

Effects of cyanoacrylate on leakage pressures of cooled canine cadaveric jejunal enterotomies

Jamie-Leigh Thompson BSc (Hons), BVM&S, MRCVS, FHEA¹  |

Lucy Miller BVSc² |

Kelly Bowlt Blacklock BVM&S, DipECVS, SFHEA, PGCert, PhD, FRCVS¹ 

¹Department of Small Animal Surgery,
The Royal Dick School of Veterinary
Studies, Edinburgh, Scotland

²Department of Anesthesia and Analgesia,
The Royal Dick School of Veterinary
Studies, Edinburgh, Scotland

Correspondence

Jamie-Leigh Thompson, Department of
Small Animal Surgery, The Royal Dick
School of Veterinary Studies, Easter Bush
Campus, Edinburgh EH259RG, Scotland.
Email: jamie-leigh.thompson@hotmail.co.uk

Funding information

Advanced Medical Solutions Group Ltd.

Abstract

Objective: To compare the intraluminal initial and maximal pressures of enterotomies closed using three different techniques (single-layer appositional continuous closure; closure with cyanoacrylate; a single-layer appositional closure augmented with cyanoacrylate) in a cooled canine cadaveric jejunal model and to report the initial leak location in all samples.

Study design: Experimental, ex-vivo study.

Sample population: Grossly normal chilled small intestine segments from three canine cadavers.

Methods: A total of 45 chilled jejunal segments ($n = 15$ segments/group) were assigned to a handsewn group (HSE), a cyanoacrylate only group (CE) and a handsewn and cyanoacrylate group (HS + CE). A 2 cm antimesenteric enterotomy was performed and closure with one of the above techniques. Initial leakage pressures (ILP), maximal intraluminal pressures (MIP) and initial leakage location were recorded by a single observer.

Results: Handsewn enterotomies leaked at higher ILP when augmented with cyanoacrylate (83.3 ± 4.6 mmHg, $p < .001$) compared to both the HSE group (43.8 ± 5.3 mmHg) and the CE group (18.6 ± 3.5 mmHg). Those sealed with cyanoacrylate only leaked at a lower MIP compared with the other groups ($p < .001$). Maximal intraluminal pressures did not differ between handsewn enterotomies, whether augmented or not ($p = .19$).

Conclusion: Reinforcement of a sutured enterotomy closure with cyanoacrylate was easy to perform and resulted in significantly increased initial leak pressures in cadaveric jejunum.

Clinical significance: The increased leakage pressures achieved by reinforcing enterotomies with cyanoacrylate could consequently reduce the incidence

Abbreviations: CE, cyanoacrylate enterotomy; HSE, handsewn enterotomy; HS + CE, handsewn and cyanoacrylate enterotomy; ILP, initial leakage pressure; ILL, initial leakage location; MIP, maximal intraluminal pressure.

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of postoperative intestinal leakage following an enterotomy and may result in reduced patient morbidity or mortality.

1 | INTRODUCTION

Intestinal enterotomies are commonly performed within the companion animal population for several diagnostic and therapeutic reasons and they have a reported overall dehiscence rate of 12%–16%.^{1,2} Dehiscence ultimately leads to leakage of the gastrointestinal contents into the peritoneal cavity and the subsequent development of septic peritonitis and potentially, systemic sepsis and death. This serious complication has substantial associated morbidity in dogs and cats alike, with published mortality rates of up to 50%, even after prompt repeat surgical interventions.^{3–5} Leakage following intestinal surgery can be attributable to technical error or associated with risk factors including the presence of biochemical abnormalities preoperatively (e.g., hypoalbuminemia, hypoproteinemia) to the presence of intestinal foreign material.^{6–8} Given the significance of the sequelae following intestinal leakage, ensuring proper enterotomy closure is crucial. A number of closure techniques have been described experimentally but only handsewn and stapled techniques are routinely used in clinical practice. Experimentally, leakage pressures of cadaveric specimens are typically used to assess the integrity of intestinal closure methods and differing abilities to withstand physiological and supraphysiological peristaltic pressures. Small intestinal intraluminal pressures in healthy dogs are reported to range from 15 to 34 mmHg in live, conscious dogs and experimentally intact, fresh or cooled jejunal segments can withstand pressures of approximately 50 mmHg.^{9–11}

Tissue sealants have evolved in both veterinary and human medicine, as an alternative or adjunct to sutures for the closure of surgical incisions. Various sealants have been proposed for use in human surgery, divided broadly into three categories: synthetic glues, biological products, and biomimetic sealants. Synthetics, including the cyanoacrylate-based sealants, are most widely used in humans for the closure of cutaneous wounds.¹² They fix wound edges rapidly, favor hemostasis, and form a seal to prevent external contamination.¹³ Biological sealants, such as fibrin, collagen or polysaccharide-based products, are inherently biodegradable and nonimmunogenic. They are insoluble in water, which lends them to be used in biomedical applications, primarily for general hemostasis during surgery. Development is still underway for biomimetic sealants; they are designed to mimic the

naturally occurring adhesives that are seen in marine life and organisms (e.g., mussels, barnacles and gelatine) and form a gel in situ which can theoretically serve to bond tissues or seal leaks.¹⁴

The strongest sealants are the cyanoacrylate-based adhesives but due to concerns regarding potential cytotoxicity, they are not widely accepted for intracorporeal use.¹⁵ This argument has been countered by studies finding no evidence of cytotoxicity and instead presenting promising clinical properties favoring their use.¹⁶ Several publications review the intracorporeal use of *n*-butyl-2-cyanoacrylate and fibrin adhesives in humans as a means of laparoscopic mesh fixation in abdominal and inguinal hernia repairs. Collectively, these studies found a shorter overall surgery time, short hospital stays and reduced postoperative and chronic pain compared to traditional open methods used for hernia repair, promoting their use intracorporeally.^{17–20} As a result of conflicting evidence and opinion, the use of cyanoacrylate in a medical setting continues to be researched. Cyanoacrylates are generally low-cost and easy to apply and consequently they are found in most veterinary practices where they are used principally for skin closure with few reported complications and good overall outcome. One study using tissue adhesive only (*n*-butyl-2-cyanoacrylate) for the closure of 695 laparoscopic port sites in 289 dogs found no hypersensitivity relating to their use, nor any long-term adverse reactions and a second study endorsed cyanoacrylate use in reconstructive surgery.^{21,22} Cyanoacrylates are not currently routinely used in clinical veterinary practice for any other purpose and ongoing research within the veterinary field is limited.

Given the potential catastrophic outcome resulting from intestinal leakage or dehiscence, the authors questioned whether the application of cyanoacrylate to a sutured enterotomy would prevent or reduce the incidence of intestinal leakage during the postoperative period and consequently reduce the incidence of associated morbidity or mortality. The first step in answering this question is to perform *ex vivo* biomechanical testing to ensure the proposed technique is deemed effective under controlled conditions before piloting *in vitro* research evaluating practical technique and clinical safety.

The primary objective of this study was to compare intestinal initial leak pressures (ILP) and maximal intraluminal pressure (MIP) after enterotomy closure in a

cooled canine cadaveric model, using three closure techniques; handsewn enterotomy (HSE), cyanoacrylate enterotomy (CE) or handsewn and cyanoacrylate enterotomy (HS + CE) and to report initial leak location (ILL). It was hypothesized that the ILP would be higher in the HS + CE group compared to the HSE, CE groups and that the ILL would differ between groups. It was also hypothesized that the leakage pressure achieved for the HSE group would be in line with previously published literature.

2 | MATERIALS AND METHODS

The research received ethical approval from The Royal (Dick) School of Veterinary Studies Institutional Review Board, reference VERC 112.20.

2.1 | Sample collection

Three mature male intact research Beagles, weighing between 10 and 15 kg, were euthanized humanely for reasons unrelated to our study. The cadavers were obtained from the Charles River laboratories, Edinburgh. The jejunum was harvested in these dogs within 1 h of euthanasia, from just aborad to the caudal duodenal flexure to the ileum. The dogs had no history of gastrointestinal disease, and no gross abnormalities were present within the intestinal tract or in the intestinal segments used for this study. The intestine was cut into 10-cm long segments using Metzenbaum scissors next to a calibrated ruler and the mesentery was excised to prevent bunching of intestinal segments. Segments were milked to clear luminal ingesta, flushed with balanced electrolyte solution until the solution ran clear, placed in a sterile saline solution (0.9% NaCl) and stored flat at 4°C for 12 h before group assignment and experimental testing was performed.

2.2 | Study groups

Prior to testing, jejunal segments were randomly assigned to one of three equally sized experimental enterotomy groups using a random number generator (Research Randomizer; <https://www.randomizer.org>). The treatment groups consisted of HSE, CE, or HS + CE and there were a total 15 segments per treatment group. Equal numbers of intestinal segments ($n = 5$) from each dog were placed in each group. Three segments from each cadaver were also randomly assigned into a control group ($n = 9$), using the same random number generator.

2.3 | Enterotomies

All jejunal segments were occluded using Doyen intestinal forceps 1 cm from the intestinal ends. Centrally, a full thickness antimesenteric enterotomy was made using a No. 11 scalpel blade to make a stab incision which was then extended using Metzenbaum scissors to a measured length of 2 cm using a ruler. Once the enterotomy was complete, the length was remeasured using a metric ruler to ensure consistency. The HSE group was then closed conventionally with a full-thickness, single-layer continuous suture pattern using absorbable monofilament suture (4-0 polydioxanone; PDS, Ethicon, New Jersey), by a single residency-trained surgeon (JLT). The surgeon ensured engagement of the submucosa on either side of the enterotomy when closing the enterotomy and sutures were placed 2–3 mm from the cut edge and 2–3 mm apart. The continuous suture line was started and terminated with a square knot followed by three throws and suture ends were cut to a length of 3 mm using mayo scissors. The CE group was closed using *n*-butyl-2-cyanoacrylate only applied using the LiquiBand®FIX8™ open hand piece (Advanced Medical Solutions Ltd, Plymouth, UK). The surgeon placed gentle pressure on either side of the jejunal segment, aiding apposition of the enterotomy before applying 37.5 mg which is equivalent to 0.03 mL of cyanoacrylate (3 triggers at 12.5 mg per trigger) directly over the enterotomy site. This volume allowed for application of a thin single layer of cyanoacrylate which covered the incision entirely and set within 1-s of deployment. The HS + CE group was closed initially as per the HSE group, followed by augmentation with cyanoacrylate as per the CE group.

2.4 | Evaluation of leakage from the enterotomy sites

Following enterotomy closure, the segments were suspended on a clear mount to allow monitoring of leakage. Two 18-gauge, intravenous catheters were placed in an oblique direction through the jejunal wall into the lumen, 3.5 cm distal from the suture knots at both ends of the enterotomy. A 5-L bag of Hartmann's solution (Aquapharm 11; Animalcare, York, UK) containing 20 mL of methylene blue (Flexipharm Austrading Ltd, Buckinghamshire, UK) was connected to a fluid line and a fluid pump and the first catheter. The second catheter was connected to a pressure transducer and a multiparameter monitor (Figure 1). The pressure transducer was zeroed at the level of the intestinal segment at the start of each test. Fluid was infused through the first catheter at rate of 999 mL/h while the enterotomy closure site was



FIGURE 1 Photograph to show the leakage testing design. Two 18-gauge intravenous catheters were inserted into the lumen at either ends of the enterotomy site. One catheter was connected to a pressure transducer, and another connected to the fluid infuser.

monitored for leakage by a single study investigator (JLT). After identification of leakage, the ILP was recorded in mmHg by a second observer (LM) and was defined as the intraluminal pressure at which the solution was first observed to visibly leak extraluminally. Leakage location was recorded to occur at level of the knots (either side of the enterotomy), from suture holes (along the length of the enterotomy), or from the incisional line itself. After the ILP was recorded, pressure testing was continued until there was complete failure (MIP) of the enterotomy site, determined by either a sudden drop in pressure or when the intraluminal pressure plateaued and sustained for at least 5 s in duration. The same experimental procedure was performed using the control segments (without an enterotomy). The multiparameter monitor read a maximum of 318 mmHg.

2.5 | Statistical analysis

A power analysis was performed with results from an ex vivo study performed by Duffy et al.¹¹ assessing ILP and MIP in canine intestines following enterotomy closure. A sample size of at least 11 paired intestinal segments per group was calculated to detect a difference of 10 mmHg leakage between study groups with a standard deviation of 8.4 mmHg by using a power of 0.8 and a confidence level of 95%.

Continuous numerical variables were assessed for normal distribution using a Shapiro–Wilk test. Results for ILP (mmHg) and MIP (mmHg) are reported as mean \pm standard deviation (SD). A one-way repeated-measures analysis of variance accounting for cadaver as a sample source was performed to assess for differences between

sample means from the different experimental groups. A one-way analysis of variance was performed to assess results among experimental groups. p -values $\leq .05$ was considered statistically significant. Statistical analysis was performed on a commercially available software (SPSS, v.28.9, IBM Corp, Armonk, New York). Results for ILL are also reported as observed.

3 | RESULTS

Data in the control group was found not to be uniformly distributed, all other data was uniformly distributed when tested with a Shapiro–Wilk test ($p < .001$). All enterotomies were successfully created, and leakage and pressure testing was performed without technical error in all specimens.

3.1 | Initial leakage pressures

The ILP in intact control segments were higher (greater than 318 mmHg) than in all test groups ($p < .001$). Mean ILP for the HSE, CE and HS + CE groups were 43.8 ± 5.3 mmHg, 18.6 ± 3.5 mmHg, and 83.3 ± 4.6 mmHg, respectively (Table 1, Figure 2). The CE group leaked at a lower ILP compared with the HSE and HS + CE groups ($p < .001$). The handsewn and cyanoacrylate group leaked at higher ILP compared to the HSE group ($p < .001$).

3.2 | Maximal intraluminal pressures

The handsewn group (HSE) revealed a mean \pm SD MLP of 133.4 ± 13.0 mmHg. Mean MIP for the cyanoacrylate

TABLE 1 Initial leakage pressure and maximal intraluminal pressure measured of handsewn enterotomies, cyanoacrylate enterotomies, handsewn and cyanoacrylate enterotomies and the control segments.

Intraluminal pressures	Control	HSE	CE	HS + CE
ILP, mean \pm SD, mmHg	314 \pm 7.5	43.8 \pm 5.3	18.6 \pm 3.5	83.3 \pm 4.6
MIP, mean \pm SD, mmHg		133.4 \pm 13.0	22.7 \pm 2.0	159.2 \pm 6.0

Abbreviations: CE, cyanoacrylate enterotomy; HS + CE, handsewn and cyanoacrylate enterotomy; HSE, handsewn enterotomy; ILP, initial leakage pressure; MIP, maximal intraluminal pressure; SD, standard deviation.

FIGURE 2 Outlier plot with initial leakage pressures (ILPs) of handsewn enterotomies (HSE), cyanoacrylate enterotomies (CE), handsewn and cyanoacrylate enterotomies (HS + CE) and the control segments.

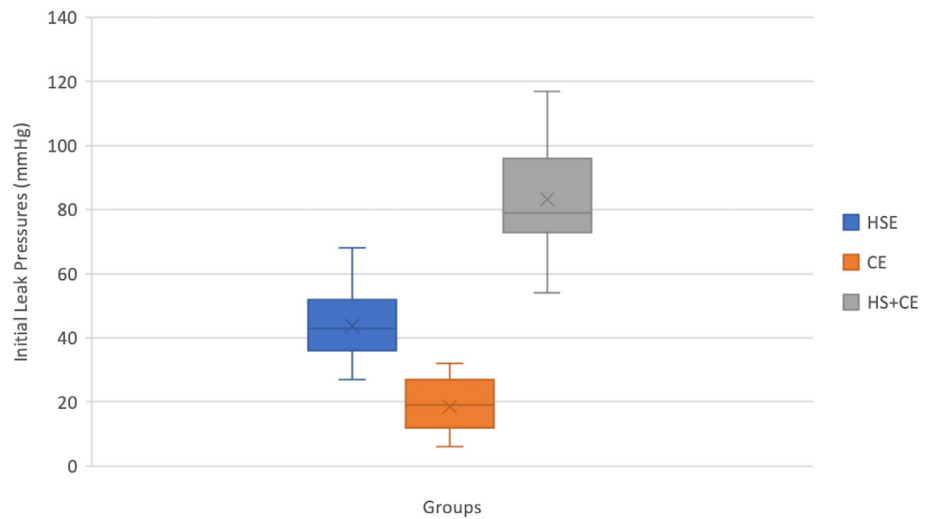
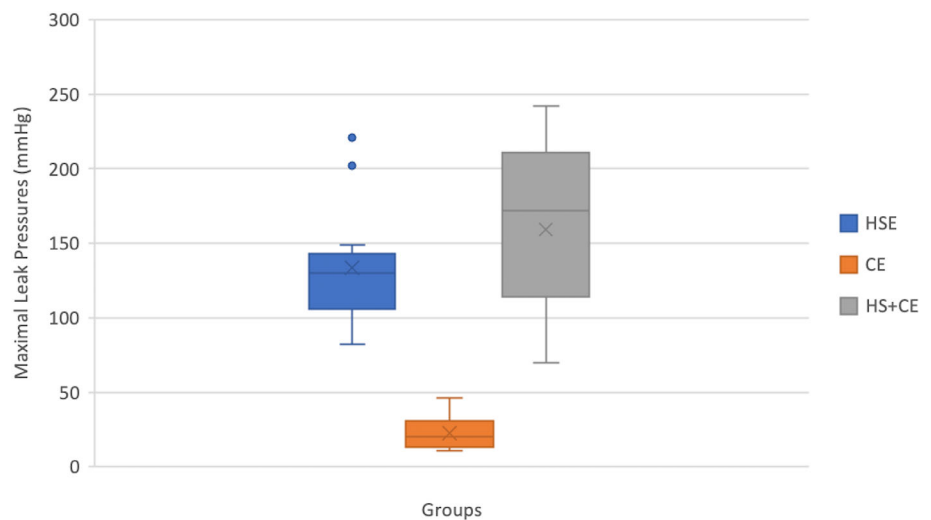


FIGURE 3 Outlier plot with maximal intraluminal pressures (MIPs) of handsewn enterotomies (HSE), cyanoacrylate enterotomies (CE), handsewn and cyanoacrylate enterotomies (HS + CE) and the control segments.



groups (CE) was 22.7 ± 2.0 mmHg, and for the handsewn and cyanoacrylate group (HS + CE) MIP was 159.2 ± 6.0 mmHg (Table 1, Figure 3). The CE group leaked at a lower MIP compared with the HSE and handsewn and cyanoacrylate groups ($p < .001$). There was no significant difference in the MIP between the HSE and the handsewn and cyanoacrylate groups ($p = .19$).

3.3 | Location of leakage

Leakage was observed at the suture holes in nine of 15 (60%) of HSE constructs, the incisional line in five of 15 (33%) of HSE constructs and the knot in one of 15 (7%) of HSE constructs. Leakage was observed at the incisional line in all (100%) of the CE constructs. Leakage

was observed at the incisional line in nine of 15 (60%) of HS + CE constructs, the suture holes in six of 15 (40%) of HS + CE constructs. All control segments failed by serosal tearing.

4 | DISCUSSION

Intraluminal leak pressure testing is a well-recognized and commonly used technique to compare intestinal integrity following experimental closure or anastomosis. The methodology used in the present study replicates previous studies with similar aims; the pressure testing device was easily constructed using accessible materials.¹¹ Sutured enterotomies reinforced with cyanoacrylate were able to withstand a significantly higher ILP in cadaveric jejunum, compared to enterotomies closed with suture alone or surgical sealant alone. The MIPs were comparable in the suture only (HSE) and suture and cyanoacrylate (HS + CE) groups. The ILP reported for the HSE group in this study were in line with previous literature looking at *ex vivo* cadaveric leakage pressures in sutured enterotomies and the above study found that the addition of surgical sealant increased the ILP beyond those previously published.¹¹ Physiological small intestinal intraluminal pressures of live, unanesthetized dogs are reported to range from 15 to 25 mmHg.⁹ However, recent research using wireless motility capsule technology reports a higher intraluminal contraction pressure in the small intestine with a mean of 34 mmHg in the conscious dog with a significant reduction in pressures when anesthetized.¹⁰ Both the HSE and HS + CE groups produced supraphysiological ILPs compared to published literature and the CE did not and consequently, would not be recommended as a closure technique for an enterotomy. The MIP did not significantly differ between groups which again, appears to be in line with published literature. The suture holes along the enterotomy accounted for 60% of the ILL in the HSE group in the above study which is a lower percentage than that previously published for sutured enterotomies in chilled cadaveric samples, whereby 100% of the leakage was from the suture holes.¹¹ In the HS + CE group, only 40% of samples leaked from the suture holes and most samples instead leaked from the suture line. The authors hypothesize that this difference in ILL is due to the cyanoacrylate “plugging” the suture holes when in the viscous state at the time of application, essentially waterproofing that area, and reducing the leakage seen from the needle holes. Although the waterproofing properties of synthetic sealants have previously been reported, their ability to also create an airtight seal has only recently been demonstrated in canine cadavers, following partial lung

lobectomies.^{23,24} As the suture continues to be the key-stone in holding the incision together, this suggestion may explain the difference in the leakage location for the HS + CE group.

Decreasing the risk of intestinal leakage and dehiscence is pertinent to a good clinical outcome in companion animal practice. Intestinal dehiscence is well documented but not fully understood. Dehiscence is often seen at day 3–5 after surgery and is presumed to be associated with the lag phase of healing where the strength of the site is reduced by approximately 85% compared to immediately postoperatively.²⁵ Risk factors reported to be associated with dehiscence include hypotension, hypoalbuminemia, septic peritonitis at the time of surgery, inflammatory bowel disease and the presence of foreign material in the intestinal tract.^{6–8,26} Reinforcement of enterotomy sites with additional procedures and biological tissue using techniques such as serosal patching and omental wrapping is favorable in apparently compromised intestine or in patients which are higher risk for dehiscence, as they have been shown to increase the construct leakage pressure.²⁷ Oversewing is another reinforcement technique that has been shown to be effective in reducing the incidence of postoperative dehiscence following gastrointestinal surgery in dogs.²⁸ Experimental studies demonstrate that oversewing successfully increases leakage pressures following stapled gastrointestinal anastomoses; however, the authors believe that the size of the canine small intestine limits the ability to perform oversewing techniques following a simple enterotomy.^{29,30} The fact that oversewing did increase leakage pressures experimentally and this has then been associated with a reduction in the incidence of intestinal dehiscence clinically, supports the general theory that interventions, such as cyanoacrylate, which increases leakage pressures experimentally may result in a reduction in dehiscence and leakage clinically. A similar experimental study did not find a significant difference in leakage pressures when stapled gastrointestinal anastomoses were oversewn with a Cushing pattern; however, the combination did yield the highest leak pressures from the constructs tested.³¹ It is important to note that dehiscence occurring within the first 24 h of surgery typically reflects technical error, such as failure of the suture to engage with the submucosa or intestinal necrosis and in this circumstance reinforcement techniques may be ineffective.²⁶ Jones et al. (2017) investigated the use of a bio-polymer adhesive in combination with suture for enterotomy in caprine cadavers. The application of sealant following routine enterotomy closure was not only shown to be feasible and technically easy but was also shown to significantly increase the intraluminal leakage pressures of the intestinal segments.³² This is congruent

with the findings of this study, that reinforcement of enterotomies with a synthetic cyanoacrylate surgical sealant increases the initial leak pressures.

Cyanoacrylates polymerize when they meet moisture forming a strong bond between tissues and making them resistant to the flow of most liquids and air. They also have high antibacterial properties which makes them appealing for the use in gastrointestinal surgery and due to their strong adhesive properties only small quantities of sealant are often needed to create a watertight barrier.^{23,33} In 2009, a group compared closure of small intestinal enterotomies by double layer suture or synthetic sealant in 10 dogs and reported no intestinal leakage, a shorter procedural length, and a lower macrophage response with the sealant, concluding it was an effective enterotomy closure technique.³⁴ Synthetic sealants have also been assessed as a closure technique following partial resection of the caecum in laboratory rats with micro- and macroscopic histological findings and postoperative outcomes supporting the use of sealants in cecal surgery.³⁵ The addition of biological sealants to canine cadaveric enterectomies also significantly increased experimental leakage pressures; however, biological sealants are inherently more expensive and are not licensed for veterinary use globally which limits the clinical applicability of this study.³⁶ In the human field, Kotzampassi and Eleftheriadis (2015) used sealants in the management of intestinal anastomotic leakage following gastrointestinal surgery in people for over 25 years. Within that period, the authors describe its use in 63 patients with a clinical and technical success rate of 96.8%; glue application was concluded to be a valuable clinical tool, and its use avoided reoperation in the study population and had no negative effects.³⁷ The use of Bioglue (CryoLife Europa Ltd, Hampshire, United Kingdom) in the attenuation of post-thoracotomy alveolar leaks was evaluated and its use was found to be associated with a shorter duration of air leakage and shorter overall hospitalization, further showcasing its sealant properties.³⁸ Another study demonstrates the hemostatic properties of cyanoacrylates during laparoscopic partial nephrectomies.³⁹ Interestingly, Nandakumar et al.⁴⁰ report that surgical adhesives were successful in reinforcing both intact and defective stapled gastrojejunostomies which begs the question as to whether surgical sealant could also be effective in reinforcing defective or incomplete sutured gastrointestinal closure.

In vitro studies using cell cultures have shown mild formaldehyde production because of the hydrolytic degradation of the alkyl chains of the sealant. This is reported to accumulate within the tissues and promote an inflammatory response. As a result, cyanoacrylates have not been readily utilized or accepted for use in

intracorporeal surgery in veterinary medicine. However, in vivo studies are ongoing, and results are showing no evidence of cytotoxicity and moreover show that cyanoacrylates have good tissue integration, effective short-term biocompatibility, and a low macrophage response in animal and human subjects.¹⁶ There are also increasing reports of the use of cyanoacrylate in vascular surgery or in the treatment of fistulae, varices, and ocular conditions within human medicine.^{41–44} Evidence promotes their use in dentistry and oral surgery, with closure of intraoral mucosal incisions being deemed easier and faster with synthetic sealants when compared to sutures, with equivalent overall outcome.⁴⁵ Veterinary publications review their use in urogenital surgery with successful cystotomy closures seen in porcine models, supported by an experimental study evaluating bladder closure in canines, showing a faster, effective closure.^{46,47}

Despite research showing no difference between leakage pressures after enterotomy closure when comparing in vitro and ex vivo models, limitations inherently include the ex vivo nature of the study.⁴⁸ Additionally, the ex vivo design means that information pertaining to any possible inflammatory responses and consequent short- or long-term side effects remains unknown. Another limitation of the study was the use of cadaveric intestine which is likely to behave differently to live or diseased tissue. In an attempt to limit the impact of this, the authors chilled and stored the cadaveric tissue as per Duffy et al.¹¹ who found no difference between pressure testing in chilled and fresh cadaveric samples. All sutured enterotomies were performed by a single residency-trained surgeon to allow for uniformity across samples; however, there was likely subtle variability which cannot be accounted for.

To the best of the authors knowledge, no previous studies have looked at the effect of cyanoacrylate augmentation of canine enterotomies with leakage pressures. The results of this study show that the mean ILP for the HS + CE was significantly higher than the HSE, and both were superior to the CE alone. Both the HSE and HS + CE groups withstood pressures that would be expected clinically, and the CE group did not. For this reason, the authors would not recommend using cyanoacrylate only to close enterotomies. Although the authors do not believe cyanoacrylate should replace suture for enterotomy closure, these results suggest that under clinical conditions, synthetic sealants may have the potential to decrease postoperative intestinal leakage or dehiscence which could subsequently reduce the incidence of associated patient morbidity and mortality. The authors propose that the use of cyanoacrylate would likely be most appropriate in circumstances where patients are deemed high risk for postoperative dehiscence. The conclusions of this study set the foundations for further research

exploring the clinical safety of surgical sealant enterotomy reinforcement with in vitro models and investigating the consequent impact on postoperative leakage.

AUTHOR CONTRIBUTIONS

Thompson JL, BVM&S, MRCVS, FHEA: Participated in the conception of this study, literature review and writing and editing of the manuscript. Miller L, BVSc: Participated in the equipment construction and data collection. Bowlt Blacklock K, BVM&S, DipECVS, SFHEA, PGCert, PhD, FRCVS: Participated in the conception of the study and critical review of the manuscript.

FUNDING INFORMATION

No monetary funding or grants were received to aid completion of this study; however, the surgical sealant handpieces (LiquiBand®Fix8™) were provided by Advanced Medical Solutions Group Ltd.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest relate to this study. All authors contributed equally to this study.

ORCID

Jamie-Leigh Thompson  <https://orcid.org/0000-0002-6634-7926>

Kelly Bowlt Blacklock  <https://orcid.org/0000-0001-6482-7224>

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How to cite this article: Thompson J-L, Miller L, Bowl Blacklock K. Effects of cyanoacrylate on leakage pressures of cooled canine cadaveric jejunal enterotomies. *Veterinary Surgery.* 2024; 53(2):367-375. doi:10.1111/vsu.14059