Received: 2 May 2023 | Revised: 20 October 2023 | Accepted: 3 December 2023

DOI: 10.1111/vsu.14067

WILEY

CLINICAL RESEARCH

Research funding by the ACVS Foundation

Use of real-time near-infrared fluorescence to assess gastric viability in dogs with gastric dilatation volvulus: A case-control study

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Funding information
American College of Veterinary Surgeons Foundation, Grant/Award Number: Surgeon-In-Training Grant; Doberman Pinscher Club of America; Great Dane Club of America; Poodle Club of America

Abstract

Objective: To describe near-infrared fluorescence (NIRF) for assessment of gastric viability and describe NIRF's influence on the surgeon's operative strategy in dogs with gastric dilatation and volvulus (GDV).

Study design: Prospective clinical trial.

Animals: Twenty dogs with GDV and 20 systemically healthy dogs.

Methods: Following gastric derotation, the surgeon's subjective assessment of gastric viability was recorded prior to near-infrared imaging. Changes in the surgeon's initial assessment of viability based on the visual pattern of gastric fluorescence was recorded. If nonviable (lack of defined vessels), a partial gastrectomy was performed and submitted for histopathology. The stapled gastrectomy line was imaged. Viable (defined vessels) and nonviable fluorescence intensities were compared with healthy dogs undergoing surgery for nongastrointestinal disease.

Results: Subjective assessment diagnosed 17 viable and three nonviable GDVs (2 fundi; 1 cardia). Near-infrared imaging demonstrated nonviable gastric fluorescence in 4 dogs (3 fundi/cardia; 1 fundus). The surgeon's margins for resection were altered in 3/20 dogs. Fluorescence intensity (cardia, fundus, body, pylorus) was lower in GDV viable (30.59%, $p = .04$; 38.17%, $p < .01$; 51.18%, $p < .01$; 44.12%, $p = .01$) and nonviable (11.00%, $p < .01$; 4.33%, $p < .01$; 57.67%, $p = .22$; 54.33%, $p = .72$) dogs compared to healthy controls (44.7%, 70.05%, 84.00%, 63.95%). Fundic fluorescence was less in nonviable

Abbreviations: CRI, Continuous rate infusion; ECG, Echocardiogram; GDV, Gastric dilatation and volvulus; ICG, Indocyanine green; NIR, Near-infrared; NIRF, Near-infrared fluorescence; PCV, Packed cell volume; TA, Thoracoabdominal; TS, Total solids.

The results of this study were presented at the American College of Veterinary Surgeons (ACVS) Summit in Portland, Oregon on October 15, 2022. An abstract for this study has been accepted for presentation at the Society of Veterinary Soft Tissue Surgery (SVSTS) in Jacksonville, Florida on June 17, 2023.

gastric tissue in comparison with viable gastric tissue ($p = .03$). Fluorescence of the gastrectomy staple line approximated that of viable tissue.

Conclusion: Near-infrared fluorescence can identify histologically confirmed nonviable gastric tissue.

Clinical significance: These results provide enough evidence to support the implementation of NIRF as an adjunct to gross examination of the gastric wall in dogs with GDV.

1 | INTRODUCTION

During emergency abdominal surgery, one of the main goals is to have a rapid and reliable means of determining tissue viability. In dogs with gastric dilatation volvulus (GDV), gastric necrosis has been reported in up to 35% of cases.[1] Attempts to assess gastric viability beyond conventional subjective parameters have been sparse in veterinary medicine. The first attempt was performed in 1986, where intravenous fluorescein dye injection was accurate in predicting gastric viability in only 58% of dogs.[2] In 1991, administration of technetium pertechnetate was found to be 91% accurate in detecting experimentally induced gastric necrosis;[3] this accuracy was reduced to 79% in clinical GDV cases.[4] Due to its radioactive nature, this modality is not safe for routine intraoperative use. Additionally, the emitted gamma rays do not define precise anatomic landmarks for gastrectomy. Most recently, in 2006, laser Doppler flowmetry was validated as a quantitative means of detecting variations in gastric blood flow intraoperatively.[1] However, this modality does not permit visualization of the vasculature by the surgeon and quantitative guidelines to differentiate viable from nonviable tissue have yet to be established.

At present, surgeons must rely on the subjective assessment of gastric viability (color, pulsations, arterial bleeding, palpable thickness, and motility), and accurate judgment is largely dependent on the surgeon's experience.[5] While visualization of the superficial macrovasculature may be possible, deeper interrogation of small arterials and venules is precluded by visual assessment alone, as visible light is almost entirely absorbed or reflected by tissues.[6] When deciding whether gastric tissue requires resection, full-thickness interrogation of the gastric wall vascular health is imperative. The deepest layer, the mucosa, is most metabolically active, consuming approximately 70% of the total blood supply to the stomach.[7] Unlike in visible light, photons in the near-infrared (NIR) spectrum are minimally absorbed or reflected, a property utilized by near-infrared fluorescence (NIRF) imaging.[8] Near-infrared fluorescence employs light in the NIR spectrum (~780 nm) to excite intravenously administered indocyanine green (ICG), which is rapidly distributed in the perfused vasculature.[6,9] Upon excitation, ICG also emits NIR light, but of a longer wavelength (~830 nm), which is subsequently detected by a NIR camera.[6,9] Unlike radionucleotides, ICG can be excited repeatedly, permitting repeated interrogation of organ vascular health.[10] In contrast to visible light, whose penetration is limited to microns, NIR light has been validated to penetrate as deep as 1.5 cm.[11] Given that the normal canine gastric wall is ~4 mm thick,[12] NIRF is capable of full-thickness interrogation of the intramural gastric vasculature.

In humans, NIRF has become an increasing popular intraoperative imaging modality, with literature demonstrating its utility in gastroesophageal[13] and colorectal resections[14] and during emergency abdominal surgery.[15] Specifically, use of NIRF in emergency surgery modified the surgeon's operative strategy in 32% of patients. Sixty-seven percent of patients did not receive a gastrointestinal resection that was initially planned, whereas 33% required a larger resection or a resection that was not originally planned.[15] This endorses the substantial variability that exists with subjective bowel assessment alone, particularly in inexperienced surgeons and in the emergent situation.

The primary objective of this study was to describe gastric wall NIR imaging findings in normal dogs, dogs with GDV, and a subset of dogs with GDV that were assessed to have gastric necrosis. The influence of NIRF imaging on operative strategy was also described. A secondary objective was to assess the degree of vascular compromise induced by surgical staples in dogs requiring a partial gastrectomy. We hypothesized that quantitative NIRF parameters of gastric tissue in healthy dogs would not differ from GDV dogs with healthy-appearing gastric tissue, that gastric necrosis would be associated with reduced NIRF intensity relative to healthy gastric tissue in dogs with GDV, that use of NIRF would modify the surgeon's operative strategy when compared to subjective assessment alone in dogs with GDV, and that fluorescence of the staple line would not differ from unstapled gastric tissue in dogs with GDV requiring gastric resection.

2 | MATERIALS AND METHODS

2.1 | Animals

Twenty client-owned dogs presented to the University of Florida Small Animal Hospital with GDV were enrolled.

Dogs were enrolled irrespective of age, breed, or sex. Twenty systemically healthy client-owned dogs undergoing a celiotomy were also enrolled as controls. Control dogs were excluded if they had gastrointestinal disease or were undergoing a gastrointestinal procedure, were systemically unstable, or had bloodwork abnormalities consistent with systemic disease. The present study was approved by the institutional animal care and use committee of the University of Florida (No. 202011069).

2.2 | Diagnosis

Gastric dilatation and volvulus was diagnosed via abdominal radiographs read by a board-certified radiologist. Sedation for imaging included butorphanol (0.1–0.5 mg/kg IV) or methadone (0.2–0.3 mg/kg IV), if needed. The systemic health of all dogs was evaluated at minimum with a venous blood gas, packed cell volume, and total protein. All dogs were hemodynamically optimized preoperatively with bilateral cephalic intravenous access, intravenous fluid therapy (lactate Ringers solution), and gastric decompression via trocarization and/or orogastric intubation. Blood lactate was rechecked following initiation of fluid resuscitation.

2.3 | Anesthesia and Monitoring

The anesthetic protocol was selected on a case-by-case basis at the discretion of the attending anesthesiologist. Use of dexmedetomidine and acepromazine as premedications were avoided due to possible effects on peripheral vascular perfusion. Anesthesia was maintained with inhalant (isoflurane or sevoflurane) or total intravenous anesthesia (propofol CRI). All dogs were administered perioperative antibiotics (cefazolin 22 mg/kg IV or amoxicillin-clavulanic acid 30 mg/kg IV). Continuous heart rate, respiratory rate, ECG, hemoglobin-oxygen saturation, end-tidal carbon dioxide, and blood pressure monitoring via an arterial line was implemented. In the event of persistent hypotension despite adequate heart rate, intravenous fluid resuscitation, and lowering of the anesthetic inhalant, a vasopressor (norepinephrine 0.1–0.5 mcg/kg/min or dopamine 5–10 mcg/kg/min) was initiated.

2.4 | Subjective Viability Assessment

Once the patient was under a stable plane of anesthesia, a ventral midline celiotomy was performed. The stomach was derotated to its normal anatomic position and excessive gaseous distention was relieved via passage of an orogastric tube. A routine abdominal explore was performed while allowing for gastric reperfusion. The surgeon's subjective assessment (viable or nonviable) of gastric viability of the cardia, fundus, body, and pylorus was made 10 min following derotation on the basis of serosal color, palpable thickness, gastric motility, and gastric and gastroepiploic arterial pulsations.

2.5 | Near-Infrared Fluorescence Assessment

Once the surgeon's subjective assessment was recorded, 0.1 mg/kg of indocyanine green (ICG; 2.5 mg/mL)[16] was administered IV followed by 3 mL of 0.9% NaCl flush by the anesthetist. All room and operating lights were turned off. A SPY Elite (Stryker, Kalamazoo, Michigan) handheld NIR camera with associated SPY-Q Analysis postprocessing software was used to visualize the onset of fluorescence of the intra-abdominal vasculature. The camera cord was covered with a sterile, disposable plastic sheath (Universal Medical, Oldsmar, Florida) and the lens with a cover designed for the SPY Elite camera. The imaged organs were minimally manipulated to avoid alterations in tissue perfusion. Qualitative and quantitative assessment of fluorescence started 1 minute following intravenous ICG administration.

Real-time qualitative images of the fluorescing gastric vasculature in the cardia, fundus, body, and pylorus were obtained. Based on the distribution of vascular fluorescence alone (presence or absence of visualization of defined blood vessels), the cardia, fundus, body, and pylorus were each recorded to be viable (Figure 1A) or nonviable (Figure 1B).

The quantification "QP" setting of the SPY Elite camera (Stryker) was then activated, displaying a box centered on the imaging screen. The box was placed over the left medial liver lobe to set the liver's quantitative fluorescence at 100%. Thus, all subsequently obtained quantitative parameters of fluorescence were relative to the liver's fluorescence. The liver was chosen as the reference point as ICG is eliminated via the hepatobiliary system. Thus, in the absence of decreased hepatocellular function, liver fluorescence should remain constant between dogs. The quantitative fluorescence of the cardia, fundus, body, and pylorus were recorded. The intensity directly over macroscopic blood vessels was not recorded to avoid misrepresentation of the degree of perfusion of the parenchyma. A single measurement for each part of the stomach was obtained as the entire cardia, fundus, body, or pylorus appeared grossly uniformly affected (i.e. there was a uniform pattern of fluorescence). The qualitative NIR assessment of each anatomic portion of the stomach was compared with the surgeon's initial subjective assessment of viability. Any change in the surgeon's assessment of viability was recorded.

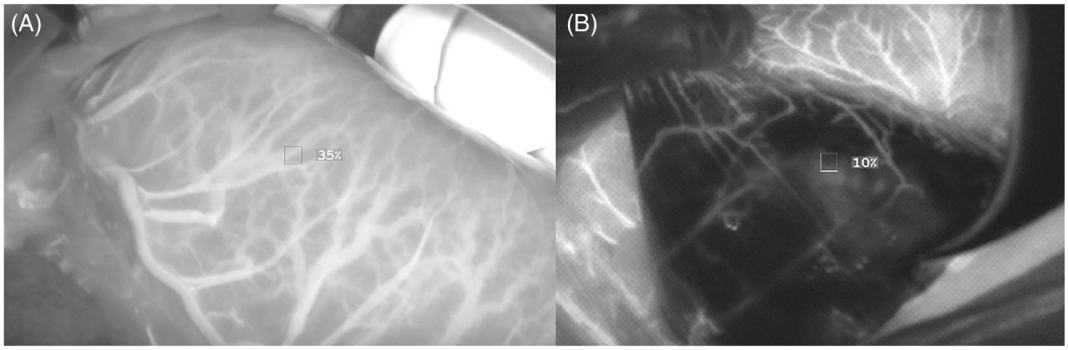

FIGURE 1 Near-infrared fluorescence images showing a fluorescence pattern with defined blood vessels (A) in a viable gastric dilatation volvulus and a fluorescence pattern with a lack of defined blood vessels (B) in a nonviable gastric dilatation volvulus.

2.6 | Incisional Gastropexy (NIRF Viable)

If all portions of the stomach were assessed to be viable based on visualization of defined fluorescing blood vessels, a routine incisional gastropexy was performed. The abdomen was closed routinely in the absence of open or closed peritoneal drainage.

2.7 | Partial Gastrectomy or Humane Euthanasia (NIRF Nonviable)

If one or more portions of the stomach were assessed to be nonviable based on a lack of visualization of defined blood vessels or absence of fluorescence of the parenchyma, a partial gastrectomy was performed, if possible. The margins of the nonviable tissue were demarcated using stay sutures and a thoracoabdominal (TA) 90 mm stapler (Medtronic, Minneapolis, Minnesota) with a green cartridge (4.8 mm open staple height, 2.0 mm closed staple height) was deployed, ensuring that the tissue was compressed for 10 seconds prior to staple firing. The nonviable tissue was sharply transected using a No. 15 scalpel blade and submitted for histopathologic assessment of necrosis. The SPY Elite camera (Stryker) was then used a second time to interrogate the staple line fluorescence qualitatively and quantitatively (Figure 2). The abdomen was closed routinely in the absence of open or closed peritoneal drainage.

If gastrectomy was not possible without introduction of substantial morbidity (e.g. gastroesophageal anastomosis), owners were contacted intraoperatively, and humane euthanasia recommended. Prior to euthanasia, the nonviable tissue was biopsied and submitted for histopathologic assessment of necrosis.

All surgeries were performed by a single surgery resident (KM) under the direct supervision of a board-certified surgeon. Operation of the SPY Elite NIR camera (Stryker) was performed solely by one investigator (KM) with previous training in device operation. All interrogations were performed with the camera approximately 40 cm from the organ of interest.[16]

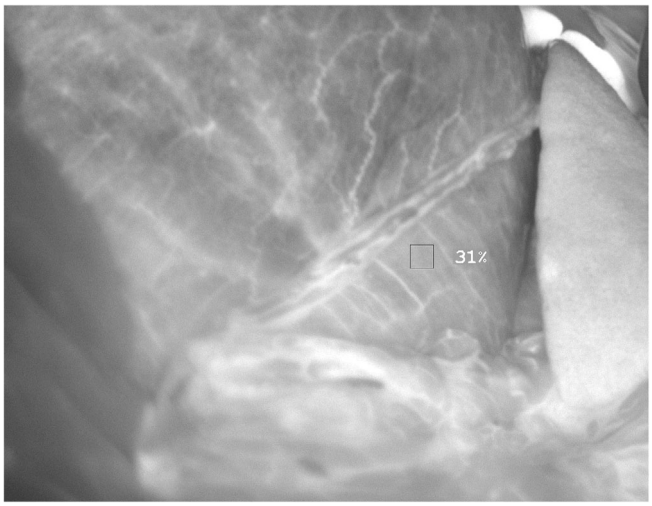

FIGURE 2 Near-infrared fluorescence image showing fluorescence to the thoracoabdominal green staple line with a fluorescence intensity similar to that of the remaining viable gastric tissue.

2.8 | Postoperative Monitoring

All patients were monitored in the intensive care unit on IV fluids (LRS 45–90 mL/kg/day), IV pantoprazole 1 mg/kg IV every 12 h, IV analgesics (ketamine 2–4 mcg/kg/h, lidocaine 25–50 mcg/kg/h, fentanyl 2–5 mcg/kg/h, and/or methadone 0.2 mg/kg every 6 h) prior to transitioning to oral gabapentin (8–10 mg/kg orally every 8 h) upon initiation of eating. Telemetry was used to monitor patients' heart rates and rhythm, and appropriate antiarrhythmics (lidocaine 2 mg/kg IV, procainamide 7 mg/kg IV) were

administered, if necessary. Additional medications, including promotility (metoclopramide 2 mg/kg/day IV or cisapride 0.3 mg/kg orally every 8 h) and antinausea agents (maropitant 1 mg/kg IV every 24 h or ondansetron 1 mg/kg IV every 12 h) were administered if regurgitation or nausea were noted, respectively. Immediate postoperative feeding was initiated either orally or via a nasogastric tube placed intraoperatively.

2.9 | Follow up

All dogs were discharged at the time of initiation of oral eating and transition to oral analgesics. Dogs were evaluated 2 weeks postoperatively for an incision recheck, or sooner if the animal declined at any point.

2.10 | Healthy (control) dogs

All control dogs had a ventral midline celiotomy performed for a nongastrointestinal elective procedure (e.g. cystotomy). 0.1 mg/kg ICG[16] (2.5 mg/mL) was administered IV followed by 3 mL of 0.9% NaCl flush by the anesthetist immediately upon entry into the abdominal cavity. Near-infrared fluorescence interrogation was performed as described previously, with the liver's fluorescence set at 100% to which the fluorescence of all other organs were compared to. The fluorescence intensities of the cardia, fundus, body, and pylorus were recorded prior to completion of the elective surgery (Figure 3).

2.11 | Statistical Analysis

A prestudy power analysis was performed, and a sample size of ≥13 dogs per group (control and GDV) was required to detect a difference with a power of 90%, $\alpha = 0.05$, and effect size of 0.5. Descriptive statistics were used to describe the distribution of all continuous variables among the groups. All comparisons between control and GDV (viable and nonviable) dogs and viable and nonviable GDV dogs were performed using one-way ANOVA with post hoc analysis using the Kruskal–Wallis method if significant for continuous variables and the two-tail Fisher's exact test for categorical variables. Significance was set at $p \leq .05$ for all analyses. All statistical analyses were performed with commercially available software (SAS, Cary, North Carolina).

3 | RESULTS

3.1 | Signalment

Twenty dogs diagnosed with GDV and 20 systemically healthy dogs met the inclusion criteria between March 2021 and March 2023. Of the healthy control dogs, fifteen underwent surgery for a cystotomy, three for a routine

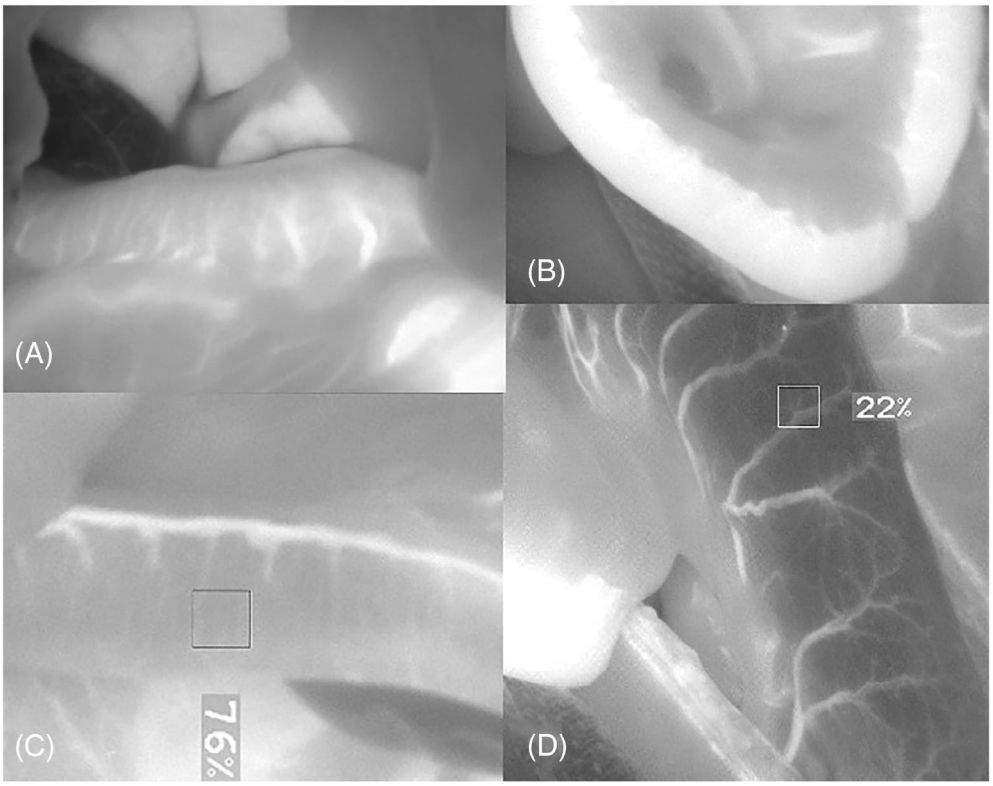

FIGURE 3 Near-infrared fluorescence images of the stomach (A), jejunum (B), ileum (C), and colon (D) from a control dog.

spay, one for a cystopexy and colposuspension for persistent urinary incontinence following ectopic ureteral ablation, and one for placement of a hydraulic urethral occluder device for incontinence.

The most common breeds in the GDV group were mixed ($n = 5$), Labrador retriever (3), standard poodle (3), German shepherd dog (3), and one each of a great Dane, Briard, boxer, corgi, boerboel, and Bernese mountain dog. Breeds in the control group included mixed (4), shih tzu (3), and one each of golden retriever, black mouth cur, Siberian husky, miniature schnauzer, English bulldog, Yorkshire terrier, Australian shepherd, American pit bull terrier, pug, basset hound, Havanese, boxer, and Chesapeake Bay retriever. Ten dogs in the GDV group were neutered females, eight were neutered males, and two were intact males. Five dogs in the control group were neutered females, six were neutered males, five were intact females, and four were intact males. The distribution of sex status did not differ between groups ($p = .06$).

3.2 | Preoperative lactate

The mean ± SD lactate (mmol/L) following initial fluid resuscitation of all GDV dogs was 5.34 ± 3.52. When divided by GDV group, the mean ± SD lactate of GDV viable dogs (4.89 ± 2.57) was less than that of GDV nonviable dogs (8.55 ± 5.44, $p = .03$).

3.3 | Clinical assessment

Fluorescence intensity was lower in dogs with GDV in comparison with controls (Table 1). Within the group of GDV dogs, only fundic fluorescence intensity was lower in the nonviable cohort when compared with the viable cohort (Table 2, Figures 4–7). In total, the stomachs of 17 GDV dogs were assessed to be subjectively viable and portions of the stomach in three GDV dogs were subjectively nonviable (Table 3). These included the fundus in two dogs (dog #7 and 15) and the cardia in 1 dog (dog #12). Near-infrared fluorescence interrogation (based on the qualitative fluorescence pattern) revealed a greater area of devitalization in dogs #7 and 12 (both cardiac and fundic involvement) and nonviable cardia and fundus in a dog (#20) that was assessed to be subjectively viable. Dogs #7 and 12 were euthanized intraoperatively due to the extent of cardiac involvement. Dog #15 underwent a fundectomy using a TA 90 stapler (Medtronic) with green cartridge. The staple line had a NIRF fluorescence of 30%. Dog #20 underwent partial resection of the cardia and complete fundus using 3–0 PDS; use of a stapler was not possible given the cardiac involvement. The dog died 4 days postoperatively from suspect disseminated intravascular coagulation. Histopathology was performed for dogs #7, 12, 15, and 20 and was consistent with complete loss of tissue architecture and transmural necrosis. All GDV nonviable dogs received vasopressor support intraoperatively (norepinephrine 0.1–0.3 mcg/kg/min in 2 dogs and dopamine 5–8 mcg/kg/min in 2 dogs) whereas only one dog in the GDV viable group required vasopressor support (norepinephrine 0.1 mcg/kg/min). In total, 17 of 20 GDV dogs survived to discharge.

4 | DISCUSSION

We described the qualitative and quantitative NIRF imaging findings in normal dogs, dogs with GDV that were assessed to have viable gastric tissue, and dogs with

TABLE 1 Clinical parameters (mean ± SD) for GDV ($n = 20$) and control ($n = 20$) groups.

Clinical parameter	GDV (all)	Control	*p*
Weight (kg)	33.06 ± 13.28	17.23 ± 10.69	<.01
Age (years)	8.01 ± 3.59	6.85 ± 4.05	.28
PCV (%)	48.05 ± 9.71	43.79 ± 6.06	.05
TS (g/dL)	7.70 ± 1.02	6.88 ± 1.00	.02
Heart rate (beats per minute)	111.50 ± 33.16	80.25 ± 27.46	<.01
SpO2 (%)	99.30 ± 1.26	99.10 ± 1.89	.52
Systolic blood pressure (mmHg)	103.20 ± 18.71	117.15 ± 25.15	.03
Diastolic blood pressure (mmHg)	63.45 ± 11.73	72.00 ± 19.65	.04
Mean arterial pressure (mmHg)	74.85 ± 11.75	86.10 ± 22.06	.04
Cardia fluorescence (%)	27.65 ± 13.02	44.7 ± 20.95	.01
Fundus fluorescence (%)	33.10 ± 19.44	70.05 ± 24.34	<.01
Body fluorescence (%)	52.15 ± 1597	84.00 ± 31.05	<.01
Pylorus fluorescence (%)	45.65 ± 16.57	63.95 ± 22.44	<.01

Abbreviations: PCV, packed cell volume; SpO2, oxygen saturation; TS, total solids.

TABLE 2 Clinical parameters (mean ± SD) for GDV viable ($n = 16$), GDV nonviable ($n = 4$), and control ($n = 20$) groups.

Clinical parameter	GDV (viable)	GDV (nonviable)	Control	*p* (overall)	*p* (control vs. viable)	*p* (control vs. nonviable)	*p* (viable vs. nonviable)
Weight (kg)	33.55 ± 13.84	30.27 ± 11.42	17.23 ± 10.69	<0.01	<0.01	0.21	0.90
Age (years)	7.90 ± 3.62	8.67 ± 4.17	6.85 ± 4.05	0.53	0.70	0.73	0.95
PCV (%)	46.59 ± 8.87	56.33 ± 12.06	43.79 ± 6.06	0.07	0.53	0.03	0.13
TS (g/dL)	7.52 ± 0.91	8.67 ± 1.20	6.88 ± 1.00	0.02	0.12	0.01	0.16
Heart rate (beats per minute)	105.06 ± 31.36	148.00 ± 15.10	80.2 ± 27.46	<0.01	0.03	<0.01	0.06
SpO2 (%)	99.18 ± 1.33	100.00 ± 0.00	99.10 ± 1.89	0.34	0.99	0.64	0.70
Systolic blood pressure (mmHg)	106.35 ± 18.45	85.33 ± 6.43	117.15 ± 25.15	0.02	0.30	0.06	0.28
Diastolic blood pressure (mmHg)	65.18 ± 11.84	53.67 ± 4.04	72.00 ± 19.65	0.04	0.41	0.17	0.50
Mean arterial pressure (mmHg)	76.59 ± 11.81	65.00 ± 5.00	86.10 ± 22.06	0.04	0.24	0.14	0.55
Cardia fluorescence (%)	30.59 ± 10.90	11.00 ± 13.08	44.7 ± 20.95	0.01	0.04	<0.01	0.17
Fundus fluorescence (%)	38.17 ± 16.32	4.33 ± 1.15	70.05 ± 24.34	<0.01	<0.01	<0.01	0.03
Body fluorescence (%)	51.18 ± 16.37	57.67 ± 15.04	84.00 ± 31.05	<0.01	<0.01	0.22	0.91
Pylorus fluorescence (%)	44.12 ± 15.99	54.33 ± 20.74	63.95 ± 22.44	0.01	0.01	0.72	0.69

Abbreviations: PCV, packed cell volume; SpO2, oxygen saturation; TS, total solids.

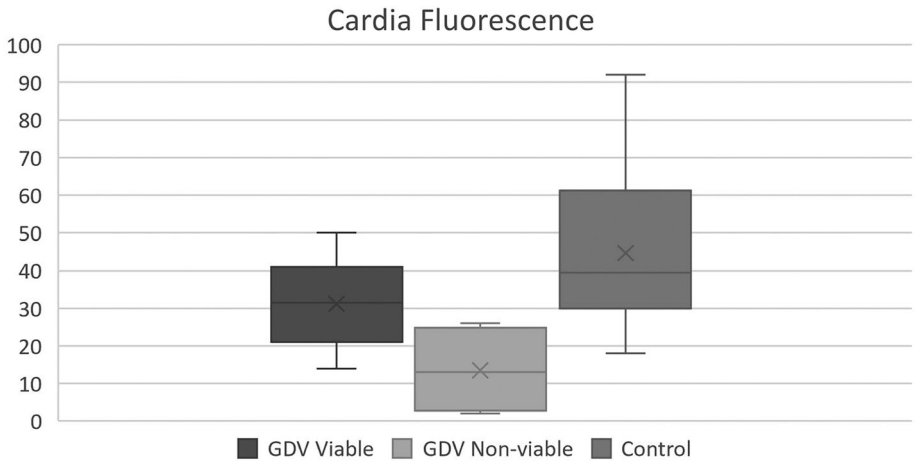

FIGURE 4 Side-by-side boxplot of cardiac fluorescence intensity between viable gastric dilatation volvulus, nonviable gastric dilatation volvulus, and control groups. Each box represents the 25th to 75th percentiles, the horizontal line is the median, the whiskers represent the range, and the X is the mean. The circles identify potential outliers.

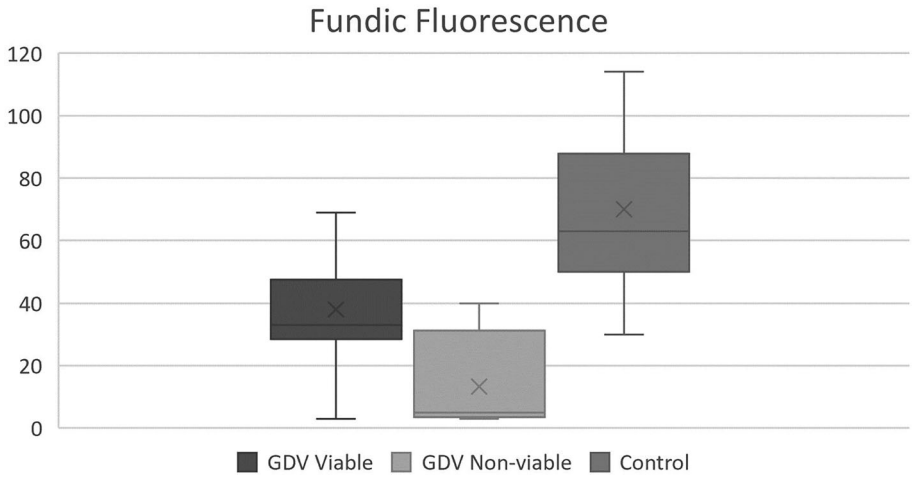

FIGURE 5 Side-by-side boxplot of fundic fluorescence intensity between viable gastric dilatation volvulus, nonviable gastric dilatation volvulus, and control groups. Each box represents the 25th to 75th percentiles, the horizontal line is the median, the whiskers represent the range, and the X is the mean. The circles identify potential outliers.

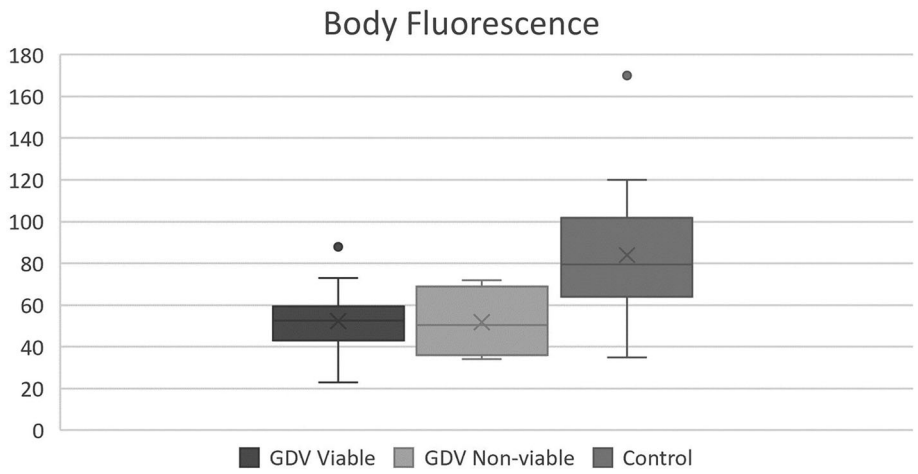

FIGURE 6 Side-by-side boxplot of body fluorescence intensity between viable gastric dilatation volvulus, nonviable gastric dilatation volvulus, and control groups. Each box represents the 25th to 75th percentiles, the horizontal line is the median, the whiskers represent the range, and the X is the mean. The circles identify potential outliers.

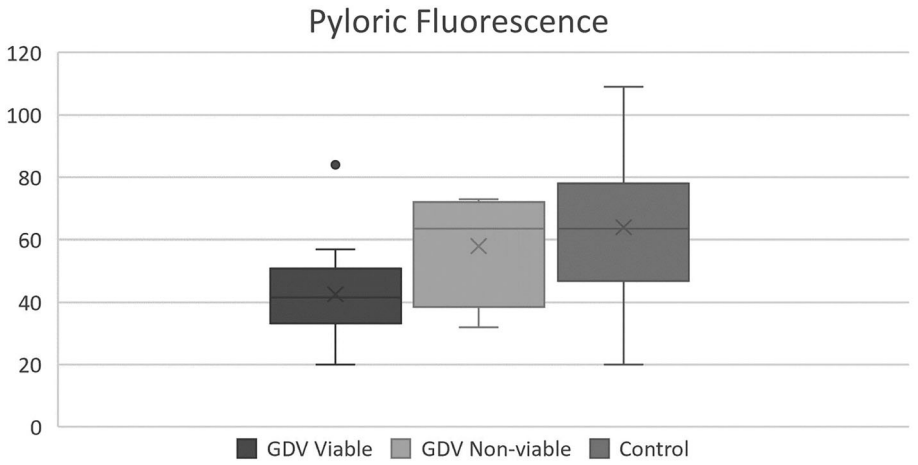

FIGURE 7 Side-by-side boxplot of pyloric fluorescence intensity between viable gastric dilatation volvulus, nonviable gastric dilatation volvulus, and control groups. Each box represents the 25th to 75th percentiles, the horizontal line is the median, the whiskers represent the range, and the X is the mean. The circles identify potential outliers.

GDV that were assessed to have gastric necrosis. The first hypothesis was rejected because gastric fluorescence intensity in GDV viable dogs was lower than the control population despite a gross pattern of fluorescence that closely resembled that of the control population. The second hypothesis was accepted as the fundic fluorescence intensity in GDV nonviable cases with histopathologic-confirmed fundic necrosis was lower than GDV viable cases. Our third hypothesis was accepted as use of the qualitative pattern of NIR fluorescence altered the surgeon's operative strategy in three of 20 GDV cases. Finally, our fourth hypothesis evaluating the effect of staple size on gastric perfusion was unable to be assessed due to an insufficient number of stapled fundectomies.

Using the properties of NIR light, this study described the use of fluorescence quantification of the intramural gastric vasculature as a means of identifying aberrations in gastric perfusion that may not be grossly appreciated. Specifically, the gross pattern of fluorescence observed in the gastric tissue of control dogs was subjectively similar to that of GDV dogs with viable gastric tissue. However, the cardiac ($p = .04$), fundic ($p < .01$), body ($p < .01$), and pyloric ($p = .01$) quantitative fluorescence were all greater in control dogs, rejecting our first hypothesis. Such disparity in gross and quantitative findings is likely a reflection of impaired vascular perfusion of deeper tissues (i.e. mucosa) and/or detection of decreased perfusion of microvessels that are not readily identified by the unaided eye. Previous studies[17,18] have established that damage to gastrointestinal microvasculature (e.g. capillaries) precedes damage to the macrovasculature (e.g. arteries and veins), potentially contributing to the inferior nature of the surgeon's subjective assessment of gastrointestinal viability. While identification of decreased microvascular perfusion may not prompt resection in an otherwise grossly viable appearing stomach, such findings may prompt the surgeon to initiate supportive measures in the postoperative period for presumptive mucosal sloughing/partial gastric ulceration or more intensive patient monitoring for sequelae of free radical generation from reperfusion injury.

While reduced fluorescence intensity in the face of visualization of defined fluorescing blood vessels suggests reversibly diseased gastric tissue (viable), the results of this study also support that reduced fluorescence intensity with a fluorescence pattern demonstrating a lack of visualization of defined blood vessels suggests

TABLE 3 Surgeon's subjective and NIRF viability assessment for all GDV cases ($n = 20$).

Case #	Subjectively viable	NIRF viable
1	Y	Y
2	Y	Y
3	Y	Y
4	Y	Y
5	Y	Y
6	Y	Y
7	N (F)	N (C, F)
8	Y	Y
9	Y	Y
10	Y	Y
11	Y	Y
12	N (C)	N (C, F)
13	Y	Y
14	Y	Y
15	N (F)	N (F)
16	Y	Y
17	Y	Y
18	Y	Y
19	Y	Y
20	Y	N (C, F)

Abbreviations: C, Cardia; F, Fundus; N, No; Y, Yes.

irreversible diseased gastric tissue (nonviable). As such, our second hypothesis was accepted as NIRF was able to differentiate viable from nonviable gastric tissue in some dogs. In this study, gastric histopathology was performed in four GDV dogs with a fluorescence pattern that failed to identify defined blood vessels, confirming full-thickness gastric necrosis. Histopathology was not performed in the remaining 16 GDV dogs with a fluorescence pattern demonstrating defined blood vessels but the survival of all 16 dogs for a minimum of 14 days postoperatively supports the absence of irreversible gastric wall necrosis in these dogs. Similarly, the lower preoperative lactate in GDV viable dogs supports improved gastric perfusion relative to the GDV nonviable group. In the four dogs with confirmed gastric necrosis, the pattern of fluorescence was consistent with a nonviable cardia and fundus in three cases and fundus only in one case. While the cardiac ($p < .01$) and fundic ($p < .01$) fluorescence intensity in these dogs was less than that of healthy gastric tissue (the control population), only the fundic fluorescence intensity was lower (4.33%) when compared to the fundic fluorescence in viable GDV dogs (38.17%, $p = .03$). Failure to identify a significant difference between cardiac fluorescence intensity in the three nonviable GDV cardia (11.00%) and viable GDV cardia (30.59%, $p = .17$) is attributed to type II error.

Although identification of nonviable gastric tissue qualitatively and quantitatively may reassure operating surgeons in their decision making process, the visual pattern of fluorescence with NIRF imaging may also modify the surgeon's operative strategy when compared to subjective unaided assessment alone. While larger scale controlled studies are necessary to assess the influence of such modifications on clinical outcomes, in the present study three dogs were subjectively identified to have nonviable gastric tissue based on the surgeon's gross observations of color, thickness, pulsations, and peristalsis. Two of these dogs were assessed to have a nonviable fundus only and one dog had a subjectively nonviable cardia only. Upon interrogation of the fluorescence pattern with the NIRF camera, a lack of defined fluorescing blood vessels was also identified in the cardia in one of the fundus-only dogs and in the fundus in the cardia-only dog. Moreover, one dog with subjectively appearing viable gastric tissue had NIRF imaging findings consistent with a nonviable cardia and fundus (lack of defined fluorescing blood vessels to absent fluorescence).

While use of NIRF imaging to define surgical margins for resection has been reported in the human literature,[15] this is the first report that NIRF may alter the surgeon's operative strategy in gastrointestinal procedures in veterinary medicine, accepting our third hypothesis. Clinically, identification of a greater area of gastric devitalization in two dogs resulted in intraoperative euthanasia. Failure to identify the true extent of gastric necrosis in these dogs may have resulted in inhumane postoperative patient suffering and owner expenses and permitted allocation of limited critical care resources to patients with survivable diseases. In addition to NIRF expanding or reducing the surgeon's proposed areas of viable and nonviable gastric tissue in GDV dogs, more precise borders of tissue resection may also be established. In this study, the two dogs that were euthanized intraoperatively had diffuse cardiac involvement. While a third dog also had cardiac involvement, the pattern of fluorescence that did not contain defined blood vessels abutted but did not span the lower esophageal sphincter, prompting the surgeon to pursue partial gastrectomy rather than intraoperative euthanasia. Ultimately, although the visual pattern of fluorescence may augment the surgeon's assessment of gastric viability, the authors caution against sole use of NIRF without consideration of the entire patient's stability. Cardiac output and systemic vascular resistance determine global vascular perfusion. While persistent hypotension can occur secondary to tissue necrosis and an ensuing systemic inflammatory response, use of inotropes to correct intraoperative hypotension for other

reasons may artifactually affect tissue fluorescence. Further study on the effects of hypotension and inotropic use on fluorescence pattern and intensity are warranted.

When a partial gastrectomy is deemed necessary, a handsewn or stapled gastrectomy may be performed. The anesthetically unstable GDV patient subjected to hypotension, reperfusion injury, and cardiac arrhythmias may benefit from a shorter operating time associated with stapled resections.[12] Use of a TA stapler (Medtronic) with a green cartridge (2.0 mm closed staple height) has traditionally been used for a partial gastrectomy. Although the normal gastric thickness (approximately 4 mm)[12] exceeds that of the closed green staple height (green), NIRF interrogation of the single stapled partial gastrectomy in this study and patient survival provides preliminary support that green TA staples may adequately preserve microvascular perfusion to the stapled edge with the B-shaped nature of the staples. However, interrogation of a greater number of gastrectomies performed with a TA green stapler (Medtronic) is warranted before definitive conclusions can be drawn.

The main limitation of this study is its underpowered nature with respect to nonviable GDV (four dogs) and stapled partial gastrectomy (one dog) cases. A prestudy power analysis determined a minimum of 13 viable and 13 nonviable GDV cases were needed to establish a significant difference in fluorescence intensity with an effect size of 0.5. Specifically, we failed to identify a difference in body and pyloric fluorescence between healthy and nonviable GDV dogs. Although the body and pylorus were viable in all "nonviable" GDV cases, we would have expected to obtain results similar to those of the "viable" GDV cohort. Moreover, while we attained a significant difference in fundic fluorescence, failure to identify a difference in cardiac fluorescence intensity despite histopathologic confirmation of necrosis is likely due to the study being underpowered. Another limitation is the arbitrary selection of time points for assessment of gastric fluorescence as the present study is the first report describing this imaging modality in dogs with GDV. While timing of interrogation was controlled among all subjects (10 minutes after derotation and 1 minute after ICG administration), interrogation of the gastric vasculature at different timepoints may result in different fluorescence measurements. Prior to committing a dog to a gastric resection, the author's recommend serial interrogation of the stomach to ensure improvement in the pattern of fluorescence of fluorescence intensity is not witnessed. A final limitation is the failure to use control dogs that were undergoing surgery solely for collection of control data. Rather, these dogs had a nongastrointestinal surgical disease that was not immediately life threatening. However, the majority of the dogs underwent a cystotomy, where the vascular supply (cranial and caudal vesicular arteries) differs from that primarily supplying the gastric tissue (celiac artery). Thus, inflammation and increased blood flow to the bladder presumptively has minimal consequences for gastrointestinal blood flow.

In conclusion, there is a paucity of veterinary literature addressing means of assessing gastric viability intraoperatively. Near-infrared fluorescence offers a relatively novel alternative that allows for precise mapping of tissue vascularity while also providing quantitative measures of fluorescence. Utilization of this modality has the potential to reduce the morbidity and mortality associated with emergent GDV surgery in dogs by altering the surgeon's operative strategy and providing improved confidence in intraoperative decision making. Further studies investigating NIRF in a larger population of GDV patients are warranted to establish quantitative reference ranges for viable and nonviable gastric tissue and assess the influence of modifications to operative strategy on clinical outcomes.

ACKNOWLEDGMENTS

Author Contributions: Mullen KM, DVM, MS: Study design, data collection, manuscript composition. Regier PJ, DVM, MS, DACVS (Small Animal): Study design, data collection, manuscript composition. Perez-Rodriguez V, BS, MS: Data collection, manuscript review. Fox-Alvarez WA, DVM, MS, DACVS (Small Animal): Study design, manuscript review. Bertran J, DVM, MS, DACVS (Small Animal): Study design, manuscript review. Colee J, PhD: Study design, data interpretation, manuscript review.

The authors would like to thank the American College of Veterinary Surgeons, and Great Dane, Doberman Pinscher, and Poodle Clubs of America for their generous support for this study.

CONFLICT OF INTEREST STATEMENT

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

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How to cite this article: Mullen KM, Regier PJ, Perez-Rodriguez V, Fox-Alvarez WA, Bertran J, Colee J. Use of real-time near-infrared fluorescence to assess gastric viability in dogs with gastric dilatation volvulus: A case-control study. *Veterinary Surgery*. 2024;53(4):684-694. doi:10.1111/vsu.14067