Report on the VOHC Digital Scoring Project - Session at the May 20, 2017 Malaga ECVD

(European Veterinary Dental Forum)

And Report on Discussions during Colin Harvey's visit to Waltham, May 29-30.

Malaga Meeting Report:

1. **Colin Harvey, VOHC Director**, gave a brief overview of the purpose of the project and activities to date.

2. **Purpose** is to make available an objective plaque and calculus scoring method that will reliably record extent and thickness.

3. **Quantitative Light-induced Fluorescence** (QLF) has been developed and marketed for use in human dentistry by **Inspektor Research Systems, BV,** (IRS) based on adaptation of a digital caries scoring system.
   a. The research group at the Waltham Centre for Pet Nutrition, led by **Dr. Lucy Holcombe, and represented by Dr. Corrin Wallis** (Wallis) at this meeting, has worked with Inspektor to adapt the IRS QLF for use in dental trials requiring scoring of dental plaque and calculus.
   b. The Malaga session was the fourth such session at the American and European Veterinary Dental Forums in the last two years. During that time, no alternative to QLF as an objective system for scoring dental plaque and calculus in dogs and cats has been identified. (There was initial interest in a thermal imaging system, but that was not pursued because of difficulty in developing the necessary software).

4. **Tooth sub-sets:** Data obtained in VOHC trials using the well-established 'VOHC Tooth Set' were reviewed. Three companies that have each conducted several VOHC trials in dogs and cats were asked to run correlation coefficient analyses using the VOHC tooth set as the standard, compared with various bilateral and unilateral subsets to determine validity of the sub-sets.
   a. Harvey presented data provided by one company, which showed a very high correlation coefficient (r_i) for some subsets ($p > 0.95$ – [r_i of >0.90 is considered highly significant]). Harvey asked the participants whether anyone had problems accepting that r_i of <0.90 validated use of subsets that met that statistical standard; no negative comments were made.
   b. Wallis then provided similar data from QLF studies conducted at or on behalf of Waltham, again showing a very high r_i with similar tooth subsets. Data from the third company were not received prior to the meeting.

c. There was discussion of the tooth subset data. A comment had been made to Harvey previously that use of unilateral tooth sets may risk credibility of the data. At the meeting, Harvey noted that, if indeed unilateral subsets are validated, this would be ideal for VOHC trials that use a split-mouth model to evaluate the effectiveness of a mechanical device; VOHC policy permits use of split-mouth trials.
d. While the two data sets now available to date indicate that this analysis will result in validation of particular sub-sets, no conclusion was reached pending review of correlation coefficient analysis results from the third company. Independent corroboration from three sources agreeing on use of a particular subset will be a powerful validating argument.

5. **VOHC Minimal Effectiveness Standard**: As Wallis showed her data to explore the potential use of tooth sub-sets, Harvey commented that one of the plaque trials in dogs shown had a plaque reduction of >20% when scored by Logan/Boyce, and slightly <20% when scored by QLF scores; the VOHC 'minimum effectiveness' standard is ≥ 15% in any one trial and ≥ 20% in the mean of the two required trials. The % plaque reductions derived from the data in the cat trial shown by Wallis between current and QLF scores were very similar. Harvey briefly commented on how the current VOHC % reduction efficacy standard was derived (including that a major change was made about 10 years ago to increase the minimum reduction efficacy to 20% from 10%, based on the standard adopted by the American Dental Association), and noted that if a recommendation is made to move scoring to a digital system, it will be necessary to ensure that doing so does not 'change the minimum VOHC standard'. Harvey also commented on the difference in % reduction in plaque (lower) and calculus (higher) in the same trials (depending upon the type of product, the % reduction in calculus may be twice as high as for plaque). This prompted a comment as to whether the current VOHC minimum % reduction for calculus should be higher. Having different % reduction standards for plaque and calculus may make the VOHC system confusing to consumers….. Harvey offered to tabulate results from several trials on record with VOHC to see how prevalent this difference is before making any further comment.

   The meeting then recessed for an hour to allow participants to attend Wallis' scheduled presentation on plaque bacteriology in another room. The session reconvened at about 11.15 am.

6. Wallis gave a presentation on use of the QLF system to **score calculus**. Plaque is scored by obtaining QLF images; the teeth are gently rinsed with water, the disclosing dye is applied, then the teeth are rinsed again with water before the plaque QLF image is taken. For calculus, the teeth are then brushed and rinsed to leave only mineralized calculus in place before a second QLF image is obtained. There was discussion about what causes calculus to fluoresce following brushing; the consensus was that plaque bacteria trapped in the mineralized calculus are responsible. This led to discussion of the reliability of trapped bacteria or porphyrins in bacterial debris as sufficient to score calculus coverage (yes) and thickness (validation needed).

7. **Elbert Waller, representing Inspektor Research Systems**, BV, gave a presentation of the **physics behind the QLF system**, and described recent work to develop the Inspektor C4 software program that includes analysis of intensity of fluorescence of **plaque** pixel by pixel.

8. **Intensity of fluorescence/Thickness of Plaque or Calculus.** The C4 version of the QLF software presents data in three DeltaR bands of color intensity. It is assumed that depth of intensity of the color of the fluorescence induced by the presence of porphyrins in dental plaque is indicative of the thickness of the plaque that is fluorescing – see additional comments below regarding potential validation.
    a. No work has been done to date to determine whether **calculus** (indicated as areas that continue to fluoresce following brushing of the tooth surface to remove plaque) thickness can also be indicated by depth of coloration of the fluorescence.
    b. There was discussion of the potential methodology required to validate correlation of intensity of color with actual thickness of plaque and calculus. There was brief discussion of obtaining QLF images, then extracting an appropriate tooth, using a diamond wheel to section the tooth surface to allow calibrated measurement of plaque or calculus on the cut surface in both the least color depth and deepest color depth areas.

9. **Disclosed vs. undisclosed plaque/calculus.** There was some discussion of whether the QLF data are best obtained from disclosed or undisclosed plaque.
    a. It is clear from comparing undisclosed and disclosed QLF images from the same tooth that disclosed plaque results in a far wider extent of coverage than does the undisclosed image (see Fig. 9 in the Wallis et al publication on QLF in dogs (http://www.vohc.org/Wallis_QLF_in_Dogs_JVetDent_2016.pdf).
    b. Inspektor does not recommend using a disclosing solution when obtaining QLF images, and does not know what is in disclosed plaque that causes it to fluoresce so much more obviously and over a larger area than undisclosed plaque. Given that QLF measures the extent of fluorescence resulting from presence of porphyrins produced by plaque bacteria in the undisclosed plaque, it may be that the area of fluorescence shown in the disclosed image may be something other than the plaque biofilm. It is not just the pellicle, given that it is assumed that the pellicle covers the entire crown surface, and there are areas of the tooth that do not fluoresce in the QLF image.
    c. The general trend of the discussion at the May 20 session was that use of a disclosing solution was not necessary, but that it provides a powerful visual confirmation of the extent of plaque present.

10. Waller then gave a **demonstration of the use of the QLF camera** to obtain fluorescent-light images of the plaque- and calculus-laden surface of a suitable model. Once the positioning was validated, two clicks and a line drawn by mouse on-screen to indicate the specific tooth surface areas to be scored produced an image with the C4 indication of intensity of fluorescence shown as three differently colored bands, with automated capture in a data-base.

11. **Effective use of a depth standard** would require determination of the maximum thickness of plaque and calculus likely to be included in a VOHC scoring image. This would not be a challenge for an actual VOHC trial, because of the Day 0 clean-tooth requirement and minimum trial duration of 28 days, though there is the challenge of how to deal with trials that may extend beyond 28 days - there is no stated maximum duration of VOHC trials, and some trials include an interim scoring session at 28 days and a repeat scoring session at 56 days, and very occasionally longer. (Note: VOHC initially had a minimum plaque trial duration of 7 days, based on data that show that dental plaque plateaus after several days, and a minimum duration for calculus of 21 days (plateauing of calculus takes longer). When the gingivitis score requirement was added (as an indicator of harm to oral tissues mechanically or chemically), the minimum trial period for both plaque and calculus was extended to 28 days.

12. More work is needed on **confirmation of the extent of calculus as opposed to plaque**. Elbert Waller stated that Inspektor considers the two synonymous – plaque adheres to the salivary-fluid-derived pellicle that is laid down on the exposed tooth immediately following scaling and polishing; then as the plaque thickens over the next 2-3 days, salivary calcium salts are deposited in the plaque milieu which is recognized as calculus because of its hard nature. Inspektor differentiates 'young plaque' from 'mature or old plaque'. Harvey explained that differentiating plaque from calculus is essential in VOHC trials because VOHC awards its Accepted Seal for both Plaque and Tartar (calculus) claims – there are products that contain anti-plaque chemical agents without any anti-calculus agent or mechanism, and vice-versa.

13. **Dirty-tooth vs Clean-tooth Model**: There was discussion about application of the QLF system to a 'dirty-tooth model' trial protocol. It was noted that, while some companies would prefer a 'dirty tooth model' (no dental scaling on Day 0, to allow their product to be sold with a claim that it reduces plaque or calculus without need for professional scaling or brushing), it would be a lot more challenging to assess thickness in a 'dirty-tooth' trial, as calculus on the maxillary fourth premolar tooth can reach at least 0.5 cm or more in thickness. Harvey noted his experience with a dirty tooth model many years ago – thick calculus often cracks off in large chunks when a product with a potential mechanical effect such as a biscuit is given to dogs, and these 'craters' are covered by a thin layer of calculus within a few days. He also noted that the 'clean tooth model' was adopted by VOHC for several reasons. One was to reduce variability of the data. A second one was to reduce the trial period to the minimum necessary, and the third was to accentuate the American Veterinary Dental College (which owns VOHC and whose Board appoints VOHC members and adopts VOHC policies and procedures from recommendations from VOHC Council) point of view that prevention of periodontal disease is most effective when periodic professional examination is combined with professional scaling as needed, followed by daily oral hygiene using an effective product or set of complimentary procedures and products (e.g. a dentifrice, brush and dental diet and chews).

14. **Cost of the QLF System**: Waller indicated that the QLF camera as demonstrated costs about US$25,000, which includes the C4 software program that is provided on a thumb drive for use with a laptop in the field. Harvey commented that the data from both dog and cat trials shown and published by the Waltham research group showed that, because the data are objective and thus less variable, a far smaller number of subjects per group is needed to show statistical significance; this would reduce the cost of conducting a VOHC trial significantly, with the result that the QLF camera-software package would be effectively paid for after use in 1-2 VOHC trials.

Summary: The session included lively discussion, with most participants making comments or asking questions. The session finally ended at about 1 pm. While no vote was taken on anything specific, the general consensus from comments was that the QLF equipment, combined with the C4 software package, and following additional work to determine how best to use the ability of the software to record intensity of plaque as indicative of thickness, plus validation of QLF scoring of calculus, appeared to be a realistic option that should be pursued further

Following the Malaga meeting, Harvey visited Corrin Wallis and colleagues at Waltham, to observe demonstrations of the use of the QLF camera in awake trained dogs, and to discuss in more detail the current status of digital scoring using QLF.

Waltham Meeting Report, May 29-30, 2017

Participants: VOHC Director Harvey and Waltham Oral Research group leader Lucy Holcombe, Senior Research Scientist Corrin Wallis and other Waltham staff.
There was agreement in principal to adopt a phased approach with respect to digital scoring using QLF for plaque and calculus extent of coverage and thickness scoring.

1. **Extent of coverage of disclosed plaque in anesthetized subjects:**
    - **a.** Shown in published Waltham studies to be practical and validated for use in cat & dog.
    - **b.** While most recent VOHC submissions use a plaque scoring index that consists of a combination of extent of coverage and thickness, VOHC protocol policy does not prohibit use of extent of coverage only when scoring plaque.
    - **c.** Waltham has requested and VOHC Council and the AVDC Board have approved use of QLF image analysis to score extent of coverage of plaque in VOHC trials in dogs and cats.

2. **Extent of coverage of disclosed plaque in awake subjects**:
    a. Shown in published Waltham studies to be practical for use in dogs.
    b. No published data currently available in cats.

3. **Extent of coverage of undisclosed plaque in anesthetized subjects**:
    a. Shown in published Waltham studies to be practical for use in dogs.

b. Shown in published Waltham studies to be practical for use in cats.
c. The same teeth undisclosed and disclosed in QLF images in dogs and cats in the Waltham publications show that disclosed plaque enables a larger area of plaque to be visualised than does undisclosed plaque.
    i. What impact would use of undisclosed vs. disclosed plaque have on % reduction in plaque in a VOHC trial?
    ii. Is the peripheral 'disclosed plaque' actually equivalent to undisclosed plaque (with regard to inclusion of bacterial-produced porphyrins) or is the fluorescence due to staining of e.g. pellicle that is not covered by porphyrin-producing bacteria, and therefore is overstating the actual bacterial plaque area? Wallis et al suggest that the difference is due to the larger area being composed of immature plaque that does not contain porphyrins. Need input from Inspektor on cause of fluorescence of disclosed 'plaque'.
    iii. What is the impact of use of disclosing solution on viability of bacteria (i.e. in a staged study, would disclosed plaque result in a lower subsequent plaque score because some bacteria are killed by disclosing solution)? Would the blue light emitted by the QLF camera affect viability of plaque in a staged study? Input from e.g. Sue Higham at Liverpool University would be helpful.
    iv. Would a different VOHC standard be necessary when using QLF images of undisclosed plaque images?

4. **Extent of coverage of undisclosed plaque in awake subjects**:
    a. Shown in published Waltham studies to be practical for use in dog.
    b. No published data currently available in cats.
    c. Same issues as in item 3 c above.

5. **Extent of coverage of calculus following removal of disclosed plaque in anesthetized subjects**:
    a. Data from Waltham studies in dogs are in preparation for publication. It is not known whether QLF fluorescence actually identifies calculus, in that there is plaque embedded in calculus, and it may be the embedded bacterial porphyrins that fluoresce. Whatever the reason, it does seem that QLF identifies the limits of calculus when plaque is brushed off the surface of the teeth. A study in either germ-free dogs (impractical) or of sterile calcium carbonate/calcium phosphate mixture laid on a sterile tooth surface would be needed to determine what actually fluoresces when superficial plaque is brushed off.
    b. No published data currently available in cats, but in principle should be practical as in dogs.

6. **Extent of coverage of calculus following removal of undisclosed plaque in anesthetized subjects**:
    a. No published data currently available in dogs or cats but in principle should be practical.

b. Do undisclosed calculus QLF images mirror plaque as in item 3 c, above? Not known. Comparing calculus extent on QLF images before and after using a disclosing agent would be practical, but has not yet been done.

7. **Extent of coverage of calculus following removal of disclosed plaque in awake subjects**:
    a. No published data currently available in dogs or cats.
    b. Whether brushing teeth of an awake dog produces the same extent of removal of plaque overlaying areas of the crown covered by calculus as brushing in an anesthetized dog needs to be considered.

8. **Extent of coverage of calculus following removal of undisclosed plaque in awake subjects**:
    a. No published data are currently available in dogs, but in principle should be practical No published data currently available in cats.
    b. Do the images of the same teeth undisclosed and disclosed in QLF images following brushing in dogs and cats show that disclosed calculus enables a larger area of the tooth to be visualised than does undisclosed calculus, as measured following brushing and rinsing?

9. **Intensity of fluorescence in individual pixels as an indicator of the thickness of plaque and calculus.**
    a. The current (C4) Inspektor QLF software analyses the fluorescence pixel by pixel, and reports the intensity in each pixel in the area that is fluorescing as being in one of thirteen bands (DeltaR), which results in a combination extent of coverage x intensity score. The C4 software visualization pane shows the data in three bands, as currently used in Inspektor's caries QLF program; the data for all 13 bands can be downloaded to an Excel file.
    b. Is the actual intensity score per pixel saved as part of the QLF image data set, from which the band data are calculated? In theory, a far larger series of bands could be used, or the combination score could be calculated as the sum of the raw intensity scores in all pixels showing any fluorescence. Need discussion with Inspektor to clarify this and determine whether raw scores could be used (and if so, whether a calibration intensity grid would need to be included in the image to validate uniformity of data collection from one image to the next). Set up electronic meeting of Inspektor, Waltham and Harvey, and then produce a proposal with expected cost and timeline for a collaborative effort. ?
    c. Validation for correlation of pixel color intensity with actual thickness of plaque and calculus could be attempted using cadaver teeth. Harvey offered to take this on using cadaver teeth.
    d. If there is a valid correlation of actual depth to intensity of fluorescence, there would be need to establish a standard based on a (presumed) maximum possible depth/intensity, so that the per pixel data can be reported as % of the standard maximum. The plaque or calculus combination extent of coverage x thickness score would be the sum of the intensity scores for each fluorescing pixel divided by the total number of pixels encompassing the crown.

10. **Use of weighted teeth area data** to provide a more balanced score.
    - **a.** The QLF images capture the number of pixels of the entire captured crown surface. This would allow data from images of different teeth to be weighted based on area so that the mean mouth score actually represents an accurate summary of the extent/intensity of plaque and calculus in the full tooth set.
    - **b.** While practical, is this relevant? The canine tooth crown is narrower but higher than the fourth premolar and first molar teeth, and thus has a smaller gingival margin to tooth height ratio…… Plus there may be a problem if more than one image is used to capture the tooth-set QLF data. Waltham and Harvey have calibrated data from teeth of dogs and cats captured on single images that would allow calculation of a generic index for calculating the contribution of individual teeth to sub-set data collection even if the teeth are captured on more than one image. Waltham has published data on mean of all teeth scored vs. weighted teeth in cats.

11. **Tooth subsets.** Data has been provided by the third company. A summary of the three data sets may be available in time for presentation at the VOHC Update session at the Nashville Forum. The Waltham data show validity when using QLF.

12. **Camera and software:**
    - **a. Inspektor**:
        - **i. Camera**: Harvey to suggest to Inspektor that potential camera developments are needed to improve reliability if the system is to be recommended for VOHC consortium use (specifics of problems associated with use of Inspektor camera will be provided by Waltham).
        - ii. **Software:** See comments above about further development of intensity recognition.
        - iii. **Crevice areas:** The Inspektor QLF images of teeth with coronal grooves resulting in a sharp crevice fail to count the dark crevice area. This will be reviewed with Inspektor. May not be an issue because all e.g. maxillary P4 and mandibular M1 teeth have the same coronal anatomy. May be more of an issue in cats because of the length of the groove in the feline canine teeth.
    - **b. Bottom line:** As things stand, if Inspektor is willing to listen to and work on problems identified by Waltham while using the QLF camera, it makes sense to work with Inspektor. The combination of high initial cost (US $25,000/set), and risk of making a whole trial unusable if the system acts up late in the final scoring session, means that reliability is a must if VOHC is to be willing to recommend that trial resources (VOHC product companies and lab animal facilities) purchase the system. VOHC would expect that Inspektor would provide updated software at no expense to any purchaser associated with VOHC; equipment updates for out-of-warranty cameras would be priced at Inspektor cost.

13. **Obtaining images:**
    a. **Positioning**. Depending on result of sub-set analysis, it **may** be possible to obtain a single image with all of the required subset teeth present.
        i. Unlikely in awake dogs because of how far the jaw would have to be opened to obtain an unobstructed view of the maxillary M1. Also, due to overbite, images have not been obtained of mandibular teeth in awake animals although this may be possible with appropriate training.
        ii. Difference in positioning resulting from use of separate images for each tooth is not considered to be a major problem for extent of coverage because coronal-apical or mesial-distal foreshortening or elongation would not change the ratio of the fluoresced area from the unfluoresced area of the crown.
        iii. Angulation of teeth. Preliminary assessment of sub-set data suggests that maxillary I3 can be deleted from the VOHC tooth set; this one of the teeth likely to be angulated in the whole-mouth view. The other is the maxillary first molar, which is the most difficult tooth to fully expose because of the lip, and because it is angled differently medially from the maxillary fourth premolar tooth.
    b. **Background light.** Intensity of fluorescence may be more of a problem if separate images of several teeth are analysed to develop the mouth mean score – background light, and distance from camera lens to the tooth surface may result in some images from the same mouth being lighter/darker than others. Include a calibration chart in each image, with the software set to automatically adjust the intensity to match a pre-set specification? The Waltham group takes the QLF images with the room darkened, and the camera lens placed one finger's width from the surface of the dog or handlers hand; this combination may be sufficient to avoid the include a calibration chart in the image field.

14. **Mechanics of plaque** - follow up with Dr. Sue Higham, University of Liverpool Dental School, about where there is any input they could bring to this discussion from their research on human dental substrates.