exact percentage of tooth area in the mouth that is covered by plaque. This would give a total mouth plaque score that is not biased by differences in tooth area.

Through a series of studies in conscious and anesthetized dogs, we have demonstrated that QLF is a highly repeatable, reproducible, and accurate technique for the measurement of plaque coverage. Therefore, QLF analysis of disclosed teeth in anesthetized dogs is a potential alternative method to the modified Logan and Boyce Index, as the method showed good agreement with respect to reductions in plaque accumulation when dogs were fed an OC chew compared to no chew. In addition, we have shown that QLF images of undisclosed teeth can be acquired and product performance can be differentiated in conscious dogs. Furthermore, QLF has many advantages over current plaque scoring methods as it is less subjective, faster, requires less training, and the images can be stored to provide a permanent database for future use. In addition, fewer animals are required to measure the same size effect in dental product efficacy trials. The use of fewer animals and the ability to undertake studies in conscious dogs supports two of the

guiding principles underpinning the humane use of animals in scientific research: (1) reducing the number of animals used and (2) refining experiments to improve animal welfare.

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Author Contribution

Corrin Wallis and Yadvinder Gill contributed equally to this work. They both contributed to the design and co-ordination of the studies and the preparation of the manuscript. Alison Colyer participated in the design of the studies, performed all statistical analyses and reviewed the manuscript. Ian Davis and Judi Allsopp were involved in the acquisition, analysis and interpretation of the data. Gleb Komarov and Sue Higham contributed to the conception and design of the studies, data analysis and revision of the manuscript. Stephen Harris conceived and participated in the design and co-ordination of the study and reviewed the manuscript. All authors approved the final article.

Declaration of Conflicting Interests

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Supplementary Material

The online [appendices/data supplements/etc] are available at http://jov.sagepub.com/supplemental

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