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## **Biomechanical comparison of four techniques for pelvic flexure enterotomy closure in horse**

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**Objective:** To compare 4 techniques for pelvic flexure enterotomy closure in horses.

**Study Design:** Ex-vivo study

**Sample Population:** Cadaveric ascending colon specimens (n=48 horses).

**Methods:** Pelvic flexure enterotomies of different lengths (5 cm, 10 cm) were performed and closed with 1 of 4 techniques: handsewn 2 layer (HS2); handsewn 1 layer (HS1); skin staples (SKS); or TA90 stapling device (TA90). Time to close each enterotomy, bursting pressure, luminal reduction, and cost were calculated and compared.

**Results:** HS2 was significantly more time consuming to perform in the 5 cm group whereas in the 10 cm group, only the HS1 and SKS were faster than the other techniques. Luminal reduction was not different between techniques in either group. HS2 resulted in consistently higher bursting pressure compared with SKS and TA90 in the 5 cm group and compared to all other techniques in the 10 cm group.

**Conclusion:** The TA90 technique had the lowest bursting pressure and highest cost. The HS2 technique was strongest.

Handsewn and stapled techniques are used to close enterotomies of the ascending colon pelvic flexure in horses.<sup>1-5</sup> A 2 layer closure (full thickness, simple continuous pattern oversewn with a Cushing pattern) is superior in terms of layers alignment and healing to a 1-layer pattern.<sup>2</sup> We reported a pelvic flexure enterotomy closure technique in horses using skin staples<sup>3</sup> and use of an intestinal stapling device (Autosuture TA90) for pelvic flexure enterotomy closure has been reported in over 200 horses with excellent results.<sup>4</sup> Our purpose was to evaluate 4 pelvic flexure enterotomy closure techniques (handsewn 1 layer; handsewn 2 layer; skin staples; TA90 stapling device) to assess which one would prove more effective by comparing construction time, luminal reduction, bursting pressure, and cost.

### **Materials and Methods**

Intestinal tract specimens containing the distal aspect of the left ventral colon, pelvic flexure, and proximal aspect of the left dorsal colon were collected from 48 slaughtered horses, aged 12–18 months, without apparent gastrointestinal disease. Specimens were collected immediately after death, emptied of ingesta, rinsed with tap water, and stored in lactated Ringer's solution for transport at room temperature. Enterotomy closure and testing were completed within 4 hours of

death. Specimens were assigned to 2 groups (5 cm enterotomy [group 1]; 10 cm enterotomy [group 2]) of 24 specimens each. Before performing enterotomy, a metal cannula was connected to a compressed air tank and inserted into the lumen at one end for inflation and a similar cannula inserted at the other end was connected to a mercury manometer. Intestines were inflated with air to a pressure of 10 mmHg. A line (line A) was drawn perpendicular to the axis of the bowel at the level of the pelvic flexure with a permanent marker. A 2nd line (line B), perpendicular to the 1st line, was drawn on the antimesenteric border to identify the enterotomy site (Fig. 1). Two skin staples were applied to mark line A at 1/3 and 2/3 of the distance between mesenteric and antimesenteric borders, to serve as markers for digital radiography measurements. Digital radiographs of the inflated specimens were taken, centering the beam on line A. Images were digitally manipulated to measure luminal reduction (Agfa Mimosa Vips 1206 proprietary software; Agfa Gevaert N.V/S.A 96, Munich, Germany). A line was drawn between the 2 skin staples to measure the luminal diameter at the same point before and after performing the enterotomy. This measurement was repeated 3 times by the same person to obtain a mean value (Fig. 2). Then, templates of standardized length (5 cm or 10 cm) were centered on line A and their extremities marked on line B. These marks were then used to mark the enterotomy, made with a #20 scalpel blade. Because the enterotomy marks could be of different thickness from ink expansion on the bowel surface (Fig. 1), we used the outside edges of these marks to delimit the enterotomy site. Each group was divided in 4 subgroups (handsewn 2 layer [HS2]; handsewn 1 layer [HS1]; skin staples [SKS]; or TA90 stapling device [TA90]) of 6 specimens each.

### *Closure Techniques*

For consistency, the same person applied tension either to stay sutures or Allis forceps used to control tissues being sutured or stapled. To facilitate radiographic identification, liquid barium sulfate was applied to the suture material just before enterotomy closure.

HS2. A handsewn 2 layer closure<sup>2</sup> was achieved by a simple continuous full thickness layer oversewn with a Cushing seromuscular suture using 2-0 polyglactin 910.

HS1. The enterotomy was closed with a single Cushing serosubmucosal suture pattern using 2–0 polyglactin-910.

SKS. An inverting closure using skin staples was performed by initially placing 2 inverting (Cushing) stay sutures, taking care to invert the enterotomy edges. Skin staples (Visistat Regular Size 5.7mm\_3.9mm skin stapler, Weck, Teleflex Medical, Kenosha, WI) were applied 1–3mm apart until complete closure was achieved.

TA90. Two Allis tissue forceps were placed at the ends of the stoma making sure to include the seromuscular and mucosal layers and paying particular attention to place the teeth of the forceps as close as possible to the edges of the enterotomy. Another 1–3 Allis tissue forceps (depending on enterotomy length) were placed \_2.5 cm apart to align the edges. An Autosuture TA90 stapler (Covidien Italia, Milano, Italy) was then placed across the bowel wall adjacent to the Allis forceps, closed, and fired. Protruding intestine was trimmed with Mayo scissors before removing the stapler. For the 10 cm enterotomy, the stapling device was applied once more in the same fashion, taking care to cross the 1st staple line with the 2nd one. Again, excess bowel was trimmed before removing the stapler.

#### *Gross Appearance*

After enterotomy completion, specimens were inspected for inversion/eversion of intestinal wall, presence of exposed suture material, and shape of the intestine.

#### *Leak Test*

Before further testing, all enterotomies were leak tested by infusing 1 L water colored with methylene blue into the lumen and then gently milking the bowel segment by hand, to spread the colored fluid toward and beyond the enterotomy site.

#### *Construction Time*

Construction time (minutes) was calculated from application to removal of the Allis forceps or trimming of excess tissue (TA90) or suture thread (HS1, HS2, SKS).

#### *Radiographic Examination and Luminal Diameter*

Each specimen was again inflated to 10mmHg and positioned with an x-ray beam centered on line A. Radiographs were taken and luminal diameter measured. Luminal diameter reduction was calculated as a percentage using the formula:  $100-(D2/D1-100)$ , where D1 is the initial diameter and D2 the diameter measured at the same point after enterotomy and closure.

### *Bursting Pressure*

After radiographic examination, all enterotomies were leak tested by air filling and immersion in a water tank. Bursting strength was tested with a modified gas inflation tank test.<sup>6</sup> Briefly, a metal cannula connected to a mercury manometer was inserted at one end of the specimen and a similar cannula at the opposite end connected to a compressed air tank. The entire specimen was submerged in water and inflated with air at 1 L/min until gas leakage occurred. Luminal pressures were continuously measured with a mercury manometer and recorded by a digital camera.

Reviewing the recordings allowed determination of the peak pressure at specimen failure. Failure was confirmed by presence of gas bubbles leaking from the submerged intestine, together with a decline in luminal pressure.

### *Cost*

Cost (Euros) was calculated according to pricing from local surgical supply distributors. Because of need for aseptic techniques in vivo, the cost of the skin stapler was considered in full, although not all the staples were needed to close a single enterotomy. For the same reason, 2 sutures were used to perform HS2.

### *Statistical Analysis*

Mean $\pm$ SD were calculated and compared for construction time, luminal diameter reduction, and bursting pressure by using a 1-way ANOVA with Bonferroni post-test. Exact values were compared for cost. All statistical analyses were performed using statistical software (GraphPad InStat®, La Jolla, CA). Significance was set at  $P \leq .05$ .

## **Results**

### *Gross Appearance*

Both handsewn techniques resulted in a smooth inverted surface with exposed suture material only where the initial and final knots were tied. Intestinal shape was maintained with very little deformation at the enterotomy site. SKS produced an inverted surface with all staples exposed and moderate deformation of the intestinal wall. The TA90 produced a linear everted suture with total mucosal exposure lined by 2 rows of staples, and marked deformation of the intestinal profile especially in 10 cm enterotomies (Fig. 3).

#### *Leak Test*

None of the enterotomies leaked.

#### *Construction Time*

Group 1. Mean<sub>SD</sub> time for HS2 (9.55±0.64 minutes) was significantly longer (P<.001) than for HS1 (5.41±0.34 minutes), SKS (4.1±0.23 minutes) and TA90 (3.62±0.64 minutes) techniques.

Construction time was not significantly different between HS1, SKS, and TA90 techniques.

Group 2. Mean time for HS2 (12.00±0.71 minutes) was significantly longer (P<.05) than for HS1 (6.31±0.43 minutes) and SKS (6.15±0.62 minutes) techniques but not compared with TA90 (9.77±0.21 minutes).

#### *Luminal Diameter*

In Group 1, the mean (±SD) luminal diameter reduction expressed in percentage was not significantly different among HS2 (7.44±3.04%), HS1 (7.41±4.3%), SKS (8.82±3.23%), and TA90 (10.05±1.6%) techniques. In Group 2, the mean (±SD) luminal diameter reduction expressed in percentage was not significantly different among HS2 (8.46±3.75%), HS1 (8.29±4.15%), SKS (10.35±4.01%), and TA90 (14.69±5.15%) techniques.

#### *Bursting Pressure*

Group 1. Mean bursting pressure for HS2 technique (176.43±12.35 mmHg) was significantly higher than for SKS (100.71±21.3 mmHg; P<.05) and TA90 (85.71±13.54 mmHg; P<.01) but not for HS1 (117.86±20.1 mmHg). Difference in bursting pressure was not significantly different between HS1, SKS, and TA90 techniques.

Group 2. Mean bursting pressure for HS2 (182.86±14.5 mmHg) was significantly higher than for HS1 (115.00±16.31 mmHg; P<.05), SKS (95.71±8.36mmHg; P<.01), and TA90 (69.21±15.01mmHg; P<.001) techniques. Bursting pressure was not significantly different between HS1, SKS, and TA90 techniques.

#### *Cost*

Costs excluding VAT (value added tax) were: HS2= 110.44 euros; HS1=5.22 euros; SKS=9.35 euros; and TA90= 198–396 euros using a reusable stainless steel Autosuture TA90 stapler considering the cost for re-sterilization to be negligible.

#### **Discussion**

We found that the TA90 closure technique was most expensive and least strong. The HS1 and SKS techniques could be considered for pelvic flexure enterotomy closure in horses after further in vivo study. The HS2 technique provided the most secure closure despite a longer construction time. Previously use of this 2 layer technique was associated with inflammation likely linked to use of catgut and to contamination of less reactive suture material by ingesta<sup>2</sup>. Surgical titanium alloy or stainless steel staples are inert, and have been associated with lower complication rates or better intestinal healing than with sutures<sup>7</sup>. Use of skin staples or intestinal staplers to close pelvic flexure enterotomies could reduce bowel handling and diminish the amount of reactive material in the intestinal wall, although eversion caused by the TA90 technique could be problematic in vivo. Clinically, this technique resulted in good healing without increased complication rates.<sup>4,5</sup> Skin staples have been evaluated for use in intestinal surgery in pigs,<sup>8,9</sup> dogs,<sup>10–12</sup> people,<sup>13–15</sup> and in horses.<sup>16</sup>

We found closures using skin staples easy to perform with minimal bowel manipulation and producing an inverting pattern compared with the everting pattern of the TA90 technique.

With the HS2 technique, contaminated suture is handled during closure of the 1st layer but this does not seem to be a critical point in vivo.<sup>2</sup> With skin staples, manipulation of contaminated suture material is avoided by application of 2 stay sutures at each end of the incision, causing edge



inversion and allowing stapling an intact, clean serosa. It could be argued that leaving such a large amount of material exposed on the serosal surface could prove problematic, but intraluminal migration of staples has been demonstrated during enterotomy healing process in dogs<sup>10</sup>; however, this needs to be demonstrated in horses.

In the TA90 technique, the contaminated mucosa is held by Allis tissue forceps away from the stapling line so there little manipulation of infected material, although, as previously described, it does protrude from the closed enterotomy.

The HS2 technique took significantly longer to perform than the other techniques in the 5 cm group but was only longer than the HS1 and SKS techniques in the 10 cm group. The TA90 technique does not provide any advantage in terms of construction time when used for enterotomies of sufficient length that require 2 applications of the stapler. Furthermore, during surgery where large colon enterotomy is needed, closure time may be less critical than bursting pressure, reduction in luminal diameter, or mucosal eversion.

Because of the anatomic and physiologic characteristics of the pelvic flexure, the most important feature of enterotomy closure is the ability to avoid leakages. Methylene blue testing has been used to assess anastomotic leakage in people<sup>17</sup> and to reduce complications. None of our techniques leaked after completion in vitro, meaning that all can provide a tight seal, the overarching objective of any closure technique.

Another important criterion for an enterotomy closure pattern is to minimize luminal constriction. This is true in the early postoperative period when motility impairment from underlying pathology may occur, and longer term, when high density ingesta passes through. Luminal reduction was not significantly different between techniques in either the 5 or 10 cm group, although the TA90 technique did create odd shapes at the enterotomy site after 2 applications of the linear stapler (Fig. 3). The HS2 technique resulted in consistently higher bursting pressures compared with SKS and TA90 techniques in the 5 cm group and compared to all techniques in the 10 cm group, although the

pressures withstood by all closures were well above those recorded in vivo and reported to cause intestinal wall damage (up to 58 mmHg).<sup>18,19</sup> Cost could be a factor in choice of an intestinal closure technique and was similar for HS1, HS2 and SKS but high for the TA90 when considering the low bursting pressure, greater luminal reduction, and gross appearance of the bowel after closure.

Hemorrhage control is a crucial factor that often dictates choice of suture pattern. In a previous study on pelvic flexure enterotomies,<sup>2</sup> formation of hematomas was a consequence of poor bleeding control at the enterotomy site with a 1-layer technique. Persistent bleeding at the time of closure was probably caused by starting the closure soon after incision, without allowing enough time for coagulation of small vessels.

In another study,<sup>20</sup> hemorrhage was a serious consequence of large colon enterotomies, although these are the only cases reported in horses. Because the HS1 and SKS technique do not include the mucosa, they may not achieve effective bleeding control and certainly this can be a critical point that needs further in vivo assessment.

There are reports of complications arising after application of incomplete staple lines or the presence of loose staples in the abdomen.<sup>21-23</sup> With the SKS technique, staples could become loose when applied to edematous colon because of decreased wall thickness when the edema subsides. This could also be a problem with the TA90 technique (Fig. 4), although it is unlikely that these partially applied staples in the pelvic flexure could reach other abdominal structures. Theoretically, considerations like cost, low bursting pressure, and mucosal eversion would suggest this technique is a poor choice in pelvic flexure enterotomy closure; however, 2 studies report clinical use without complications in 200 horses<sup>4</sup> and complications comparable to a 2 layer handsewn technique in 84 horses.<sup>5</sup>

Those results should be interpreted with caution because of lack of a control group and objective evidence of absence of complications (only 10 horses were necropsied)<sup>4</sup> and a control group that was probably too small compared to the treatment group. Furthermore the reported rate of complications appears very high (31%).

Our results contrast those of a study comparing TA90 closure with a 2 layer handsewn closure technique<sup>24</sup> in vitro, but there are study design and methodologic differences that make direct comparison difficult. It is not clear how measurements taken before and after enterotomy and closure were controlled without prior placement of a marker, whereas we consistently measured the distance between the same points before and after performing the enterotomy and closure.

Bursting pressure was measured with a manometer that could record a maximum value of 120 cm H<sub>2</sub>O (88.27mmHg) and 4 of 7 handsewn and 4 of 6 stapled enterotomy closures burst at pressures >120 cm H<sub>2</sub>O and concluded there was no significant difference in bursting pressure between techniques. Because most bursting pressures exceeded the range of the manometer, this conclusion maybe erroneous. Finally, comparing our in vitro results with clinical outcome<sup>4,5</sup> an argument could be made that bursting pressure, luminal reduction, and construction time as we evaluated may not be valid methods to define potential clinical efficacy. In vitro evaluation of a surgical technique can provide insight into potential problems, but randomized controlled in vivo studies with adequate follow up are necessary for understanding of further pitfalls or complications. We concluded that the HS2 technique remains the best option to close a pelvic flexure enterotomy in vitro. One layer techniques, both handsewn or stapled, could prove satisfactory but further vivo studies are needed before general clinical use.

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**Figure legends:**

**Figure 1:** Landmarks for enterotomy measurements and luminal diameter measurements 817mm x534mm (72x72 DPI).

**Figure 2:** Measurement of luminal diameter of intact bowel 130mm x167mm (72x72 DPI).

**Figure 3:** TA90 closure on a 10 cm enterotomy. Note shape of the staple line 112mm x 84mm (180x180 DPI).

**Figure 4:** Incompletely placed staple after closure with the TA90 stapler 440mm x 258mm (180 x180 DPI)