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CLINICAL RESEARCH

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Near-infrared fluorescence cholangiography in dogs: A pilot study

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Abstract

Objective: To determine the effect of indocyanine green (ICG) dose and timing of administration on near-infrared fluorescence (NIRF) imaging of the normal canine biliary tree.

Study design: Preclinical prospective study.

Animals: Eight purpose-bred beagles.

Methods: The dogs were randomized to receive two of four intravenous ICG dose (low [L]:0.05 mg/kg or high [H]:0.25 mg/kg)/time (0 and 3 h prior to NIRF) combinations. NIRF images were collected every 10 min for 120 min. Target (cystic duct)-to-background (liver) ratios were calculated for all time-points and compared.

Results: ICG cholangiography was successful in all dogs. The contrast ratio was above 1 in the L0 group by 20 min and reached its peak at 100 min. In the H0 group, the ratio was above 1 by 60 min and reached its peak at 90 min. Contrast ratios above 2 (fluorescence twice as bright in the cystic duct compared to the liver) were maintained from 180 to 300 min for L3 and H3 and was achieved after 80 min for L0.

Conclusion: Low dose ICG provided better ratios early after injection compared to the high dose which remained highly concentrated in the liver tissue after injection. Both doses provided excellent visualization of the biliary tree at 3 h post injection, low dose ICG provided better ratios from 3 to 5 h post injection. Based on these results, 0.05 mg/kg of ICG administered at anesthetic premedication, or as early as 3 h prior to laparoscopic surgery should yield optimal fluorescence images.

Clinical significance: This study provides guidelines for NIRF cholangiography in clinically normal dogs.

Abbreviations: BD, bile duct; BDI, bile duct injury; CBD, common bile duct; CD, cystic duct; CDLR, cystic duct-to-liver ratio; EHBO, extrahepatic bile duct obstruction; FI, fluorescence intensity(ies); GLM, general linear model; HR, heart rate; IBD, invasive blood pressure; ICG, indocyanine green; IOC, intraoperative cholangiography; IV, intravenous; LC, laparoscopic cholecystectomy; MAP, mean arterial pressure; NIR, near-infrared; NIRF, near-infrared fluorescence; NIRFC, near-infrared fluorescence cholangiography; ROI, region of interest; TBR, target-to-background ratio.

1 | INTRODUCTION

Laparoscopic cholecystectomy (LC) has widely replaced open cholecystectomy in humans. However, LC is still very new to veterinary medicine with only 165 cases reported.[1–6] Laparoscopic cholecystectomy is currently recommended for more elective procedures for benign conditions such as mucocele, cholelithiasis and cholecystitis without evidence of rupture, extrahepatic bile duct distension or obstruction (EHBO).[3,6] Recently, Kanai et al. also reported on 76 dogs that underwent LC with signs of jaundice, gall bladder rupture, abdominal effusion, or EHBO.

Complication rates associated with LC in humans are low (0.5%–6%), with bile duct injury (BDI) occurring in 0.18%–1.5% of cases.[7–16] Bile duct injury typically results from misidentification of the common bile duct (CBD) or cystic duct (CD) anatomy.[12,17–19] Such complications can have devastating consequences and are associated with a mortality rate of up to 21%.[20] Conversion rates of 0%–30% and BDI rates of 0%–5% have been reported in dogs.[1,3,5,6]

Intraoperative cholangiography (IOC) allows visualization of the intra- and extra-hepatic bile ducts, aids in identification of cholelithiasis and has been shown to reduce the incidence of, and increase identification of BDI.[21–23] However, in humans, bile duct (BD) catheterization for IOC poses a risk for BDI, it increases surgical and anesthetic times, it exposes the patient and personnel to unnecessary radiation and the ensuing images can be difficult to interpret.[7,21,24] Near-infrared fluorescence (NIRF) imaging has recently been used in both open and laparoscopic procedures. It is recommended for its ease of use, lack of ionizing radiation, excellent imaging properties, and has been shown to increase the identification rate of BDI in people undergoing LC.[24]

Following intravenous administration, ICG is rapidly cleared from the plasma and exclusively excreted, unaltered by the liver into the bile which allows visualization of the biliary tree anatomy using a NIRF laparoscope.[16,25–28] The near-infrared (NIR) light emitted is invisible to the human eye and does not alter the appearance of the surgical field in white light.[16] Dose and timing of administration are most important to optimize the target-to-background (bile duct-to-liver) contrast ratio and therefore enhance visualization of the biliary tree.[7,16,19,27,29,30] The exact dose and timing of administration for identification of the BD in dogs is unknown.

The objective of this study was to determine the effect of ICG dose and timing of administration on NIRF imaging of the biliary tree in dogs to help guide clinical use of this technique during LC in dogs. It was hypothesized that all dose/time combinations would provide fluorescence of the CD in dogs and that a longer time between injection and surgery would lead to improved visualization and contrast of the CD.

2 | MATERIALS AND METHODS

2.1 | Animals and ICG administration

This study was conducted in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Institutional Animal Care Committee at the University of Guelph, AUP #4383. Eight purpose-bred beagle dogs, four neutered males and four spayed females, assessed to be healthy on the basis of a physical examination, preoperative complete blood count, biochemistry profile and urinalysis were used in this study. Dogs were randomized in two stages using SAS Proc Plan (SAS Institute Inc. 2015. SAS/STAT® 14.1. Cary, North Carolina) to receive two dose/time combinations. First, each dog was randomized to a dose group (low [L] or high [H] dose) and then randomized to a sequence of time of administration (0 h followed by 3 h, or 3 h followed by 0 h) in a split plot incomplete crossover design (Table 1 and Figure 1). Dogs received either low dose (0.05 mg/kg ICG IV) ICG at time 0 h (L0) and 3 h (L3) or high dose (0.25 mg/kg ICG IV) ICG at time 0 h (H0) and 3 h (H3) given via an IV catheter placed in a cephalic vein. A minimum of 72 h washout period was obtained between each experiment as earlier studies have shown an average of 97.3% of ICG was excreted in bile within 6 h following injection with minimal enterohepatic circulation.[27] Dogs were fasted 12 h prior to their surgery.

2.2 | ICG administration and histamine levels

Indocyanine green (25 mg vials; Diagnostic Green GmbH, Farmington Hills, Michigan) was reconstituted to a 2.5 mg/mL solution according to manufacturer instructions and was given at the studied doses intravenously within 6 h as per the manufacturer's recommendations. Awake animals were subjectively assessed for signs of adverse effects. Heart rate only and HR and MAP were monitored immediately prior to, and at 5 and 10 min following ICG injection in conscious and anesthetized animals, respectively. Peripheral blood samples were collected immediately prior to, and within 3 min of ICG administration, transported on ice, centrifuged to collect plasma and conserved, frozen at −80°C. Plasma histamine levels were measured using a histamine enzyme immunoassay (Immunotech EIA Histamine, Prague, Czech Republic) in a triplicate fashion to generate a

TABLE 1 Individual patient characteristics.

Dog	Age (years)	Gender FS: female spayed MN: male neutered	Weight (kg)	Body condition score	Dose group	Procedure order randomization (0 h;3 h or 3 h;0 h)
1	5.4	MN	11.6	6/9	Low	0 h;3 h
2	2.7	MN	12.2	7/9	Low	3 h;0 h
3	2.7	FS	9.6	4/9	Low	0 h;3 h
4	5.4	MN	11.2	6/9	Low	3 h;0 h
5	2.7	FS	9.4	4/9	High	3 h;0 h
6	2.7	FS	10	5/9	High	0 h;3 h
7	2.7	FS	11.3	6/9	High	3 h;0 h
8	2.7	MN	14.8	9/9	High	0 h;3 h

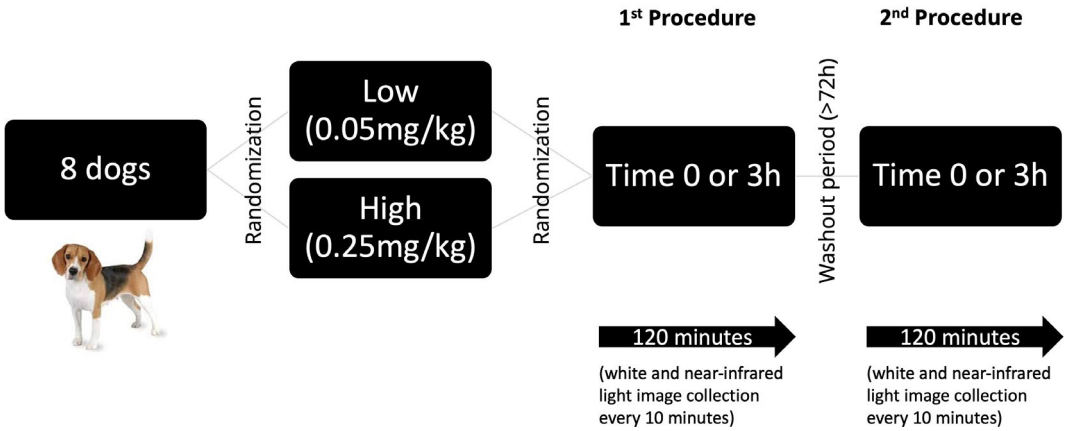

FIGURE 1 Schematic of study methodology.

mean histamine level for each sample. Plasma histamine levels below <0.8 ng/mL were considered normal.[31]

2.3 | Procedure

Dogs were premedicated with hydromorphone (0.05 mg/kg IV), induced with propofol (IV, to effect), maintained using isoflurane on 100% oxygen, and ventilated using intermittent positive pressure ventilation. A multiparametric monitor (Datex-Ohmeda S/5 Anesthesia Monitor; GE Healthcare, Chicago, Illinois) was used to measure direct arterial blood pressure (IBP), heart rate (HR) and rhythm, end-tidal CO_2, end-tidal isoflurane, pulse oximetry, and core body temperature, every 5 min. Perioperative antibiotics (cefazolin 22 mg/kg IV q. 90 min) were administered. Dogs were instrumented for laparoscopy using a modified Hasson technique with one 6 mm (instrument) and one 10 mm (camera) laparoscopic trocars (Karl Storz Veterinary Endoscopy, Goleta, California). Pneumoperitoneum was established with carbon dioxide insufflation maintained at 8 mmHg intraabdominal pressure. For time 0 h groups, dogs were instrumented for laparoscopy prior to injecting ICG intravenously at time 0 h allowing for pre-ICG images to be collected. For time 3 h groups, dogs were anesthetized ~2 h after ICG injection and images obtained starting exactly at the 3 h mark. For all dose/time combinations, images of the biliary tree under white light and NIRF light were collected every 10 min for 120 min throughout the procedure. The portal sites were closed in a standard three-layer closure.

Dogs were recovered and given a dose of hydromorphone (0.05 mg/kg subcutaneously) and meloxicam (0.1 mg/kg subcutaneously followed by orally, every 24 h) for 2 days. Dogs were returned to the colony after a minimum of 72 h of monitoring.

2.4 | Near-infrared imaging system and image collection

A laparoscopic NIRF imaging system (IMAGE1 S, Karl Storz Veterinary Endoscopy) was used to collect white and NIR light images throughout the procedures.

Images were collected with the 0-degree, 10 mm laparoscope perpendicular to the area of interest and maintaining a 5 cm distance (measured using a graduated laparoscopic probe) from the target. Conventional and NIR images were obtained successively by switching light modes (white light vs. NIR) using a foot pedal while maintaining anatomical orientation. Near-infrared images were recorded and displayed in a blue overlay on a Karl Storz 4 k monitor.

2.5 | Qualitative assessment of intraoperative fluorescence

Postoperative surgeon and assistant qualitative assessments of the intraoperative fluorescence and contrast were performed after each procedure (Appendix A). Both were blinded to the dose but were aware of timing of administration due to methodology.

2.6 | Quantitative analysis of NIRFC images

OsiriX software (https://www.osirix-viewer.com/; Pixmeo SARL, Bernex, Switzerland) was used to measure fluorescence intensities (FI) of regions of interest (ROIs), as previously described.[30] Fluorescence images were analyzed in a blinded (to dose and time of administration as well as order) fashion to determine cystic duct-to-liver ratio (CDLR), as previously described.[7,16,32] Cystic duct-to-background ratio was determined as the mean FI of two ROIs of the CD, divided by the mean FI of two representative background ROIs in the liver hilum; using the following formula: TBR = (FI of target/FI of background) or more specifically CDLR = FI of CD/FI of liver.[7,16,32]

2.7 | Statistical analysis

A general linear model (GLM) was fit to determine predictive equations for the change in fluorescence intensities of the liver, CD and contrast ratios over time for the four groups. Main effects included dose, time of injection and effect of time since injection. A post hoc pairwise *t*-test with Tukey adjustment was performed to compare contrast ratios between groups at selected time points. Modeled equations were used to determine the time of peak and value at peak. Histamine levels, mean arterial pressure (MAP) and heart rate (HR) were compared using a GLM with the main effects of dose and time (pre- and post-ICG injection). The interaction of dose and time were modeled for HR and MAP. Histamine was modeled with dose, time of injection and time in the model.

Interobserver agreement from the qualitative assessments was analyzed using a weighted Kappa. The individual Likert survey scores were summed (total score out of 20) and Lin's concordance correlation and a test of the bias was used to test for agreement between observers. As interobserver agreement was excellent, a single observer's scores were used in a GLM to test for main effects of dose and time period as well as their interactions. The random effect of a dog within a group was included.

All data was checked for normality using a Shapiro–Wilk test and examination of the residuals. Transformations were applied to meet the assumptions of normality. Log transformation was applied to the CDLR ratios, FI of the liver and FI of the CD. A logit transformation was applied to the summed Likert data. All analyses were performed using a commercial statistical software (SAS Institute Inc. 2013. SAS/STAT® 9.4) and $p < .05$ was considered statistically significant.

3 | RESULTS

Dogs were a mean age of 3.4 years (2.7–5.4 years), mean bodyweight of 11.26 kg (9.4–14.8 kg) and mean body condition score of 6/9 (4–9/9).

Each dog successfully underwent laparoscopic NIRFC. Only mild[33] adverse reactions associated with the use of ICG or the NIRF imaging system were identified. In patients receiving the high ICG dose prior to anesthesia (time 3 h), rapid onset and short-lived (<60 s) signs consistent with nausea (lip licking and swallowing) were noted immediately after IV injection. There was no overall effect of ICG administration on HR (pre = 124 bpm; post = 129 bpm; $p = .24$), nor was there a significant change in HR between the low (130.5 bpm) and the high dose (122.5 bpm) groups ($p = 0$). There were no significant changes in MAP associated with ICG administration (pre L: 98 mmHg; H:104 mmHg; post L:100 mmHg; H: 99 mmHg) [$p = .86$]), pre- or post-administration ($p = .341$). There were no overall differences in histamine levels pre-ICG administration (0.42 ng/mL) compared to post-administration (0.45 ng/mL) ($p = .64$), nor was there a significant change in histamine levels post-ICG injection for low (0.59 ng/mL) compared to high dose (0.32 ng/mL) ($p = .091$).

Mean anesthetic time was 198 min (165–445) and mean surgical time was 161 min (130–405). Technical difficulties with the NIRF equipment led to an exceptionally long anesthetic time during the first procedure (at time 0). After removing this outlier, mean anesthetic time was 181 min (165–210) and mean surgical time was 145 min (130–170). All dogs recovered uneventfully from their procedures. A total of 16 procedures (4 trials per time/

dose combination) were performed. The mean washout period was 113.6 h (range: 72–139 h).

3.1 | Qualitative assessments

All four dose/time combinations provided subjectively and objectively improved, repeatable, and clinically useful hepatobiliary visualization at some point throughout the studied times (0–2 h and 3–5 h post injection). Near-infrared fluorescence was readily visible within the liver less than 30 s following IV injection in the L0 and H0 groups (injection under laparoscopic NIRF visualization). For these groups, arterial fluorescence was noted to appear within seconds of IV ICG administration, first seen in the lungs through the diaphragm followed by the vasculature of the diaphragm and body wall, and hepatobiliary and intestinal vasculature. Visualization of fluorescence within the hepatic ducts was noted at ~10 min, whereas visualization within the common bile and cystic ducts was appreciated between 15 and 20 min after injection of ICG and remained visible until study completion for all cases. No evidence of hepatobiliary or other visceral abnormalities were noted on exploratory laparoscopy.

Good to excellent agreement was obtained for the two raters on the individual Likert scores. Weighted Kappa were 0.91, 0.81, 0.51 and 0.78 for questions 1, 2, 3, and 4, respectively ($p < .0006$). Overall agreement was excellent (0.88; 0.77–1) for the total score. No significant bias between raters was noted with mean difference of 0.375 (−0.44–1.19) ($p = .35$). Summed Likert data had significantly lower scores for time of administration 0 h (score 12.5; range 11–17) compared to time of administration 3 h (score 19.5; range 10–18) ($p < .0001$). There was no effect of dose ($p = .88$), or an interaction of dose with time of administration ($p = .65$). Overall, superior visualization scores were obtained at longer time points from administration.

3.2 | Quantitative assessments

3.2.1 | Fluorescence intensities

The FI of the liver and CD showed a significant positive linear slope for L0 and H0 until they reached their peak after which a significant negative quadratic slope was present ($p < .0001$) (Figures 2 and 3). The FI peak of the liver for L0 and H0 occurred at 37 and 67 min, respectively whereas the FI peak of the CD were at 77 min for both dose groups. The highest FI of the liver and CD were noted at 180 min for L3 and H3. The FI of the liver and cystic duct were greatest in the high

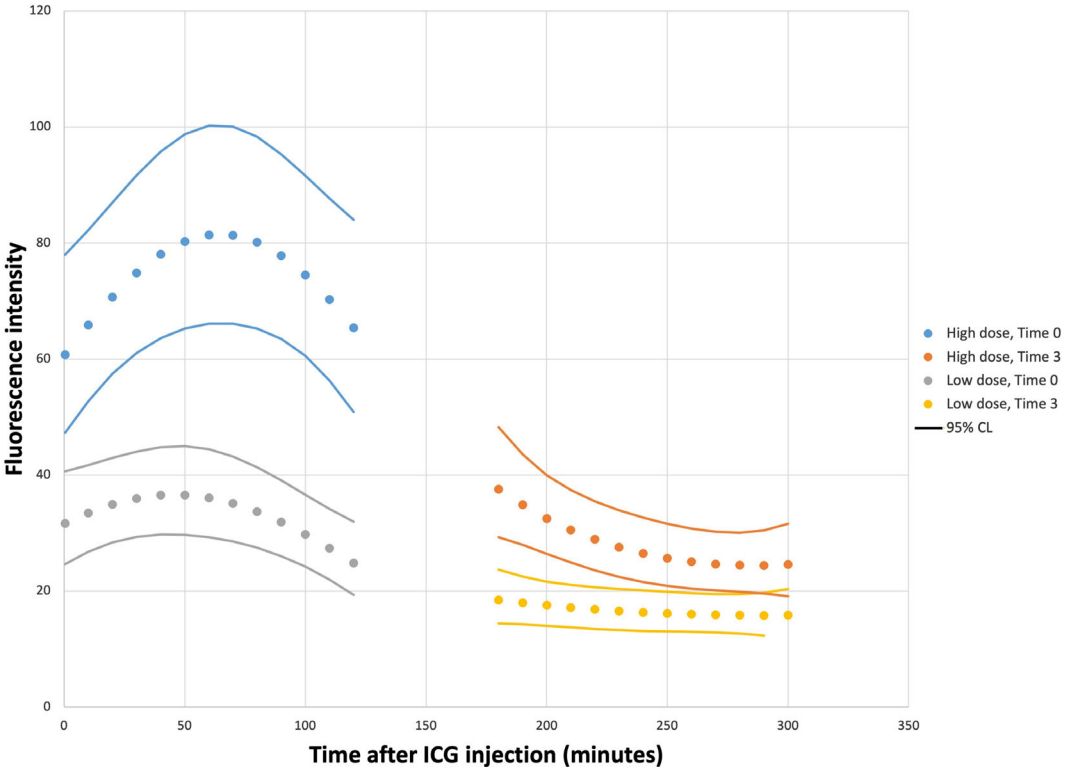

FIGURE 2 Liver fluorescence intensity at given times after indocyanine green (ICG) injection. The dotted lines represent the mean fluorescence intensity of the liver hilum for each group. The 95% confidence limits for each group are represented by the solid lines.

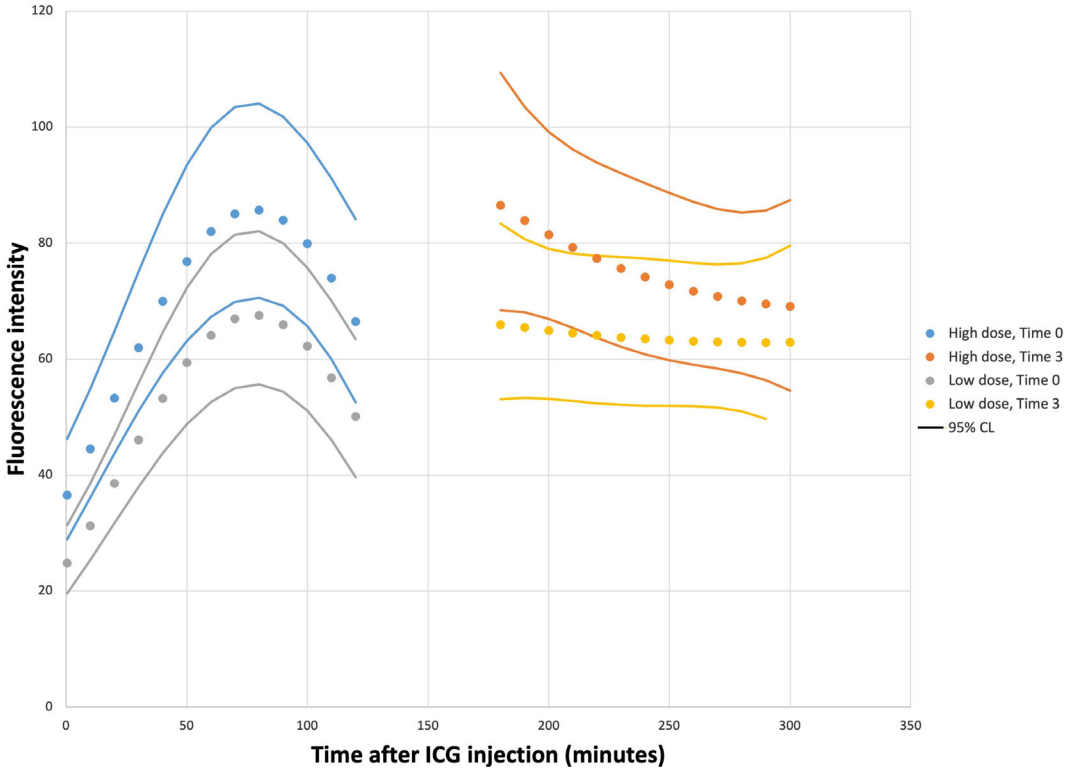

FIGURE 3 Cystic duct fluorescence intensity at given times after indocyanine green (ICG) injection. The dotted lines represent the mean fluorescence intensity of the cystic duct for each group. The 95% confidence limits for each group are represented by the solid lines.

dose groups. Faint residual fluorescence of the gall bladder and biliary tree and negligeable fluorescence of the liver was noted in two dogs that underwent H3 followed by H0 at time 0 (prior to ICG injection) during the second event.

3.2.2 | Contrast ratios (cystic duct to liver ratios)

The contrast ratio was above 1 in the L0 group by time 20 min and reached its peak at 100 min (Figure 4). In the H0 group, the ratio was above 1 by 60 min and reached its peak at 90 min. Contrast ratios above 2, (i.e., fluorescence twice as bright in the CD compared to the liver) were maintained from 180 to 300 min for L3 and H3 and was achieved after 80 min for L0. H3 appeared to have just reached plateau (linear slope $p = .086$; quadratic $p = .243$) at 270 min. This suggests that H3 ratios could still increase past 280 min and is considered a significant trend with a sample size of four dogs per dose/time group. The contrast ratios of L0 were superior to those of H0 during the entire period of visualization (0–120 min) leading to better visualization of the biliary tree (Figures 5 and 7). Both doses provided the highest contrast ratios when administered 3 h prior to surgery and offered optimal visualization of the biliary tree (Figures 6 and 8). However, the contrast ratio difference between L0 (2.003; 1.63–2.46) at 80 min was no longer significantly different from H3 at 280 min (2.86; 2.32–3.51) ($p = .078$) which was its peak value in this study. L0 at 120 min (2.017; 1.58–2.56) was significantly different from L3 at 180 min (3.57; 3.12–5.04) ($p < .0001$). The highest contrast ratios were obtained in group L3 where the CD was nearly four times as bright as the surrounding liver (3.98 [3.19–4.94]; 280 min).

4 | DISCUSSION

Near-infrared cholangiography using ICG is safe and feasible in healthy dogs and consistently improved visualization of the biliary tree in this study. The lower ICG dose provided better delineation of the biliary tree early after injection (0–2 h), while with the higher dose, ICG remained highly concentrated in the liver tissue and resulted in overall lower target-to-background ratios (Figures 5 and 7). Though both doses provided excellent visualization of the biliary tree at 3 h post injection (Figures 6 and 8), the lower dose provided better contrast between the biliary tree and liver.

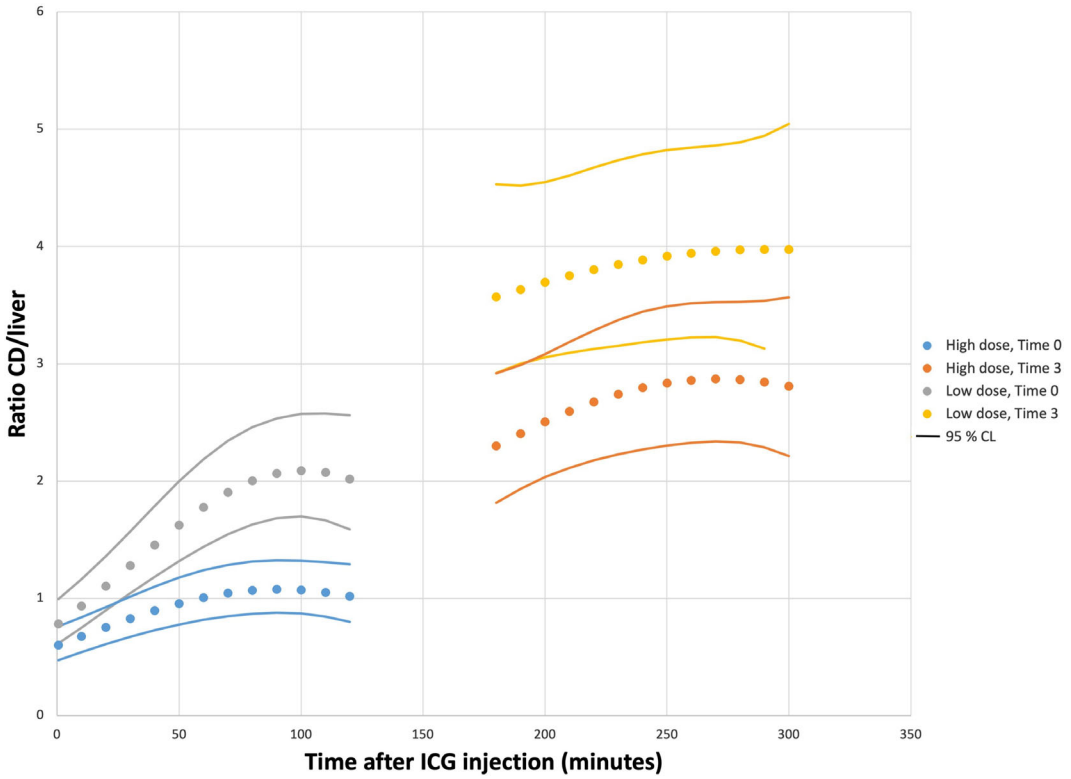

FIGURE 4 Contrast ratios between cystic duct and liver at given times after indocyanine green (ICG) injection. The dotted lines represent the mean ratios for each group. The 95% confidence limits for each group are represented by the solid lines.

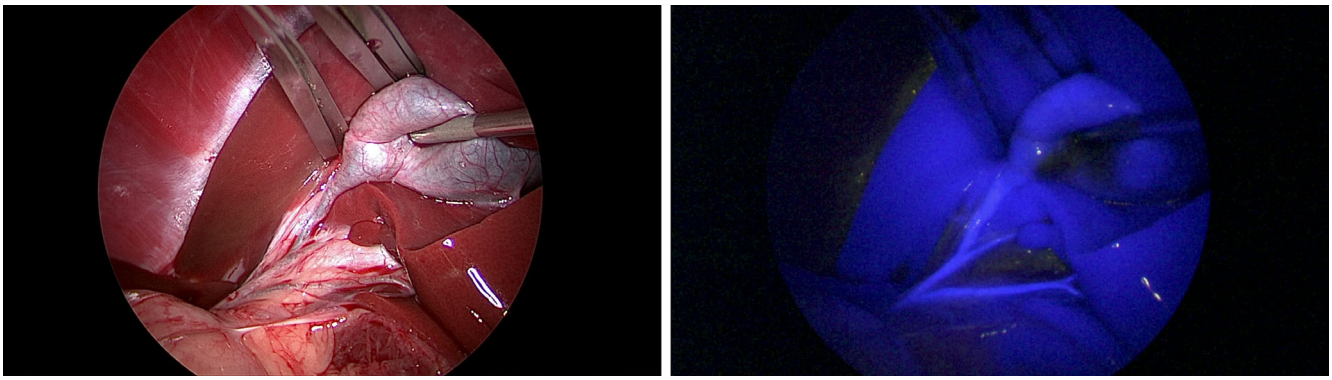

FIGURE 5 Dog 2 (low dose time 0 h): White (left) and fluorescent light (right) intraoperative images at 50 min post-injection demonstrating visualization of the biliary tree/gall bladder with high fluorescence of the liver (low contrast to background ratio).

In this study, the cystic artery was consistently visualized within 10 s of ICG injection and lasted for 30 s prior to the liver becoming fluorescent. This finding suggests that in instances where intraoperative identification of the cystic artery or aberrant vasculature is desirable, an additional dose of ICG could be administered though this would obscure BD visualization.[7] Time to fluorescence of the hepatic ducts (10 min), CBD and CD (15–20 min) were consistent with those reported in human experimental and clinical studies that range from 8 to 20 min following IV injection.[25,30]

Perceived and measured FI have been shown to depend on several factors. Increasing the distance between the tip of the endoscope and the fluorophore from 5 to 14 and 5 to 15 cm has been shown to result in a 5 to 30 times and 50% lower FI, respectively, in two ex vivo studies.[19,29] Maintaining a perpendicular endoscope angle to the ROI also leads to higher FI measurements.[19,29] Although the latter may not always be possible in a clinical setting or when using a 30° laparoscope, these factors were controlled for in this experimental study setting. Patient-related factors such as the presence of local fat and

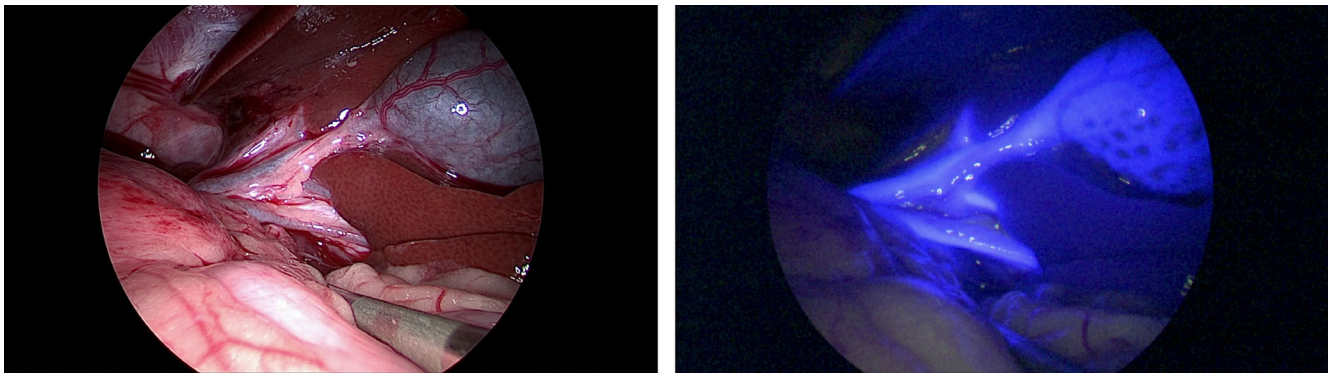

FIGURE 6 Dog 1 (low dose time 3 h): White (left) and fluorescent light (right) intraoperative images at 4.5 h post-injection demonstrating visualization of the biliary tree/gall bladder with low liver fluorescence (high contrast to background ratio).

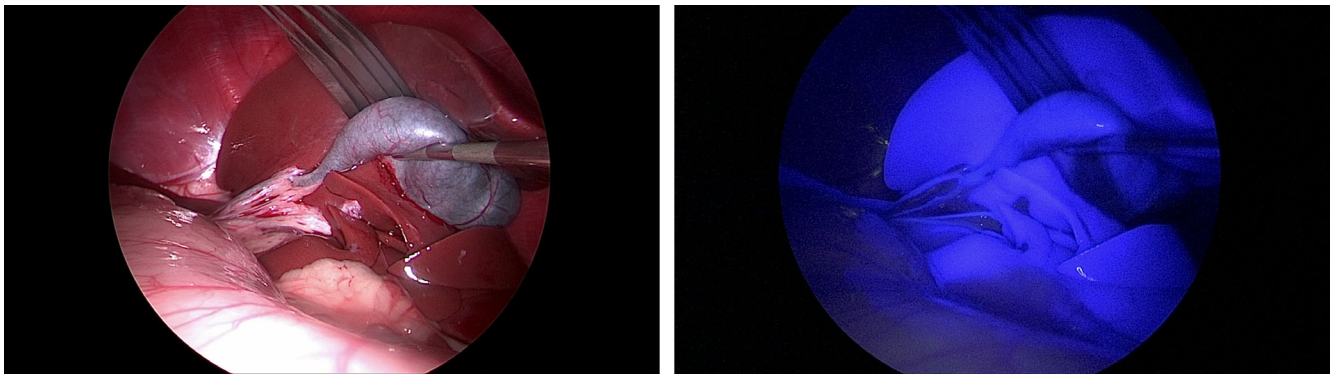

FIGURE 7 Dog 5 (high dose time 0 h): White (left) and fluorescent light (right) intraoperative images at 50 min post-injection demonstrating visualization of the biliary tree/gall bladder with high fluorescence of the liver (low contrast to background ratio).

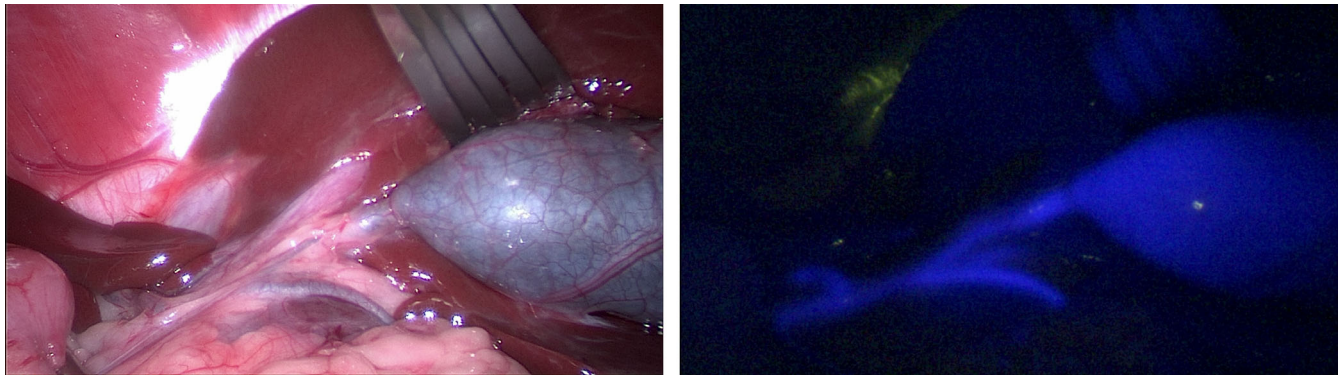

FIGURE 8 Dog 6 (high dose time 3 h): White (left) and fluorescent light (right) intraoperative images at 4.5 h post-injection demonstrating visualization of the biliary tree/gall bladder with low liver fluorescence (high contrast to background ratio). *Decreased resolution due to magnification.

inflammation can also reduce the perceived and measured FI since tissue depth of penetration of ICG NIR light reportedly ranges from 5 to 10 mm.[7,19,21,27] None of the dogs in this study had excess fat or inflammation in the area of interest. Finally, use of different NIR imaging systems complicates comparison between studies as measured signal contrast and images generated can differ significantly.[29] The Karl Storz imaging system used in this study is commonly found in clinical practice.

When measuring FI, ROIs need to be carefully chosen as subtle differences in signal intensity, light reflection and scatter can occur within the same ROI and can lead

to non-representative measurements.[34] Such artifacts were carefully excluded when selecting ROIs for FI measurements in this study. In a systematic literature review exploring software packages and formulas used to calculate FI in human LC, OsiriX and Image J (US National Institutes of Health, Bethesda, Maryland) were found to provide similar results, while Photoshop (Adobe Systems, San Jose, California) results differed significantly precluding comparison.[34] The CDLR formula used in this study was shown to correspond well with human visual perception.[34] This is supported in the current study as surgeon qualitative assessments scores reflected contrast ratio measurements with L3 and H3 having highest survey scores and highest contrast ratios.

Only five studies have reported on optimal dose and time of ICG injection for laparoscopic fluorescence cholangiography in humans. These studies administered ICG at varied doses (0.02–0.25 mg/kg and 5–25 mg) and administration times (0–24 h prior to surgery), and used inconsistent NIR imaging technology, imaging software programs and contrast ratio formulas.[7,16,32,35,36] Their reported CD- or CBD-to-liver ratios ranged from <1 to 2.3 but when ratios of <1 and >1 were obtained, qualitative scores revealed improved visualization of the structures of interest, regardless of the actual measured contrast ratio.[7,32,36] Indocyanine green doses selected for this study were based on the lower and higher end dose range utilized in human studies which provided good FI and contrast ratios. Longer delays between ICG injection and NIRF imaging led to less residual hepatic fluorescence and better contrast ratios.[7,27,32] In this study, we chose to assess two doses between 0 and 5 h following ICG injection in order to investigate timeframes that are practical for same-day procedures.

While lower BD-to-liver contrast ratios may not be ideal, they can still result in improved visualization of the biliary tree against a nonfluorescent (fat or connective tissue) background.[7,32] Nonetheless, an optimal dose and timing of administration would lead to high CD fluorescence with low liver fluorescence or high CDLR. This is especially true in dogs, where little fat is present at the hilus to provide a low fluorescence background. A more conservative dose and longer time between administration has generally met the aforementioned goals in humans.[7,16,35] Indeed, doses of 10 mg given at 10–12 h[34] or 10 mg given at 24 h preoperatively[16] have reportedly provided contrast ratios superior to 2.3, that is, the CBD was twice as bright as the liver. In our study, the CD was >2 to 4 times as bright as the liver in the L0 and L3 groups after 80 min and 180–300 min, respectively. Additionally, a CDLR nearing 3 was obtained in the H3 group at 280 min with a slope suggesting that the contrast ratios may continue to increase leading to further improved contrast beyond 5 h. These higher contrast ratios compared to the human studies likely result from species-related differences and rigorously controlled factors affecting FI in our study. Humans, notably those with a higher body mass index, have more fat covering their biliary tree and commonly present with cholangitis prior to undergoing NIRFC for LC.[19,21]

Residual fluorescence could only be assessed when the second ICG injection was at time 0 h. While residual fluorescence was not noted in dogs of the low dose group or dogs that had >92 h washout period, minimal residual liver fluorescence of the liver and gall bladder was noted in two high dose group dogs with a shorter washout period (72 h). Residual fluorescence was negligible in the liver hilum and minor in the biliary tree and did not appear to affect measurements and contrast ratios. Residual fluorescence should not be clinically relevant but longer washout periods could be considered for canine research as the ICG plasma clearance rates and excretion rates are lower in dogs when compared to humans.[37–39]

Hyperbilirubinemia, hypoalbuminemia, hypotension and reduced hepatocyte function have been shown to negatively impact ICG uptake and clearance in humans.[21,37,40] Furthermore, inflammation can lead to thickened gall bladder and bile duct walls which could affect FI. While gall bladder mucocele and associated gelatinous bile may hinder diffusion of ICG into the gall bladder, ICG should still diffuse into the CBD and possibly the CD to improve visualization and reduce conversion and BDI rates as it did in humans.[7,15,18,30,41–44] Optimal dose and timing of ICG administration may be different in dogs with more advanced disease, and this remains to be studied.

Mild adverse reactions to ICG reportedly occur in 0.15% of human patients and include nausea, vomiting, and pruritus, whereas severe reactions occur in 0.003%–0.05% of cases and are usually associated with anaphylactic shock.[33,45] Repeated doses are sometimes given for vascular angiography in humans with recommendations not to exceed 5 mg/kg/day.[46] No safety studies or recommended dosages have been described in dogs. In this study, mild transient signs suggestive of nausea developed in the four dogs that received the high dose while awake. General anesthesia may have masked signs of nausea in the other dogs as ICG was given intraoperatively for half of the procedures. It is unknown if a slow infusion could potentially mitigate clinical signs of nausea in awake patients. Although larger safety studies will be needed to corroborate these findings, ICG IV administration was considered safe in this population of dogs based on stability of cardiovascular parameters and lack of histamine release.

Limitations associated with this study are inherent to its pilot nature. The sample size is small which limits the

strength of the results. Due to contamination from hemolysis, histamine analysis could not be performed on all plasma samples limiting our conclusions. Additionally, order (first vs. second anesthetic event and drug administration) and its effects on MAP, HR or histamine levels could not be investigated due to limited power. However, the selected randomization scheme prevented unequitable distribution of the events in any group which may have resulted in biased results. Finally, the overall assessment of NIRFC was performed in a cohort of healthy dogs therefore, the results of this study as well as recommendations regarding dose and timing of ICG administration may not be generalizable to clinical patients with hepatobiliary disease.

The results of this study support that NIRFC is safe and feasible in healthy dogs. It consistently provides improved visualization of the biliary tree. An ICG dose of 0.05 mg/kg given at least 3–5 h prior to visualization should provide excellent contrast between the biliary ducts and liver as well as minimal residual liver fluorescence, improving visualization during NIRF laparoscopy. Alternatively, a 0.05 mg/kg dose given at the moment of premedication for general anesthesia is practical for emergent cases and should provide acceptable contrast and improved visualization. Additional indications could include to aid in the identification of biliary tears when bile leakage has stained the surrounding tissues. Further investigations to determine the optimal dose and timing of administration of ICG and the effects of hepatobiliary disease on NIRFC in canine patients are underway.

AUTHOR CONTRIBUTIONS

Chagnon Larose P, DVM: Data acquisition, analysis and interpretation, drafting and revision of manuscript. Brisson B, DVM, DVSc, DACVS, ACVS Founding Fellow Minimally Invasive Surgery: Conception and study design, data acquisition, analysis and interpretation, drafting and revision of manuscript. Sanchez A, DVM, DVSc, DACVAA: Data acquisition and interpretation, revision of manuscript. Monteith G, BSc: Data analysis. Singh A, BSc, DVM, DVSc, DACVS (Small Animal): Revision of manuscript. Zhang M. BSc: Data acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest related to this report.

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APPENDIX A

Postoperative surgeon qualitative assessment of intraoperative fluorescence (modified from Zarrinpar et al. 2016).

Rate the statements below from 1 to 5 according to the following grading scale:
1 = no improvement/identification not confirmed.
2 = marginally improved.
3 = sufficiently improved.
4 = well improved.
5 = greatly improved/exceeds expectations.

1. Being able to identify and distinguish the cystic duct (CD)_____.
2. Being able to identify and distinguish the common bile duct (CBD)_____.
3. Being able to identify and distinguish the CD and CBD from one another_____.
4. Being able to identify and distinguish the CD and CBD from the liver_____.

Survey completed by:_____.
Circle which is appropriate: ACVS Surgeon/Resident.