**Quantification of canine dental plaque using Quantitative Light-induced Fluorescence**

Corrin Wallis (PhD)a\*, Yadvinder Gill (PhD)a\*, Alison Colyer (MSc, CStat) a, Ian Davis (PhD)a, Judi Allsopp (RVN, BVNA Certificate in Veterinary Dentistry)a, Gleb Komarov (BDS, PhD)a, Susan Higham (PhD)a, Stephen Harris (PhD)a

\* Both authors contributed equally

aThe WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, LE14 4RT, UK.

bThe University of Liverpool, Department of Clinical Dental Sciences, Edwards Building, Daulby Street, Liverpool, L69 3GN, UK.

corrin.wallis@effem.com, Senior Research scientist, Tel: +44 1664 415103, Fax: +44 1664 415440.

Yadvinder.gill@effem.com, Senior Research Scientist, Tel: +44 1664 415098, Fax: +44 1664 415440.

ian.davis@effem.com, Senior Research Scientist, Tel +44 1664 415018, Fax: +44 1664 415440.

Judi.allsopp@effem.com, Research Scientist, Tel: +44 415457, Fax: +44 1664 415440.

Komarovg@liv.ac.uk, Research fellow, Tel: +44 151 706 5162, Fax +44 151 706 5937.

 S.M.Higham@liverpool.ac.uk, Professor of Oral Biology, Tel: +44 151 706 5251, Fax +44 151 706 5937.

Stephen.harris@effem.com, Research Manager Oral Care, Tel +44 1664 415320, Fax: +44 1664 415440.

**Summary**

The aim of this work was to evaluate Quantitative Light-induced Fluorescence (QLFTM) 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 anaesthetised dogs, QLFTM 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 QLFTM software accurately identifies areas of plaque as demonstrated by comparison to the variability of five human scorers manually marking plaque on QLFTM acquired images (*p*=0.1).. There was good agreement with the modified Logan and Boyce method in the percentage reduction of 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 two treatments, a retrospective power analysis (90%) of the data established that only seven dogs would be required, compared to 19 for the modified Logan & Boyce method. QLFTM is a reliable method for measuring dental plaque in dogs with the added advantage that it is not subjective and requires fewer animals.

*Key words:*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 aetiological factor in the development of the disease5. If allowed to accumulate and mature it leads to an inflammatory response (gingivitis) which can ultimately give rise to periodontitis involving the destruction of the periodontal ligament and alveolar bones that support the tooth. This can be painful and ultimately lead to tooth loss6. 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 colour to estimate thickness of plaque7-10. There has been much criticism of these methods with respect to their resolution, subjectivity and need for examiner training11. Planimetric analysis generally involves disclosing plaque, with subsequent photography of the tooth surfaces12-13. The images are then either traced by hand and the area of plaque calculated, or they are digitised and analysed using computer software. Studies found that computer based plaque analyses are more reliable14, more precise15 more objective14-16 and more sensitive15 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 (QLFTM), a technique initially used for detecting caries lesions in humans, has also been employed to detect dental plaque11,17-19. This method either relies on the natural fluorescence of plaque under blue light (405nm) 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 tooth20 and the gingival contour plaque index (GCPI) which focusses on plaque that accumulates along the buccal gingival margin21,22. Both of these methods have been endorsed by the Veterinary Oral Health Council (VOHC[[1]](#footnote-2)) for supporting product claims relating to plaque control. *In vivo* product efficacy trials require a clean mouth model where cats or dogs are anaesthetised 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 anaesthetic occasions per animal, decrease the duration of anaesthesia, 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 QLFTM have not been used before in dogs. Therefore, the aims of these studies were to evaluate the reproducibility and accuracy of QLFTM for quantification of canine dental plaque and to compare this to an established clinical scoring system, namely the modified Logan & Boyce20 method.

**Materials and Methods**

The dogs included in the studies detailed below were pair housed at the WALTHAM Centre for Pet Nutrition in environmentally enriched kennels and provided with a comprehensive dog-dog and dog-human socialisation programme 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 (six females and five males, weight range 7.0 to 10.2 kg), which had received a recent scale and polish and had little or no visible calculus, were recruited to the study. Dogs were tooth brushed daily for approximately one week prior to the start of the trial using TePe[[2]](#footnote-3) compact medium or soft brushes 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 QLFTM image acquisition and analysis for further details). Three repeated sets of images, two in the morning and one in the afternoon were taken of conscious dogs by a single photographer. A set of images comprised four views around the mouth; two images on both the left and right hand side of the dog’s mouth were taken to visualise the maxilla 1st premolars (P1; 105, 205), 2nd premolars (P2; 106, 206), 3rd premolars (P3; 107, 207) and 4th premolars (P4; 108, 208).

**Inter-photographer reproducibility – Undisclosed teeth**

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

The dogs were trained so that QLFTM images could be captured without the need for anaesthesia and with minimal restraint (see section on QLFTM image acquisition and analysis for further details). Each photographer took four images of each dog capturing left and right maxillary 3rd 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 (two females and five males, weight range 7.2 to 10.8 kg) were tooth 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 that was 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 QLFTM images captured without the need for anaesthesia. For imaging plaque, the teeth on the dogs right side were first washed with 3ml water using a plastic Pasteur pipette and then 1ml undiluted 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 washed off with a further 3ml of water. QLFTM images were immediately taken of the disclosed teeth by three 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 four images of the maxilla of each dog capturing the I3, C, P1, P2, P3 and P4 on each side

**Accuracy**

The ability of the QLFTM software to identify plaque correctly was determined by comparing the software results with those from five human scorers that had manually marked plaque on QLFTM acquired images in an image processing package as described below. The five human scorers (including two veterinary dentists) were trained to be able to assess plaque coverage using the modified Logan and Boyce method. A test set of QLFTM images, anaesthetised dogs with disclosed teeth, were selected to contain examples of teeth with a range of plaque coverages. This set contained 54 teeth in 30 QLFTM images from nine dogs. Raw images were opened in Adobe Photoshop® software (Version CC, Adobe Systems Inc., San Jose, CA, USA) 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 five scorers and the QLFTM software, an image projection for each tooth was rendered using Image J by stacking each plaque image from the five scorers.

**Comparison to Logan & Boyce**

A randomised cross-over trial, a study design endorsed by the VOHC, was undertaken to determine the agreement between QLFTM and modified Logan & 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 to 12.5 kg) were included in the study. They were divided into two groups where one group was fed a daily OC chew in phase one and no chew in phase two of the study and the other group received no chew in phase one and a daily OC chew in phase two. 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 CaninTM Medium Adult, which conformed to the National Research Council Nutrient Guidelines 200623;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 grams of the diet was removed from the main meal and used for the purpose of training the dogs as part of their normal socialisation routine.

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

At the end of each test phase plaque (coverage and thickness) was scored using the modified Logan & Boyce technique described by Hennet *et al*.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 1st 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 Recognised European Specialist in Veterinary Dentistry and were calibrated two weeks prior to the start of the trial to ensure consistency between examiners.

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

Data were excluded from 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 was tooth 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 QLFTM 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**TM **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[[3]](#footnote-4), to allow visualisation of the upper jaw. In addition, dogs were trained to accept the presence of the QLFTM camera.

On average it took six weeks to train dogs for QLFTM image capture when provided with 30 minute sessions each day (20-25 hours). These dogs had also received mouth handling from about four 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 SLR camera Canon 450D. The camera is equipped with an illumination tube with white and blue LED’s 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 QLFTM 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 maximise the quality of the QLFTM images captured24. 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 QLFTM images was analysed 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 (∆R%)25. The ∆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. QLFTM images were opened in Photoshop CC® and a standardised spot of clean tooth devoid of plaque was added as a reference point to each image in order to baseline the algorithm. Images were analysed in QA2 software with the additional tooth spot included in the contouring. The additional spot added a negligible increase in pixel counts.

Images were scrutinised for quality in terms of focus, parts of teeth obscured, illumination, or any other artefacts which 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 (REML) were used to estimate variance components of the percentage plaque coverage, using repeat nested within dog as random effects. Firstly a model for an average mouth, (maxillary P1, P2, P3 and P4)) was used, followed by assessment of each tooth type. The percentage variability that was accountable to repeatability and the percentage 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 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 nine dogs, as assessed by five human scorers, were analysed 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 five human scorers). The probability of the QLFTM 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 QLFTM software score. A test level of 5% was used.

*Comparison to modified Logan & Boyce:* The percentage plaque coverage measured by QLFTM and modified Logan and Boyce, averaged for all teeth, were analysed 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 two-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.

*Comparison between conscious and unconscious imaging:* The percentage plaque coverage as measured by QLFTM of undisclosed teeth from conscious (average of upper jaw teeth) and anaesthetised (average of all teeth) dogs, was analysed 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 eleven 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% (see Figure 2).

The intra-photographer repeatability component of variability showed that the QLFTM 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% (see 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%.

**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 twelve conscious miniature schnauzers. The teeth selected were based on the teeth scored using the modified Logan & 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 are 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 seven 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 & 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 are shown in supplementary Figure 2.

**Accuracy of QLF**TM

The ability of the QLFTM software to identify plaque correctly was determined by comparison with plaque coverage levels determined by five human scorers manually marking plaque on QLFTM acquired images (see Figure 5 for a sample of images). A high agreement in identification of plaque was seen between the five scorers (‘overlay’) and in comparison to the QLFTM software (Figure 6). Agreement was seen across the entire range of plaque coverage from 0.6% to 100% (min, max). Simulations of the variance of the five scorers showed the QLFTM software was not significantly different to the human scorers with 10% of simulated human scorers having lower average percentage plaque coverage than the QLFTM software.

**Comparison with Logan & Boyce**

A product efficacy trial was undertaken to determine the agreement of QLFTM to modified Logan and Boyce. Analysis of QLFTM 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 chewcompared to no chew (see 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). QLFTM images were also taken of undisclosed teeth, whilst dogs were under anaesthesia, 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 QLFTM 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 QLFTM with disclosed and undisclosed images was seven and 14 dogs respectively (Figure 7).

QLFTM images of undisclosed teeth were also taken of ten dogs consciously at the end of each test phase of the cross-over study prior to being placed under anaesthesia. There was a significant difference between OC chew and no chew for both dogs imaged consciously (*P*<0.001) and the same ten dogs imaged unconsciously (*P*<0.001). The average plaque 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 ten dogs were imaged under anaesthesia (undisclosed) the average plaque coverage was 30.5% (25.0%, 36.1%) for no chew and 9.5% (4.0%, 15.0%) when fedan 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 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 QLFTM images taken of conscious and unconscious dogs (disclosed and undisclosed teeth) are shown in Figure 9.

**Discussion**

We have shown that QLFTM is a reliable technique for measuring the plaque coverage on undisclosed and disclosed teeth of both anaesthetised and conscious dogs. QLFTM showed good intra-photographer repeatability with a %CV of 7.5%. In the majority of teeth assessed QLFTM 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 favourably with other plaque scoring methods. For instance the whole mouth inter-grader variability of GCPI was reported as 18%21. In addition, it has previously been reported that experience is a significant factor when scoring plaque for research purposes using plaque index methods such as Logan and Boyce20. For our studies, photographers received a half day training session on how to acquire the QLFTM images and also how to interact with the dogs, which clearly demonstrates that experienced photographers are not required to obtain precise measurements using QLFTM.

Whilst 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 QLFTM software to five human scorers manually marking plaque on QLFTM 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 QLFTM 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 QLFTM and that the reduction in plaque accumulation when dogs received an OC chew compared to no chew is comparable to the results obtained using modified Logan and Boyce. A retrospective power analysis showed that fewer dogs are required to measure a reduction in plaque accumulation using QLFTM compared with the modified Logan and Boyce method: Using the modified Logan and Boyce method, to statistically show a 15% reduction in plaque accumulation when dogs are being fed an OC chew compared to no chew (with 90% power), requires 19 dogs whereas using QLFTM the number of dogs required is only seven.

It is also possible to visualise the plaque using QLFTM without the need to disclose the teeth. However, the percentage 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 species27. The fact that the mean plaque coverage for undisclosed teeth, is lower than for disclosed teeth suggests that not all the bacteria in mature biofilms autofluoresce and therefore QLFTM underestimates the amount of total plaque on undisclosed teeth. This has also been reported in a study that assessed the potential for using QLFTM for measuring plaque coverage on human teeth11. It is not yet known which canine bacterial species autofluoresce and at what stage of biofilm development undisclosed plaque can be visualised by QLFTM. Recent work indicates that *Porphyromonads* are not primary colonisers in dog plaque and do not predominate in the first 24 hours of biofilm development28. 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 18-20 hours of new plaque accumulation between the feeding of the last OC chew and the QLFTM assessments. This plaque may have consisted mainly of bacteria that do not naturally fluoresce. Therefore, whilst QLFTM analysis of undisclosed teeth is suitable for distinguishing between canine dental products the plaque needs to be disclosed to measure the efficacy of products at reducing total plaque volume.

This study has shown that QLFTM can be used on conscious dogs that have been appropriately trained: Dogs that have received regular mouth handling from an early age take on average about six weeks of training to be confident when having QLFTM images taken. In conscious dogs it is currently only possible to capture images of the upper jaw and it is not possible to visualise the 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 visualised and with the use of lip retractors it may also be possible to visualise the 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 anaesthetised animals when teeth are undisclosed (Figure 7).

One potential limitation of QLFTM, 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 colour 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 tooth29. 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 QLFTM 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 QLFTM 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 give a truer reflection of a product’s ability to reduce plaque in the mouth especially since there is evidence that the total amount of plaque in the mouth is a key predictor of oral health30. Harvey31 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. Harvey *et al.*32 later proposed a system for more accurately scoring gingivitis and periodontitis on a whole mouth basis. This system called the Total Mouth Periodontal Score (TMPS) 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 two papers to be taken to their logical conclusion, as it is facile to calculate 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 anaesthetised dogs we have demonstrated that QLFTM is a highly repeatable, reproducible and accurate technique for the measurement of plaque coverage. Therefore, QLFTM analysis of disclosed teeth in anaesthetised dogs is a potential alternative method to modified Logan and Boyce as the method showed good agreement with respect to reductions in plaque accumulation measured when dogs were fed an OC chew compared to no chew. In addition, we have shown that QLFTM images of undisclosed teeth can be acquired and product performance can be differentiated in conscious dogs. Furthermore, QLFTM has many advantages over current plaque scoring methods as it is less subjective, faster, photographers require 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; namely reducing the number of animals used to a minimum and refining the way experiments are carried out to improve animal welfare.

**References**

1Hamp S, Olsson S, Farso-Madsen K, Viklands P, Fornell J. A macroscopic and radiologic investigation of dental diseases of the dog. *Veterinary Radiology 1984;* 25:86-92.

2Butković V, Šimpraga M, Šehić M, Stanin D, Sušić V. Capak D, Kos J. Dental diseases of dogs: A retrospective study of radiological data. *Acta Veterinaria Brno* *2001;* 70:203-208.

3Kyllar M, Witter K. Prevalence of dental disorders in pet dogs. *Veterinarni Medicina-Czechoslovakia* *2005;* 50:496-505.

4Kortegaard H, Eriksen T, Baelum V. Periodontal disease in research beagle dogs - an epidemiological study. *Journal of Small Animal Practice* *2008*; 49:610-616.

5Van Dyke T E. The etiology and pathogenesis of periodontitis revisited. *Journal of Applied Oral Science 2009;* 17:1678-7757.

6Williams RC. Periodontal disease. *The New England Journal of Medicine 1990;* 322:373-382.

 7Quigley G, Hein J. Comparative cleansing efficiency of manual and power brushing. *Journal of the American Dental Association* *1962;* 65:26–9.

8Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odolltol Scalld* *1964;* 22:121-135.

9Löe H. The gingival index, the plaque index and the retention index systems. *Journal of Periodontology* *1967;* 38:610-616.

10Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamin C. *Journal of Periodontology 1970;* 41:41-43.

11Pretty IA, Edgar WM, Smith PW, Higham SM. Quantification of dental plaque in the research environment. *Journal of Dentistry* *2005;* 33:193-207.

12Söder PO, Jin LJ, Söder B. Computerized planimetric method for clinical plaque measurement. *Scandinavian Journal of Dental Research 1993;* 101:21-25.

13Staudt CB, Kinzel S, Hassfeld S, Stein W, Staehle HJ, Dörfer CE. Computer-based intraoral image analysis of the clinical plaque removing capacity of 3 manual toothbrushes.*Journal of Clinical Periodontology* *2001;* 28:746-752.

14Verran J, Rocliffe MD. Feasibility of using automatic image analysis for measuring dental plaque in situ. *Journal of Dentistry* *1986;* 14:11-13.

15Block RP, Bouwsma OJ, Howardnordan KS, Miller JM, Poore CL, Sunberg RJ. Validation of computerized photoimage analysis (PIA) measurement of plaque. *Journal of Dental Research* *1996;* 75:367.

16Shaloub A, Addy M. Evaluation of accuracy and variability of scoring-area-based plaque indices. A laboratory model. *Journal of Clinical Periodontology* *2000;* 27:16–21.

 17Pretty IA, Edgar WM, Higham SM. A study to assess the efficacy of a new detergent free, whitening dentifrice in vivo using QLF planimetric analysis. *British Dental Journal* *2004;* 197:561-566.

18Mohan N, Mahesh MR, Varghese VI, Pretty IA, Taylor AM. Evaluation of the sensitivity of a digital plaque imaging system on different tooth surfaces. *Journal of Clinical Dentistry* *2012;* 23:11-16.

19Hope CK, Wang Q, Burnside G, Adeyemi AA, Quenby S, Smith PW, Higham SM, Whitworth M. Assessing the association between oral hygiene and preterm birth by quantitative light-induced fluorescence. *The Scientific World Journal* *2014;* 2014:374694.

20Hennet P, Servet E, Salesse H, Soulard Y. Evaluation of the Logan and Boyce Plaque Index for the Study of Dental Plaque Accumulation in Dogs. *Research in Veterinary Science* *2006;* 80:175-180.

21Scherl DS, Coffman L, van Cleave M, Lowry S. Validation of a new dental plaque quantification method in dogs. *Journal of Veterinary Dentistry 2007;* 24:14-19.

22Scherl DS, Bork K, Coffman L, Lowry SR, VanCleave M. Application of the Gingival Contour Plaque Index: Six-month plaque and gingivitis study. *Journal of Veterinary Dentistry* *2009*; 26; 23-27.

23National Research Council (US). Ad Hoc Committee on Dog and Cat Nutrition. *Nutrient requirements of dogs and cats.* National Academies Press 2006.

24Pretty IA, Edgar WM, Higham SM. The effect of ambient light on QLF analyses. *Journal of Oral Rehabilitation* *2002;* 29:369-373.

25De Josselin de Jong E, Higham SM, Smith PW, van Daelen CJ, van der Veen MH. Quantified light-induced fluorescence, review of a diagnostic tool in prevention of oral disease. *Journal of Applied Physics* *2009;* 105:102031.

26Marsh PD, Martin MV. *Oral Microbiology*, 3rd ed. London, Chapman & Hall, eds 1992.

27Davis IJ, Wallis C, Deusch O, Colyer A, Milella L, Loman N, Harris S. A cross-sectional survey of bacterial species in plaque from client owned dogs with healthy gingiva, gingivitis or mild periodontitis. *PLoS ONE* *2013;* 8:e83158.

28Holcombe LJ, Patel N, et al. Early Canine Plaque Biofilms: characterisation of key bacterial interactions involved in initial colonisation of enamel. Accepted by PLoS ONE

29Hennet P. Review of studies assessing plaque accumulation and gingival inflammation in dogs. *Journal Veterinary Dentistry 1999;* 16:23-29.

30Darveau R, Tanner A, Page R. The microbial challenge in periodontitis. *Periodontology* *1997;* 14:12-32.

31Harvey CE. Shape and size of teeth of dogs and cats-relevance to studies of plaque and calculus accumulation. *Journal Veterinary Dentistry 2002;* 19:186-195*.*

32Harvey CE, Laster L, Schofer F, Miller B. Scoring the full extent of periodontal disease in the dog: Development of a total mouth periodontal score (TMPS) system. *Journal Veterinary Dentistry 2008;* 25:176-180*.*

**Acknowledgements**

The authors would like to acknowledge the WALTHAM staff who trained the dogs, volunteered to take part in the reproducibility study, helped with the accuracy assessments and specifically Mark Marshall for managing the modified Logan and Boyce trial. The authors would like to acknowledge Inspektor Research Systems BV, Amsterdam, The Netherlands for the algorithm modifications and their technical support. Finally the authors would like to acknowledge Lisa Milella and Florian Boutille for their help in scoring images for the accuracy experiments.

**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 |

**Table 2**

Comparison of QLFTM (undisclosed and disclosed) to modified Logan & Boyce for measuring the difference in percentage plaque reduction between dogs fed an OC chew compared with no chew.

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

**Figure Legends**

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).

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 1st, 2nd, 3rd and 4th premolars) as determined by QLFTM on undisclosed teeth, by dog (A-K) and repetition (1-3).

Figure 3. Inter-photographer repeatability of five photographers taking images of undisclosed teeth of conscious dogs. Variability chart of percentage plaque coverage (whole mouth average: maxillary 3rd incisors, maxillary and mandibular canines and 3rd and 4th premolars) as determined by QLFTM by dog (A-L) and photographer (1-5).

Figure 4. Inter-photographer repeatability of five photographers taking images of disclosed teeth of conscious dogs. Variability chart of percentage plaque coverage (whole mouth average: maxillary 3rd incisors, maxillary and mandibular canines and 3rd and 4th premolars), as determined by QLFTM by dog (A-G) and photographer (1-3).

Figure 5. Visual representation of plaque identified by five human scorers marking plaque in Photoshop® and plaque identified by the QLFTM software, on four sample disclosed teeth. “Overlay” is an amalgamation of the five scorers.

Figure 6. Variability chart of percentage plaque coverage identified by five human scorers marking plaque in Photoshop® (black data points) and QLFTM software (red data points): Maxillary 3rd incisors, maxillary and mandibular canines and 3rd and 4th premolars (disclosed teeth).

Figure 7. Number of dogs required to detect a 15% reduction in plaque accumulation when fed an OC chew compared to no chew in a two-way crossover trial. Solid line depicts QLFTM (disclosed teeth), dashed line modified Logan & Boyce and the dot dashed line QLFTM (undisclosed teeth).

Figure 8. Average plaque coverage of maxillary jaw only (3rd incisors, canines, 3rd and 4th premolars) of conscious dogs (blue dots) and maxillary (3rd incisors, canines, 3rd and 4th premolars and 1st molars) and mandibular (canines, 3rd and 4th premolars and 1st molars) jaw of unconscious dogs (red dots) when fed an oral care chew vs no chew. Filled dots represent average percentage plaque coverage and bars depict 95% confidence intervals.

Figure 9.Examples of QLFTM images of undisclosed teeth (2nd, 3rd and 4th premolars and 1st molars) of anaesthetised dogs receiving A) no chew and B) an OC chew, disclosed teeth of anaesthetised dogs (2nd, 3rd and 4th premolars and 1st molars) receiving C) no chew and D) an OC chew and undisclosed teeth (1st, 2nd, 3rd and 4th premolars) of conscious dogs receiving E) no chew and F) an OC chew. The plaque can be seen as red against the white tooth.

Supplementary Figure 1. Variability charts of percentage plaque coverage, as determined by QLFTM, by dog (A-L), photographer (1-5) and tooth: a) undisclosed teeth 103 to 108 (maxillary 3rd incisors, canines, 1st, 2nd, 3rd and 4th premolars) and b) undisclosed teeth 203 to 208 (maxillary 3rd incisors, canines, 1st, 2nd, 3rd and 4th premolars).

Supplementary Figure 2. Variability charts of percentage plaque coverage, as determined by QLFTM on disclosed teeth, by dog (A-G), photographer (1-3) and tooth: a) disclosed teeth 103 to 108 (maxillary 3rd incisors, canines, 1st, 2nd, 3rd and 4th premolars) and b) disclosed teeth 203 to 208 (maxillary 3rd incisors, canines, 1st, 2nd, 3rd and 4th premolars).

1. www.vohc.org [↑](#footnote-ref-2)
2. TePe Oral Hygiene Products Ltd, Bronsåldersgatan 5
213 76 Malmö, Sweden [↑](#footnote-ref-3)
3. Mirahold child’s cheek retractor, Henry Schein, 135 Duryea Road, Melville, NY 11747 [↑](#footnote-ref-4)