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Ex vivo comparison of hand-sutured versus circular stapled anastomosis in canine large intestine

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Abstract

Objective: To compare leakage pressures of colonic anastomoses performed with circular staplers to conventional hand-sewn techniques in dogs.

Study design: Ex-vivo study.

Animals: Colon from 11 canine cadavers.

Methods: Thirty-two colonic anastomoses were performed. Four segments from each colon were randomly assigned to one of four techniques: hand-sewn colonic anastomoses performed with 4-0 glycomer 631 (G) and 4-0 barbed glycomer 631 (BG), and circular stapled colonic anastomoses using 4.8 mm End-to-End Anastomosis (EEA C4.8mm) and 3.5 mm End-to-End Anastomosis (EEA C3.5mm), 21 mm diameter circular staples in cadaveric canine colon. Leakage pressure was defined as the pressure at which dye-containing solution was first observed to leak from the anastomosis site.

Results: Leakage pressures were 49.5 mmHg (range:16-72) in group G, 45.5 mmHg (range:19-80) in group BG, 5.3 mmHg (range:0-31) in group C3.5mm, and 29.5 mmHg (range:23-50.3) in group C4.8mm. Anastomoses leaked at lower pressures when stapled rather than hand-sewn (C4.8mm-G $p = .0313$, C4.8mm-BG $p = .0131$, C3.5mm-G $p = .0469$, C3.5mm-BG $p = .0313$). Two of the C3.5mm constructs leaked immediately after saline infusion with 4/6 leaking at <5.3 mmHg.

Conclusion: End-to-end colonic anastomoses closed with circular stapler leaked at lower pressures than hand-sutured anastomoses. Use of the EEA stapler with a staple height of 3.5 mm did not result in safe colonic anastomoses.

Clinical significance: These results provide evidence to support hand-suturing colonic anatomoses with G and BG in dogs. The 4.8 mm staples may be considered in anatomical locations difficult to reach.

1 | INTRODUCTION

Colonic resection and anastomosis is indicated in small animals to treat neoplastic lesions, polyps, trauma, colitis, colonic torsion, and megacolon.[1–3] Hand suturing remains the most common anastomotic technique

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although alternative options include stapled anastomosis and biofragmentable rings.[1,2,4] Relative rates of colonic leakage following colectomy are currently not well established in small animals, but a secure closure is of the utmost importance due to the high bacterial load in the large intestine.[5] Current gastrointestinal stapling techniques include the use of a gastrointestinal anastomosis (GIA) stapler with a thoracoabdominal (TA),[6–11] an end-to-end anastomosis (EEA),[2,3] or an anastomosis with skin staplers.[12] In man, studies comparing hand-suturing to stapling of colonic and ileocolic resection and anastomosis reporting conflicting results regarding the relative risks of leakage between closure techniques.[13–17] Evidence remains insufficient to establish the superiority of a method over the other in human patients.[18] Similar studies are lacking in small animals, particularly with circular stapling equipment. Two case series of canine cases have described the successful use of a circular stapler in a clinical setting.[2,3] In dogs, hand-sutured and end-to-end stapled colonic anastomoses have only been compared by Bundy et al in 1993,[19] but this study did not evaluate staples of various heights. The objective of our study was to compare the leakage pressures of hand-sutured colonic anastomoses performed with monofilament suture, unidirectional barbed suture, and a circular staple device in fresh, canine cadaveric large intestine. We hypothesized that leakage pressures would not differ between colonic anastomoses after hand-sutured or stapled closure.

2 | MATERIALS AND METHODS

Large intestine was harvested from 11 medium to large, mixed breed dogs immediately after euthanasia. All dogs had been euthanatized for reasons unrelated to the study (IACUC No. 17-7102A) and their prior medical history was unknown. The large intestines were collected from the ileocecocolic junction to the level of the rectum, where the caudal rectal artery penetrates the rectal wall. All specimens were inspected for gross pathology, and luminal contents were removed by gentle flushing with tap water. They were then divided into four equal sections based upon the total length of the large intestine harvested. Each section was then randomly assigned to one of four groups; G group, BG group, C3.5mm group, C4.8mm group; depending on the anastomosis technique applied. For the G group, 4-0 glycomer 631 (Biosyn, Medtronic, Minneapolis, Minnesota) with a CV 25 taper needle was used. For the BG group, 4-0 barbed glycomer 631 (VLoc 90, Medtronic, Minneapolis, Minnesota) with a V 20 taper needle was used. For the C3.5mm and C4.8mm groups, 21 mm diameter circular staplers (EEA DST circular stapler, Medtronic, Minneapolis, Minnesota) were used with 3.5 and 4.8 mm staple heights, respectively. Since each colon was divided into four segments, the anastomosis techniques were initially randomly assigned to a section of large intestine of each dog by a masked observer. This assignment was then rotated between dogs so that all locations along the length of the large intestine were assessed by each technique. Prior to testing, segments were stored in 0.9% NaCl solution chilled at 40°C and all anastomoses were constructed and tested within 24 h of euthanasia, as previously described.[20]

2.1 | Constructing the anastomoses

Hand-sutured closure in the G and the BG groups consisted of two, simple-continuous suture lines starting from the mesenteric and antimesenteric borders (Figure 1). All anastomoses were performed by a surgery resident (JAS) under the supervision of a board-certified surgeon (EM). Bites were placed 2-3 mm apart and 2-3 mm from the cut edge of the tissue. For the BG group, the loops were placed at the mesenteric and anti-mesenteric borders, bites were continued beyond the previous lines loop for two throws and the suture was locked by passing one bite 180° to the previous bite. The barbed suture ends were cut to leave a 0.5 cm free end. For the G group, simple continuous lines were started on the mesenteric and antimesenteric borders, traction was maintained by a surgical assistant placing equal tension on the suture tags, and the second continuous line tied to the tag of the first lines knot. A total of six and eight throws were placed to start and end the simple continuous line, respectively. Interrupted bites with 4-0 glycomer 631 were added at the surgeon's discretion for both the BG and G groups based upon visual inspection of the anastomosis line, to replicate the clinical setting. Leak testing was not performed as has been previously described.[21] The total number of suture bites, and number of additional simple interrupted sutures placed were recorded.

Stapled closures in the C3.5mm and C4.8mm groups started with placement of a purse string suture at the opening of one of the colonic segments no more than 2.5 mm from the cut edge using 2-0 polybutester (Novafil, Medtronic, Minneapolis, MN) and a purse string clamp (Furness clamp, Medtronic, Minneapolis, Minnesota). A 21 mm diameter Medtronic EEA DST circular stapler was then advanced through the lumen of the segment of colon up to the purse string suture. A purse string was applied in similar fashion to the second intestinal segment, however, prior to tightening, the detachable anvil on the EEA device was removed and placed within the lumen. The purse string suture was then tightened down around the anvil's purse string notch. The EEA

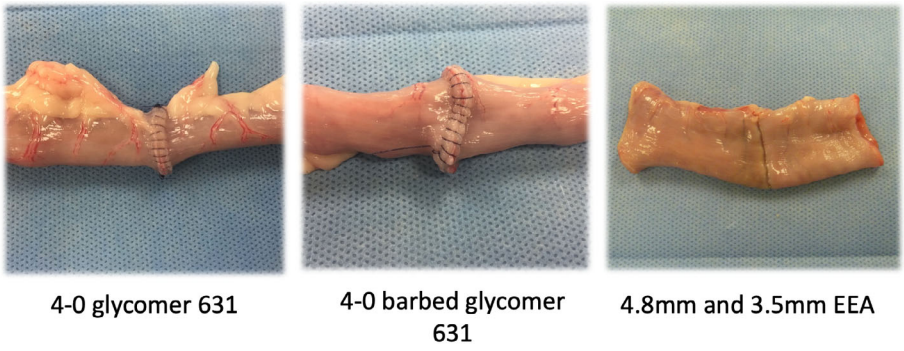

FIGURE 1 Representative appearance of each construct prior to pressure testing. For the EEA stapled construct, note the inverted stapled anastomosis line. The 3.5 and 4.8 mm constructs appeared the same after completion of the anastomosis

trocar was then advanced through the purse string suture line until the orange band on the trocar was visible. The trocar was connected to the anvil and the tissue was compressed within the device. Tissue compression within the circular stapler was confirmed using the green indicator on the stapler handle. After deploying the staples, the anastomosis was decompressed by turning the black handle on the device no more than two full turns to facilitate safe removal of the anvil. After removal of the stapler anvil, the donut of tissue within the device was evaluated to ensure that 360° of tissue purchase had been achieved (Figure 1). All constructs were completed by a surgery resident with direct supervision of a board-certified surgeon.

2.2 | Pressure testing

The constructs were elevated and sealed at one end with right agle forceps (Figure 2). On the other end of the construct, a 7Fr introducer (Arrow medical, Centennial, CO) was placed, and a single encircling suture with 3-0 nylon (Medtronic, Minneapolis, MN) was secured around the introducer to form a water tight seal. A right-angle forcep was then placed up to the base of the introducer, followed by a second mosquito hemostat to fully seal the intestinal lumen (Figure 2). A pressure transducer (Millar Mikro-Tip, Houston, Texas) was then placed through the introducer into the intestinal lumen to the level of the anastomosis site. The side arm of the introducer was connected to an injection pump (Harvard pump, Hollinston, MA) and a saline/Evans blue solution was infused at a continuous rate of 600 ml/h. The amount of Evans blue added to saline was enough to generate a dark blue suspension. Intralumnial pressure was recorded after calibration using data acquisition software (Power Lab, AD Instruments, Colorado Springs, Colorado; Figure 2). Initial leakage pressure was defined as the pressure at which the dye-containing saline solution was first observed to leak from the anastomosis site (suture line, suture hole, or staple line) by two separate observers on either side of the construct. Location of leakage was recorded.

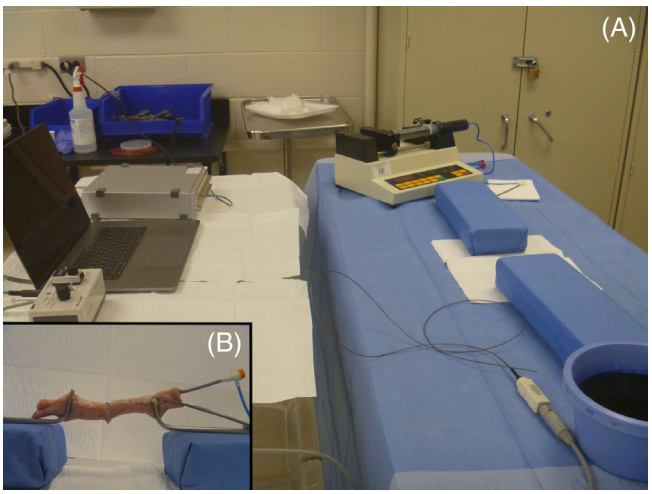

FIGURE 2 Testing for leakage. (A) Pressure testing setup including an infusion pump, pressure transducer, elevated platform, laptop with pressure acquisition software, and saline with Evans blue solution. (B) Image of a glycomer 631 construct prior to pressure testing. The pressure transducer was placed through the pressure transducer catheter to the level of the anastomosis prior to infusion with the Evans blue and saline solution

2.3 | Statistical analysis

A Shapiro-Wilk test was performed to assess normality and determined that leakage pressures were not normally distributed. A Wilcoxon matched-pairs signed rank test was used to compare leakage pressure, number of suture bites, and the number of extra suture added between groups. Data are reported as median and range (JMP, SAS Institute). A p value $<.05$ was considered significant.

3 | RESULTS

A total of 44 colonic sections were collected from 11 canine cadavers. Due to financial limitations, only six EEA staplers were obtained for each respective staple height. Two extra specimens were prepared for the C4.8mm group as two previous constructs had to be

discarded due to operator error while deploying the circular stapler, and only half of the large intestine from one dog was used for testing. A total of 32 colonic sections were randomly distributed between the G (10), BG (10), C3.5mm (6), and C4.8mm (6) groups. No difference was detected in the number of bites taken for each anastomosis between the G and the BG groups ($p = .469$). Similarly, the number of additional interrupted sutures placed did not differ between the G and the BG groups (p = 0.5; Table 1).

No difference was found in leakage pressures between the BG and the G groups ($p = .492$, Table 2 and Figure 3). Leakage pressure was lower in the C4.8mm than the BG group ($p = .0131$) and G group ($p = .0313$). Leakage pressures were also lower in the C3.5mm than the BG group ($p = .0313$) and G group ($p = .0469$).

Anastomoses in the BG group leaked at the suture line in 3/10 dogs and through a suture hole in 7/10 dogs. Leakage in the G group occurred at the anastomosis line in 4/10 dogs and through a suture hole in 6/10

TABLE 1 Median and range reported for number of suture bites as well as additional simple interrupted sutures used per construct

	Glycomer 631	Barbed Glycomer 631	*p* value
Median number of suture bites	30.5 (26-53)	30 (24-42)	$p = .469$
Median number of additional simple-interrupted sutures	0.5 (0-2)	0 (0-3)	$p = .5$

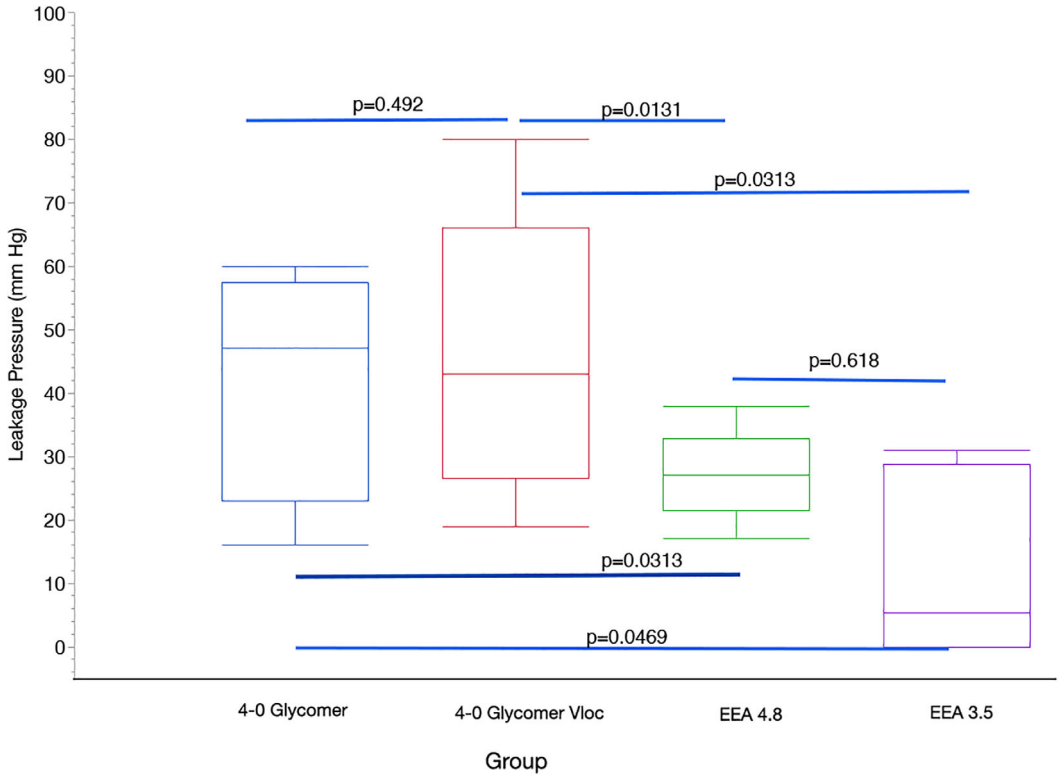

FIGURE 3 Box and whisker plot of initial leakage pressures. The blue lines connecting two anastomosis techniques lists their associated *p* value when those two techniques were compared

TABLE 2 Median leakage pressures (range) for the four groups

	Glycomer 631	Barbed Glycomer 631	EEA 3.5	EEA 4.8
Number of specimens	10	10	6	6
Leakage pressure (mm Hg)	49.5 (16-72)[a,c]	45.5 (19-80)[b,d]	5.3 (0-31)[a,c,d]	28 (17-50.3)[a,b]

Note: Numbers with the same superscript are significantly different at $p < .05$.

constructs. All specimens in the C4.8mm group leaked at the staple line. In the C3.5mm group, 2/6 constructs leaked at the anastomosis line and 4/6 constructs leaked at the anastomosis line through visibly everted tissue that became apparent immediately after dye infusion. All stapled anastomoses seemed appropriately inverted immediately after construction of the anastomoses, and the central donut of tissue inspected once the EEA device was removed.

4 | DISCUSSION

Colonic anastomoses closed with a hand-sutured technique leaked at higher pressures than those closed with circular staples. Unidirectional barbed suture did improve seal when compared to conventional monofilament suture for colonic anastomoses.

The large intestine was harvested from fresh canine cadavers and tested within 24 h of euthanasia to closely emulate the mechanical properties of in vivo colonic tissue. Fresh or chilled specimens stored at 4°C have been recommended to study intestinal anastomoses,[20] which is consistent with the methodology used in this study. A similar study looking at storage conditions and their effect on the closure of jejunal enterotomy sites closed with unidirectional barbed suture in fresh, chilled and frozen-thawed jejunal sections, concluded that fresh or chilled specimens stored at 4°C are recommended,[22] again consistent with the methodology used in this study. Testing immediately after euthanasia ideally maintains the viscoelastic and tensile properties of the colonic tissue. In addition, the pressure testing methodology used in this study was similar to previously published canine and human studies.[6,23,24]

The leakage pressure of hand-sutured colonic anastomoses did not differ when conventional or unidirectional barbed suture was used. The pressures measured in our study were lower than those reported in-vitro on human colonic anastomoses (median leakage pressure of 85 mmHg).[24] In that study, anastomoses were performed with a two-layer closure, including an inverting pattern, which may explain the discrepancy with our results.[24] The leakage pressures for canine small intestinal anastomoses have previously been found higher when unidirectional barbed suture were compared to conventional suture.[23] The authors postulated that unidirectional barbed suture maintains constant tension across the anastomosis after each bite, as the barbs engage the tissue. When the surgeon releases the tension on the suture line to make the next throw, the tension is maintained with the barbed suture whereas with conventional suture, the tension may be released. In our study, an assistant was maintaining tension on the suture line in both groups, which might explain the absence of difference between conventional and unidirectional barbed suture. Since leakage for the hand-sutured constructs most commonly occurred through suture holes in the tissue, an equal amount of tension was likely maintained with each suture type, creating an adequate seal. The use of barbed suture has also been reported in a canine prospective study, where colotomies were closed with unidirectional barbed suture, and no post-operative leakage was encountered.[25] Our results provide additional evidence that unidirectional barbed suture should be considered an appropriate suture choice for closure of resection anastomoses and enterotomies, in the large intestine of dogs.

The colonic anastomoses performed with circular staples leaked at a lower pressure than the hand-sutured anastomoses in our study. The colonic anastomoses performed with 4.8 mm staples leaked at pressures higher than reported colonic physiological pressures, justifying further consideration of this staple height. Resting colorectal pressure ranges in the dog have been estimated to be between 2-8 mmHg.[26–28] Using a staple height of 3.5 mm appears more unpredictable and unsafe as four of the six 3.5 mm EEA constructs leaked at or below a pressure of 5.3 mm Hg. Some colonic anastomoses leaked at very low pressures; we suspect that the staples could not engage both walls of the colon during the anastomosis construction, despite appropriate use of the circular stapling device. The authors acknowledge that clinically diseased or edematous bowel would cause thickening of the colonic wall and may diminish staple purchase in the tissue. This must be considered in the clinical setting, and the Medtronic recommendations for EEA stapling equipment should be reviewed. The use of 3.5 mm staple height is recommended only if the tissue can be "safely compressed to 1.5 mm", or if the surgeon would feel comfortable using a linear stapler associated with the same tissue (ie GIA or TA 3.5 mm). When correctly deployed, circular staples should form an inverting anastomosis. At the time of leakage for the C3.5mm constructs, the colonic walls were everted instead of inverted, as appropriate tissue purchase by the staples had not been achieved. A previous study has reported the successful use of a circular stapler in eight dogs and two cats with the only major complication being post-operative stricture formation in one dog and one cat, respectively. This study, however, did not evaluate staple sizes and circular stapler specifications were lacking.[3] A second study evaluated a circular stapler for trans-cecal subtotal colectomies in 15 cats diagnosed with acquired megacolon. In this study, a 25 mm diameter circular stapler was used in 13 cats, and a 21 mm diameter circular stapler was used in two cats. A staple height of 4.8 mm was used in all cases, and all cats had good to excellent outcomes.[2]

The 21 mm diameter circular stapler was selected for all specimens in our study. The circular stapler is available in several outer diameters, including 21, 23, 25, 28, 29, 31, and 33 mm, depending upon the manufacturer. Using a different diameter cartridge for this study may have affected outcomes as cartridges of a larger diameter may have stretched the wall of the colon, providing a thinner colonic wall on either side of the anastomosis for the staples to engage. Only a 21 mm circular stapler diameter was used in our study to facilitate placement of the same circular cartridge in all samples, as prior to the experiment the colonic specimen sizes were unknown. In this study, all circular staplers were comfortably introduced into the colonic lumen, and given that the specimens were not pathologically dilated we suspect using a larger diameter circular stapler would have been too large and risked serosal tearing. In the clinical setting of pathologically dilated colonic tissue, a larger diameter circular stapler may be required. In addition, the development of a fibrin seal would occur which would also be a barrier against early leakage. No difference in leakage pressure was detected between colonic anastomoses performed with a circular stapler (84 mmHg) and two-layer hand-sutured anastomoses in freshly explanted colon from human subjects undergoing wide-resection colectomy. [24] The leakage pressures reported in this study are higher than the leakage pressures reported in our current study. The circular stapler used by Schwab et al deployed a double-staggered row of staples, like the device used in our study. However, there was no mention of circular stapler diameter and staple height used in that study.

Limitations to this study include the use of cadaveric tissue and a small sample size. Ex vivo pressure testing studies do not reflect the in vivo response of the tissue following colonic anastomosis, including bleeding, tissue viability, acute healing, and potential for stricture formation. Leakage pressures were assessed in this study, which clinically reflects early leakage from the anastomosis site related to a failure in anastomosis construction or technique. This is unrelated to dehiscence, which involves the debridement phase of tissue healing and the up-regulation of matrix metalloproteinases and collagen degradation. Collagenase activity is particularly important in the colon following an anastomosis.[29,30] The number of specimens for this study were limited by the inherent challenges of obtaining fresh canine, cadaveric tissue, and the cost of the circular stapling equipment.

In conclusion, colonic anastomoses performed with hand sutures outperformed the anastomoses performed with the circular staples. The colonic anastomoses completed with the 4.8 mm staple height leaked at a pressure above reported colonic physiologic pressures making it a viable option for colonic anastomoses, particularly for anastomoses in locations not amenable to hand suturing. Additional ex vivo and prospective clinical trials are warranted to evaluate the safety of various size staple heights when using circular EEA staplers in canine large intestine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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