

# A comparison of in situ and in vitro techniques for bursting pressure testing of canine jejunum

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**Objective**—To compare bursting pressures in canine jejunum, measured by use of an in vitro and an in situ bursting pressure technique.

**Study Population**—Cadavers of 3 healthy adult dogs.

**Procedures**—54 enterotomies were performed on 3 canine cadavers immediately after euthanasia. After completion of enterotomy closure, bursting pressure was measured on 9 jejunal segments by use of an in situ technique and on 9 jejunal segments by use of an in vitro technique for each canine cadaver. Bursting pressure testing time was recorded for both in situ and in vitro techniques. Techniques were compared by means of randomized block ANOVA.

**Results**—The mean  $\pm$  SE in vitro and in situ bursting pressures were  $93.63 \pm 24.10$  mm Hg and  $141.19 \pm 38.10$  mm Hg and were not significantly different. Mean in situ testing time was 40.7 min/cadaver; mean in vitro testing time was 50.3 min/cadaver.

**Conclusions and Clinical Relevance**—The in situ bursting pressure testing technique yielded results similar to those of the in vitro method, was somewhat less labor-intensive, and may be applicable to future studies of live dogs. (*Am J Vet Res* 2010;71:370–373)

Dehiscence and enterotomy leakage are potentially devastating complications in patients that have undergone gastrointestinal surgery.<sup>1,2</sup> In vitro bursting pressure testing has been reported as a useful method for evaluation of enterotomy techniques<sup>3–5</sup> and has the advantage of the relative ease of detection of enterotomy failure at bursting pressure. However, this technique cannot be performed in vivo. In situ bursting pressure testing may offer several advantages over in vitro testing: the intestinal segment does not need to be harvested prior to testing, and this experimental technique could potentially be used in live animals that will be recovered from surgery.

The objective of the study reported here was to compare bursting pressures in canine jejunum, measured by use of an in vitro and an in situ bursting pressure technique. Our hypothesis was that an in situ bursting pressure technique would yield bursting pressures similar to those for an in vitro bursting pressure technique.

## Materials and Methods

**Sample population**—Jejunal samples were harvested from cadavers of 3 dogs euthanatized for reasons

unrelated to the present investigation. Dogs were all mixed breeds that weighed 20 to 25 kg and included 1 spayed female, 1 neutered male, and 1 sexually intact male. Prior to euthanasia, dogs were determined to be healthy on the basis of physical examination findings and PCV and serum total protein concentrations that were within laboratory reference ranges. Prior to euthanasia by IV administration of an overdose of sodium pentobarbital, dogs were cared for in accordance with institutional animal care and use requirements. Jejunal samples were obtained immediately following euthanasia, and enterotomies and pressure testing were completed within 4 hours after euthanasia.

**Experimental procedures**—Multiple enterotomies were performed in each jejunal segment at approximately 15-cm intervals. Sequential enterotomies were performed starting approximately 5 cm aborad to the duodenocolic ligament. Two atraumatic Doyen intestinal forceps were used to isolate an approximately 15-cm-long jejunal segment. In the center of each isolated jejunal segment, a transverse 4-mm-long stab incision was made at the antimesenteric surface. The stab incision was then followed by a resection of an approximately 4 × 4-mm full-thickness jejunal sample, which was obtained by use of a 4-mm laparoscopic punch biopsy forceps<sup>a</sup> inserted parallel to the jejunum in an aboral direction. The laparoscopic biopsy instrument was used to ensure full-thickness defects of uniform size. Incisions were closed with simple interrupted appositional sutures of 3-0 polyglactin 910 on a taper point needle with a 1- to 2-mm distance between sutures.<sup>6,7</sup> Four to 5 full-thickness sutures were placed to close each enterotomy, with care taken not to allow protrusion of mucosa between sutures. After 1 enterotomy

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had been sutured, another 15-cm-long jejunal section was isolated by maintaining the position of the aborad Doyen forceps, milking of ingesta proximally, and placement of the second Doyen forceps 15 cm proximal to the existing Doyen forceps (Figure 1). This procedure was repeated for each isolated segment as described until the ileum was reached. All enterotomies and suture closures were performed by 1 individual (KMC).

To minimize potential variation along the length of the jejunum, successive 15-cm-long jejunal segments were allocated to be tested by use of either the in situ or in vitro method. After allocation of specimens, all in situ specimens were tested, followed by all in vitro specimens. No portion of the jejunum previously tested in situ was used for in vitro testing. A total of 60 enterotomies were performed. Jejunal samples harvested from cadavers of dogs 1 and 3 each had 19 enterotomies, with 22 enterotomies performed on samples harvested from the cadaver of dog 2. The first 9 sequential jejunal samples from each canine cadaver tested by each technique were selected for statistical analysis. Therefore, 54 enterotomies were included in the study. In the present study, only a simple interrupted appositional suture technique was examined because pilot testing of jejunal samples from 2 canine cadavers indicated that a continuous appositional suture technique did not consistently yield a watertight closure.

**In situ pressure testing**—After all enterotomies had been closed, in situ bursting pressure testing was conducted by use of a method that has been previously described.<sup>8</sup> Commencing with the most orad enterotomy site, atraumatic Doyen forceps were placed approximately 6 cm from each end of the enterotomy. Two 18-gauge needles were inserted into the intestinal lumen approximately 5 cm from each end of the enterotomy to be tested. Saline (0.9% NaCl) solution colored with black margin marking dye<sup>b</sup> was infused through the orad needle via a fluid pump<sup>c</sup> at a rate of 999 mL/h. During infusion, intraluminal pressure was continuously monitored by use of a pressure transducer<sup>d</sup> attached to the aborad needle, which had been zeroed to atmospheric pressure prior to testing of each specimen. The enterotomy bursting pressure was defined as the peak pressure attained at enterotomy or jejunal failure (ie, visible leakage of saline solution). Site of failure was recorded as suture line, tissue failure, or the site of needle insertion (for fluid infusion or pressure measurement).<sup>3,9</sup>

**In vitro pressure testing**—In vitro pressure testing was conducted by use of a method similar to one that has been reported previously.<sup>3</sup> Ingesta were gently manually milked out of each 15-cm jejunal specimen. A 14F Foley catheter was inserted into each end of the jejunal segment, and each catheter was secured with a single ligature of 2-0 silk. Each catheter balloon was inflated with 2.5 mL of saline solution until the intestinal wall was occluded. The orad catheter was used to inject dyed saline solution at a rate of 999 mL/h. The aborad catheter was attached to a pressure transducer to record intraluminal pressure. The enterotomy bursting pressure was recorded as described for the in situ technique. The site of failure was recorded as suture line, ligature site, or tissue failure.<sup>3,10</sup>

**Time recording**—Bursting pressure testing time was recorded for both in situ and in vitro techniques. Each method was timed from the start of testing to the completion of testing of all specimens from an individual canine cadaver. For the in situ technique, testing time was defined as placement of the first Doyen forceps until failure of the last specimen. For the in vitro technique, testing time was defined as the first incision for jejunal resection until jejunal failure of the last specimen that was harvested. Consequently, in vitro testing time included the period of specimen harvesting.

**Statistical analysis**—Descriptive data were calculated with standard methods and presented as mean and SE. Bursting pressures for in situ and in vitro techniques for jejunal specimens from the different canine cadavers were compared by use of a randomized block ANOVA. On the basis of results of the Hartley test for homogeneity of variance, pressure data were transformed by use of natural log transformation. Mean values were backtransformed and reported as adjusted means. Because confidence intervals are asymmetric when logarithmic transformation is used, values for SE were not backtransformed and are not presented. Time data are presented as descriptive data only, as they consisted of too few observations for meaningful statistical analysis. A *P* value  $\leq 0.05$  was considered significant.

## Results

**Bursting pressure**—Individual in situ bursting pressure values ranged from 15 to 122, 23 to 233, and 25 to 226 mm Hg for cadavers of dogs 1, 2, and 3, respectively. Individual in vitro bursting pressure values ranged from 10 to 157, 12 to 262, and 17 to 360 mm Hg for cadavers of dogs 1, 2, and 3, respectively. The adjusted mean in vitro and in situ bursting pressures were

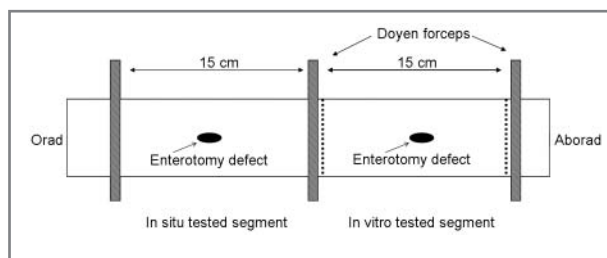


Figure 1—Schematic representation of jejunal segments with a 4 × 4-mm enterotomy defect that has been created on the antimesenteric surface in the center of each segment. After in situ testing of alternating segments, the aborad segment was transected at the dashed lines and removed from the cadaver for in vitro testing.

Table 1—Bursting pressure measured in jejunal samples from cadavers of 3 dogs by use of in situ and in vitro techniques.

	In situ			In vitro		
	n	Mean bursting pressure (mm Hg)	SE	n	Mean bursting pressure (mm Hg)	SE
Dog 1	9	41.89	11.20	9	49.67	14.49
Dog 2	9	133.78	28.40	9	166.22	32.20
Dog 3	9	105.22	20.07	9	207.67	42.34
<b>Total</b>	<b>27</b>	<b>93.63</b>	<b>24.10</b>	<b>27</b>	<b>141.19</b>	<b>38.10</b>

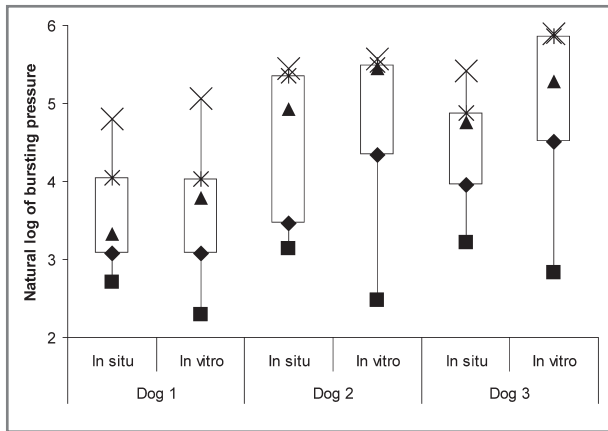


Figure 2—Whisker-and-box plot presenting the natural log of bursting pressures observed in jejunal samples from cadavers of 3 dogs measured by use of an in situ and an in vitro testing technique. Each box represents values from the first (diamond) to the third (asterisk) quartiles, whereas whiskers extend from the lowest (square) to the highest (large X) value. The triangle within each box represents the median value. Results were not significantly ( $P = 0.16$ ) different between techniques.

95.7 and 136.3 mm Hg, respectively. No significant ( $P = 0.16$ ; power, 0.25 when  $\alpha = 0.05$ ) difference was detected between bursting pressure measured by the in situ versus the in vitro testing technique. However, adjusted mean bursting pressures for each testing technique differed significantly ( $P < 0.001$ ) between cadavers (Table 1; Figure 2).

**Site of failure**—All jejunal segments tested by both methods burst at the suture line, except 1 specimen tested in situ in the cadaver of dog 1, which failed at 15 mm Hg at the infusion needle site.

**Time recordings**—The total in situ testing time for cadavers of dogs 1, 2, and 3 was 47, 45, and 30 minutes, respectively, with a mean time of 40.7 min/cadaver. The total in vitro testing time for cadavers of dogs 1, 2, and 3 was 50, 58, and 43 minutes, respectively, with a mean testing time of 50.3 min/cadaver.

## Discussion

The bursting pressures recorded in the present study with both the in situ and in vitro techniques were similar to the results of other studies in which mean bursting pressures measured with an in vitro technique ranged from 93 to 221 mm Hg.<sup>3-5</sup> The in situ jejunotomy bursting pressure technique equaled the in vitro technique with respect to mean pressure values and data variance, while being somewhat less labor-intensive. Five of the 54 enterotomies leaked at pressures lower than 20 mm Hg, with all except 1 failing at the enterotomy site.

The in situ technique used in the present study was somewhat less labor-intensive than the in vitro technique. During pilot testing, it became apparent that an assistant was required to enable efficient in vitro testing to maintain testing time within 4 hours after euthanasia. Despite the disadvantage of less staffing, the mean time for in situ testing was approximately 60 seconds faster per sample than the mean time for in vitro testing. For

the in situ technique, the intestinal segment tested was not removed but was isolated by use of Doyen forceps rather than traumatic clamps or ligatures. Therefore, the in situ method could potentially be used in live dogs, particularly if testing to failure was not required. Because maximum jejunal intraluminal pressure during peristalsis in clinically normal dogs has been shown to be 20 to 40 mm Hg,<sup>11,12</sup> bursting pressures that are slightly greater may be of clinical relevance. It is possible that intraluminal pressure may not reach 40 mm Hg adjacent to surgical sites as a result of transient postoperative ileus. However, we suggest that an enterotomy should be able to withstand peak physiologic intraluminal pressure. Peak intraluminal pressure in a study<sup>11</sup> of clinically normal dogs was approximately 20 mm Hg, and 1 report<sup>10</sup> on human patients suggests the value of bursting pressure data for the evaluation of anastomotic technique. To test canine intestines to pressures much higher than 20 to 40 mm Hg would perhaps be of limited clinical importance, as these high pressures would probably be supraphysiologic.

In the clinical setting, it has been recommended that the surgeon apply gentle digital pressure to an intestine that is moderately distended with saline solution to test for an acceptable enterotomy closure; if the saline solution is found to leak, additional sutures are placed.<sup>6</sup> In the present study, we elected to test only once, with quantification to failure, after what was subjectively considered adequate closure of the enterotomy sites. We did notice a difference between canine cadavers, with the cadaver of dog 1 having the lowest bursting pressures, compared with that of the cadavers of the other 2 dogs. This may have been a result of individual variation in small intestinal tissue strength between canine cadavers. However, this also may have resulted from the investigator not being blinded to the pressure results when performing the procedures on the subsequent 2 canine cadavers. Size of incisions and the number of sutures may also have affected the consistency of bursting pressure results. Although a laparoscopic biopsy punch was used in an attempt to make all defects of equal size, some variation was possible. Gentle pretesting of the closure with the opportunity for placement of additional sutures might have been beneficial and may have minimized the differences in bursting pressures among canine cadavers. Further studies are recommended to determine whether the in situ bursting technique is of value for studies with live dogs that are to be recovered from anesthesia.

- a. Laparoscopic punch biopsy forceps, 4mm, Karl Storz Veterinary Endoscopy, Goleta, Calif.
- b. Margin Marking Dye, American MasterTech Scientific Inc, Lodi, Calif.
- c. Baxter Flo-Gard 6201 volumetric infusion pump, Baxter Diagnostics Inc, Deerfield, Ill.
- d. OmniCare CMS 24, Hewlett Packard, Palo Alto, Calif.

## References

1. Frileux P, Attal E, Sarkis R, et al. Anastomotic dehiscence and severe peritonitis. *Infection* 1999;27:67-70.
2. Ralphs SC, Jessen CR, Lipowitz AJ. Risk factors for leakage following intestinal anastomosis in dogs and cats: 115 cases (1991-2000). *J Am Vet Med Assoc* 2003;223:73-77.

3. Hedlund CS, Fossum TW. Surgery of the digestive system. In: Fossum TW, eds. *Small animal surgery*. 3rd ed. St Louis: Elsevier Health Sciences, 2007;339–530.
4. Cimino Brown D. Small intestines. In: Slatter D, eds. *Textbook of small animal surgery*. 3rd ed. Philadelphia: Saunders Book Co, 2002;644–664.
5. Fukuzawa J, Terashima H, Ohkohchi N. Early postoperative oral feeding accelerates upper gastrointestinal anastomotic healing in the rat model. *World J Surg* 2007;31:1234–1239.
6. Semevolos SA, Reed SK, Gamble K. In vitro bursting pressures of jejunal enterotomy closures in llamas. *Vet Surg* 2007;36:64–67.
7. van Oosterom FJ, van Lanschot JJ, Oosting J, et al. Hyaluronic acid/carboxymethylcellulose membrane surrounding an intraperitoneal or subcutaneous jejunojejunostomy in rats. *Eur J Surg* 2000;166:654–658.
8. Gilbert JM, Trapnell JE. Intraoperative testing of the integrity of left-sided colorectal anastomoses: a technique of value to the surgeon in training. *Ann R Coll Surg Engl* 1988;70:158–160.
9. Gandini M, Bertuglia A. In vitro evaluation of an inverted end-to-end equine jejunojejunal anastomosis using skin staples. *Vet Surg* 2006;35:678–682.
10. Nieto JE, Dechant JE, Snyder JR. Comparison of one-layer (continuous Lembert) versus two-layer (simple continuous/Cushing) hand-sewn end-to-end anastomosis in equine jejunum. *Vet Surg* 2006;35:669–673.
11. Tasaka K, Farrar JT. Intraluminal pressure of the small intestine of the unanesthetized dog. *Pflugers Arch* 1976;364:35–44.
12. Chiba T, Sarr MG, Kendrick ML, et al. Limitations of implantable, miniature ultrasonic transducers to measure wall movement in the canine jejunum. *J Surg Res* 2004;116:219–226.