Evaluation of a laparoscopic technique for collection of serial full-thickness small intestinal biopsy specimens in standing sedated horses

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Objective—To assess a technique for laparoscopic collection of serial full-thickness small intestinal biopsy specimens in horses.

Animals—13 healthy adult horses.

Procedures—In the ex vivo portion of the study, sections of duodenum and jejunum obtained from 6 horses immediately after euthanasia were divided into 3 segments. Each segment was randomly assigned to the control group, the double-layer hand-sewn closure group, or the endoscopic linear stapler (ELS) group. Bursting strength and bursting wall tension were measured and compared among groups; luminal diameter reduction at the biopsy site was compared between the biopsy groups. In the in vivo portion of the study, serial full-thickness small intestinal biopsy specimens were laparoscopically collected with an ELS from the descending duodenum and distal portion of the jejunum at monthly intervals in 7 sedated, standing horses. Biopsy specimens were evaluated for suitability for histologic examination.

Results—Mean bursting strength and bursting wall tension were significantly lower in the ELS group than in the hand-sewn and control groups in both the duodenal and jejunal segments. Use of the hand-sewn closure technique at the biopsy site reduced luminal diameter significantly more than use of the stapling technique. In the in vivo part of the study, all 52 biopsy specimens collected during 26 laparoscopic procedures were suitable for histologic examination and no clinically important perioperative complications developed.

Conclusions and Clinical Relevance—Laparoscopic collection of serial full-thickness small intestinal biopsy specimens with a 45-mm ELS may be an effective and safe technique for use in healthy adult experimental horses. (*Am J Vet Res* 2008;69:431–439)

Collection and evaluation of full-thickness small intestinal biopsy specimens is useful for investigation of small intestine physiology in experimental horses,[1-4] and serial collection of biopsy specimens is occasionally necessary to confirm diagnosis of refractory intestinal disorders in clinical horses.[5,6] At present, full-thickness small intestinal biopsy specimens in horses are collected through flank laparotomy or midline celiotomy incisions.[5,7-9] However, these procedures are invasive and can be associated with substantial postoperative complications such as incisional infections, wound dehiscence, herniation, and development of abdominal adhesions.[10] In the authors' opinion, repeated midline celiotomy or flank laparotomy cannot be used to collect serial full-thickness small intestinal biopsy specimens in experimental horses because these procedures are associated with high risk for postoperative complications[11] and would negatively impact the horses' health.

Laparoscopy has been used to collect full-thickness intestinal biopsy specimens for diagnosis of multiple gastrointestinal diseases in humans.[12,13] This procedure is associated with a short hospital stay, less postoperative pain, and minimal complications and is considered to be a safe and efficient alternative to midline celiotomy in humans.[12,13] Automatic endoscopic stapling devices have been used for laparoscopic collection of

Abbreviation

ELS Endoscopic linear stapler

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full-thickness small intestinal biopsy specimens in humans.[12,13] In horses, use of these automatic stapling devices reduces surgical time and risk of contamination, compared with conventional hand-sutured anastomosis techniques.[14-16]

Laparoscopy is established as a minimally invasive surgical technique in horses.[17] It allows for excellent observation of and access to abdominal organs and is associated with minimal postoperative pain and complications.[17-19] A laparoscopic technique for collection of full-thickness biopsy specimens of the cecum and duodenum by use of a single-layer intracorporeal suturing technique in horses has been reported.[17-21] However, the technique is technically challenging, and its use is limited to surgeons with advanced laparoscopic intracorporeal suturing skills. Furthermore, the technique is associated with risk of abdominal contamination and postoperative septic peritonitis.[20]

The purpose of the study reported here was to evaluate a laparoscopic technique involving use of an ELS for collection of serial full-thickness small intestinal biopsy specimens in healthy experimental adult horses. We hypothesized that use of an ELS to collect intestinal biopsy specimens laparoscopically would be associated with lower bursting strength and bursting wall tension at the biopsy site, that anastomosis could be performed with less luminal diameter reduction at the biopsy site than if double-layer hand-sewn closure were used, and that the specimen derived would be suitable for histologic examination. We also hypothesized that the lower bursting strength and bursting wall tension and luminal diameter remaining at the biopsy site after serial full-thickness biopsy with an ELS would not be associated with intra- or postoperative complications.

Materials and Methods

All procedures and experimental protocols were approved by the Animal Care Committee of the University of Guelph.

Part 1—Part 1 of the study consisted of ex vivo evaluation of bursting strength and bursting wall tension of the descending duodenum and distal portion of the jejunum after collection of full-thickness biopsy specimens by use of either hand-sewn or stapling closure techniques.

Horses

Six horses (1 gelding and 5 mares; 3 Standardbreds and 3 Thoroughbreds) euthanized for reasons unrelated to gastrointestinal tract disease were used in the ex vivo part of the study. Horses ranged from 3 to 16 years of age (mean, 11 years) and weighed from 385 to 604 kg (mean, 489 kg).

Collection of intestinal segments

Sixty-centimeter segments of duodenum and distal portion of the jejunum were obtained from each horse through a right flank laparotomy performed immediately after euthanasia. Duodenal segments were collected from a point 5 cm aboral to the duodenal sigmoid flexure to the most aboral portion of the ascending duodenum. Distal jejunal segments were collected from a point 30 cm oral to the jejunoileal junction. Collected duodenal and jejunal segments were divided into three 20-cm segments; labeled as ascending, middle, and descending parts of the duodenum and as oral, middle, and aboral parts of the distal segment of jejunum; and preserved in 4°C lactated Ringer's solution until tested.[22-24] Biomechanical testing of the collected intestinal segments was performed within 3 hours after collection.

Collection of biopsy specimens

A randomized block design was used to assign each 20-cm intestinal segment to one of the following groups: control, full-thickness small intestinal biopsy by use of a double-layer hand-sewn closure technique, or full-thickness small intestinal biopsy by use of an ELS. All control groups were left intact for biomechanical testing. In the biopsy groups, a full-thickness 3-cm-long and 1-cm-wide full-thickness intestinal biopsy specimen was collected from the antimesenteric border of the middle section of the intestinal segment under simulated surgical conditions. In the hand-sewn closure group, specimens were obtained with conventional surgical instruments, and biopsy sites were closed in 2 layers with 2-0 gylcomer 631[a] suture material in a full-thickness simple continuous suture pattern followed by a Cushing continuous inverting suture pattern.

In the ELS group, a 45-mm endoscopic articulating linear stapler[b] with a 440-mm shaft was used to collect biopsy specimens. The ELS cartridges[c] contained 4 staggered rows of titanium staples with a leg length of 4.1 mm. To collect the biopsy specimens, the open jaws of the ELS were applied on the antimesenteric border of the middle section of the intestinal segment at a 10° angle to its long axis. The jaws were closed and the device fired to both activate the 4 parallel staggered rows of staples and make a full-thickness section through the intestinal wall, leaving 2 staggered rows of titanium staples on both the biopsy specimen and the biopsy site. The stapler was reloaded, and a second cut was performed crossing the first one at a 120° angle to obtain a full-thickness V-shaped intestinal biopsy specimen. In both the hand-sewn and ELS groups, the time taken to perform full-thickness biopsy was recorded. Biopsy construction time was defined as the time from the moment of incising the full-thickness biopsy specimen at the antimesenteric border of the intestinal segment to completion of the second layer for the hand-sewn group and from the moment of loading the ELS to the moment of obtaining the V-shaped biopsy specimen for the ELS group.

Determination of biomechanical variables

Bursting strength was determined by increasing the intraluminal pressure of each intestinal segment to the point of mechanical failure of the walls. During testing, each intestinal segment was placed in a plastic tub and submerged in 25 L of saline (0.9% NaCl) solution at temperatures ranging from 21° to 23°C (**Figure 1**). Both ends of the tested segment were attached to 3.8-cm barbed tube connectors so that the tip of each connector was located 2.5 cm into the intestinal segment lumen. Adherent tape[d] and 2 stainless-steel screw hose

clamps were positioned on both ends of the tested segment and tightened to provide a watertight seal between the intestinal wall and barbed connectors. One barbed connector was secured to an end cap with a bleeder valve. The second barbed connector was attached to a T-connector, which was attached to a pressurized new methyelene blue solution delivery system. This system consisted of a pressure transducer,[e] shutoff valve, and new methyelene blue solution reservoir. Pressure transducer instrumentation consisted of a 12-V direct current power supply, a strain gauge amplifier,[f] an analog-to-digital data acquisition card,[g] and a personal computer with data acquisition software.[h] System pressure was sampled at 30 Hz. Before each mechanical bursting trial, the pressure transducer[e] and associated instrumentation were calibrated with a linear scale and water column U-tube manometer operating at an ambient temperature of 21° to 23°C. The new methyelene blue solution reservoir was suspended by a winch system. When activated, the winch system elevated the reservoir so that it delivered a constant 2 mm Hg/s pressure increase to the intestinal segment being tested. Volume of solution infused into the intestinal segment during bursting trials was determined with a force plate and associated instrumentation. The force plate consisted of 3 uniaxial load cells[i] fixed between 2 sheets of 2-cm plywood. The force plate instrumentation consisted of 3 strain gauge amplifiers[f] and the same analog-to-digital data acquisition card, personal computer, and data acquisition software used to measure sample pressure increase. The force plate was positioned underneath the plastic tub used to hold the tested intestinal segment and saline solution. Prior to each bursting trial, force plate calibration factors were determined with a set of calibration weights and accompanying output voltage from the strain gauge amplifiers. For each bursting trial, the output voltage of the 3 load cells was combined with their respective calibration factors and summed to yield total force plate load. The weight of the plastic tub, saline solution, and tested intestinal segment was constant, whereas the weight of infused solution in the tested intestinal segment increased with infused volume. The weight increase measured during each bursting trial was converted to sample volume increase by dividing load change (in Newtons) by density of the new methyelene blue solution at 20°C.

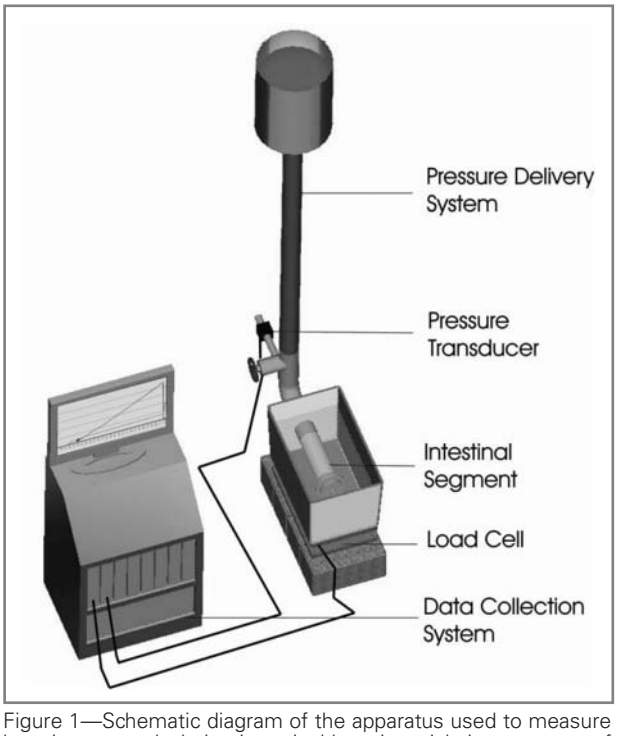

Figure 1—Schematic diagram of the apparatus used to measure bursting strength during intestinal bursting trials in segments of intestine from 6 horses by increasing intraluminal pressure in 2-mm Hg increments until the point of mechanical failure.

For each bursting trial, the data collection system was activated, the reservoir was situated adjacent to the sample, the shutoff valve was opened, and new methyelene blue solution was infused into the tested intestine segment. Air in the tested intestine segment was removed through the end cap and bleeder screw. The winch connected to the new methyelene blue reservoir was activated, and the dye solution was infused in the tested intestinal segment. Intestinal bursting strength was defined as the maximum pressure, or bursting pressure, recorded before a sudden decrease in the slope of the pressure-versus-time curve or, alternatively, the pressure recorded when leakage of new methyelene blue solution was observed on the outer surface of the intestinal segment. The volume of solution infused (in milliliters) at the time of failure, bursting pressure in millimeters of mercury at failure, and location and mode of failure (ie, leaking or bursting) were recorded. Bursting trials were video recorded, and images at failure were digitized. The video system was calibrated with objects of known dimensions. Length of the intestinal segment at failure was measured with computer software.[j] Mean radius of each intestinal segment at failure was calculated by use of the equation $r = \sqrt{v/\pi} \times l$, where r is radius of the intestinal segment at failure, $\sqrt{}$ is square root, v is volume of fluid infused at failure, and l is length of the intestinal segment at failure.[22,25] Bursting wall tension in dynes per centimenter was calculated by use of LaPlace's law: $BWT = BP \times r$, where BWT is bursting wall tension, BP is bursting pressure, and r is radius.[22,25-27]

Evaluation of reduction in luminal diameter

Digitized images of each constructed intestinal biopsy segment at the time of mechanical failure (bursting pressure) were used to compare the percentage of luminal diameter reduction between biopsy techniques. Luminal diameter measurements were obtained with computer software.[j] For the ELS group, the first measurement was taken at a site 90° from the junction at which the 2 staple lines crossed to form a V shape relative to the mesenteric border. For the hand-sewn group, the first measurement was taken at a site 90° from the center of the biopsy site to the mesenteric border. Three measurements were taken on each side of the biopsy site: 1 in the tissue adjacent to the biopsy site and 2 at 3 and 6 cm, respectively, from the beginning of the suture or staple line. The mean values of these 6 measurements were used as control measurements, and luminal diameter reduction at the biopsy site was expressed as a percentage of these control values. Percentage of lumi-

nal diameter reduction was calculated by dividing the diameter at the biopsy site by the mean control luminal diameter.

Part 2—In part 2 of the study, a laparoscopic technique in which an endoscopic stapling device was used for collection of full-thickness small intestinal biopsy specimens in standing, sedated, experimental horses was evaluated in vivo.

Horses

Seven mature horses (3 geldings and 4 mares; 5 Standardbreds and 2 Thoroughbreds) were used in the in vivo part of the study. Horses' ages ranged from 7 to 16 years (mean, 10.3 years). Horses were assessed to be healthy on the basis of physical and per rectal examinations and results of CBC, serum biochemical analyses, abdominocentesis, and abdominal ultrasonographic examination. Each horse underwent laparoscopy a minimum of 3 and a maximum of 4 times, with a 1-month interval between procedures. During each procedure, full-thickness small intestinal biopsy specimens were collected from the descending duodenum and distal portion of the jejunum by means of a laparoscopic stapling technique. Prior to each surgery, hay was withheld for 24 hours and grain was withheld for 18 hours to reduce the volume of intestinal contents. Before surgery, water (5 L, by nasogastric intubation), trimethoprim-sulfadoxine (24 mg/kg, IV), and flunixin meglumine (1 mg/kg, IV) were administered. Right flank laparoscopy was performed with neuroleptanalgesia and local anesthesia and with horses restrained standing in stocks. Acepromazine maleate (0.01 mg/kg, IV) was administered 30 minutes prior to initiation of deep sedation. Horses were sedated with a loading dose of detomidine hydrochloride (15 µg/kg, IV) and butorphanol tartrate (0.03 mg/kg, IV). Neuroleptanalgesia was maintained by constant rate infusion of detomidine hydrochloride (8.5 µg/kg per hour, IV) delivered by an electronic infusion pump.[k]

Laparoscopic surgical procedure

Skin overlying the right paralumbar fossa was clipped, aseptically prepared, and draped in a routine fashion. Three portal sites were created in the right paralumbar fossa and used to perform laparoscopic collection of full-thickness small intestinal biopsy specimens. Portal site 1 was midway between the tuber coxae and the 18th rib, just dorsal to the crus of the internal abdominal oblique muscle. Portal site 2 was placed at the level of the ventral border of the tuber coxae and either immediately caudal to the 18th rib or between the 17th and 18th ribs, depending on the length of the flank in each horse. Portal site 3 was placed immediately cranial to the ventral aspect of the tuber coxae. The full thickness of the abdominal wall at the portal sites was infiltrated with 20 mL of 2% mepivacaine hydrochloride solution. The first cannula[l] was placed in the abdomen at portal site 1 through a 10-mm skin incision, and a 10-mm 30° laparoscope connected to a fiberoptic cable and video camera was introduced through the cannula to confirm its intra-abdominal position. The cannula was connected to an automatic high-flow carbon dioxide insufflator, and intra-abdominal pressure was increased by 8 to 10 mm Hg. The abdomen was illuminated with a 300-W light source. Insertion of the accessory trocar-cannula units at portal sites 2 and 3 was laparoscopically guided to avoid visceral injury. The descending duodenum was identified dorsal to the base of the cecum.

The midportion of the descending duodenum was the designated first biopsy site for the first laparoscopic procedure. A sterile artificial insemination pipette was introduced through portal site 2, and 20 mL of 2% mepivacaine hydrochloride solution was applied to the serosal surface of the duodenum. Laparoscopic Babcock forceps[m] were inserted into portal site 3 and used to grasp and apply tension on the antimesenteric border of the duodenum. An endoscopic automatic 45-mm linear stapler[b] was inserted in portal site 2, and a full-thickness V-shaped intestinal biopsy specimen was collected on the antimesenteric border of the descending portion of the duodenum in a 2-step fashion as described in the ex vivo part of the study. Laparoscopic Babcock forceps were used to exteriorize the biopsy specimen from the abdominal cavity. The biopsy site was observed for leakage or bleeding and a swab specimen was obtained under laparoscopic guidance for bacterial culture. The site was flushed with 40 mL of sterile saline solution through the insemination pipette at portal site 2. The length of the small intestine was viewed and examined by means of laparoscopic Babcock forceps placed in portal sites 2 and 3. The time to examine the small intestine from the most oral aspect of the ascending duodenum to the ileum was recorded. A second full-thickness biopsy specimen was collected at a site 30 cm oral to the jejuno-ileal junction on the antimesenteric border of the jejunum as described, and a swab specimen was obtained and the site was flushed. After completion of the procedure, the external sheath of the abdominal external oblique muscle was closed with size 0 glycomer 631 in a simple interrupted suture pattern, and skin was closed with simple interrupted sutures in size 0 polypropylene.[n]

During subsequent laparoscopic procedures, biopsy sites and the abdominal cavity were examined for abnormalities, which were recorded. Full-thickness small intestinal biopsy specimens were collected at sites approximately 5 cm aboral to the previous biopsy sites. Bacterial culture of sites was only performed for the first 2 laparoscopic procedures. Surgical time was recorded, defined as the time from introduction of the first laparoscopic cannula to complete closure of surgical incisions.

Postoperative care and monitoring

Trimethoprim-sulfadoxine and flunixin meglumine were administered for 3 days after each laparoscopy. Horses were permitted free access to water immediately after surgery. All experimental horses were concurrently participating in a parallel nutritional study conducted by 2 of the authors (RJG and HLW). As part of the experimental protocol, all horses received 5 L of water via nasogastric intubation twice daily for 24 hours, and feeding was resumed on the morning after surgery. Horses were fed a diet consisting of grain or hay. Monitoring was performed every 6 hours for the first 7 days and twice daily for an additional 7 days. Each horse was monitored for attitude, appetite, signs of depression,

heart and respiratory rate, rectal temperature, signs of abdominal pain, gastrointestinal tract motility, fecal and urine output, and swelling or drainage associated with the incisions. Complete blood count, serum biochemical analyses, abdominal ultrasonographic examination, peritoneal fluid analysis, and abdominal ultrasonographic examination were performed on days 2, 4, 6, and 8 after surgery for the first 2 laparoscopic procedures to monitor for complications that may have developed.

Evaluation of biopsy specimens

Specimens were rinsed in cold buffered saline solution immediately after collection and were weighed and measured. A full-thickness cross section of each specimen was removed and placed in phosphate-buffered 10% formalin solution for histologic examination. Portions of each biopsy specimen were sectioned and stained with H&E stain. Biopsy specimens were examined by a board-certified pathologist who assessed suitability of the tissue for histologic examination on the basis of size, depth (presence or absence of deep mucosa and submucosa), quality (biopsy preservation with minimal fragmentation and tissue artifacts), and adequacy for diagnosis, defined overall as a well-oriented specimen of sufficient depth and quality to allow full histologic assessment and accurate diagnostic conclusions.

Follow-up information

Five of the 7 experimental horses were used 1 year after completion of the study by 2 of the authors (LPB and SGN) for development of a new laparoscopic imaging technique in standing sedated horses. Biopsy sites and the abdominal cavity were evaluated, and abnormalities were recorded.

Statistical analysis

Descriptive statistics were reported as mean ± SE. The Shapiro-Wilk test was used to test whether data were normally distributed. A log transformation was applied if data did not meet the assumptions of normality. For the ex vivo portion of the study (part 1), 3-way ANOVA was used to determine whether intestinal segments (ascending, middle, and distal portions of the duodenum and oral, middle, and aboral segments of the jejunum), intestinal sections (duodenum or distal portion of the jejunum), and groups (hand-sewn, ELS, or control) or their interactions were significantly different for mean bursting strength, bursting wall tension, or time of construction. A post hoc Tukey test was used for paired comparison of bursting strength and bursting wall tension. A paired *t* test was used for comparison of biopsy construction times. Analysis of variance for a mixed model, with the main effects of intestinal sections (duodenum and distal portion of jejunum), luminal diameter (biopsy site and control values), biopsy closure (hand-sewn and ELS), and their interactions, was used to compare the percentage of luminal diameter reduction between biopsy techniques. A logit transformation was applied to the percentage data.

For the in vivo portion of the study (part 2), variables measured (CBC, biochemical analyses, and peritoneal fluid analysis) were analyzed by use of a general linear mixed model that fit appropriate correlation structures to account for repeated measures in the same horse. The fixed effects in the model were surgery (1 or 2), day (0, 2, 4, 6, or 8), and their interaction with repeated measures made on a horse within a surgery. If ANOVA revealed an overall day effect (with $P < 0.05$) and there was no interaction between surgery and day, a post hoc Dunnett test for paired comparison was used to compare each of the days back to day 0, with mean values for the 2 surgeries used. If surgery and day interaction was significant (overall *F* test, $P < 0.05$), a post hoc Tukey test was used for paired comparisons. Statistical analyses were performed with commercially available software.[o] For all comparisons, values of $P < 0.05$ were considered significant.

Results

Part 1—Mean biopsy construction time was sigificantly ($P < 0.001$) faster in the ELS group (2.27 ± 0.31 minutes) than in the hand-sewn group (11.49 ± 0.32 minutes).

Mechanical properties of biopsy sites

Mean ± SD bursting strength and bursting wall tension were significantly lower in the ELS group than in the hand-sewn group for both duodenal segments and distal jejunal segments (**Table 1**). Intestinal segments in the control group were significantly stronger in bursting strength and bursting wall tension than those in the ELS group for both duodenal segments and distal jejunal segments and were stronger than those in the hand-sewn groups in distal jejunal segments. Given the number of horses used in the ex vivo portion of the study, no significant differences were found between mean bursting strength ($P = 0.385$) and bursting wall tension ($P = 0.123$) of the duodenum in the control and hand-sewn groups.

Mode and location of intestinal wall failure

Failure of the intestinal walls occurred at the mesenteric border in the duodenal and jejunal control groups, at the staple line in the ELS duodenal and jejunal groups, and between the interface of normal tissue and suture line in the hand-sewn duodenal and jejunal groups. All intestinal segments in the control and hand-sewn group failed by bursting, whereas all intestinal

Table 1—Mean ± SD values of bursting strength and bursting wall tension measured ex vivo in duodenal and jejunal segments from control, double-layer hand-sewn closure, and ELS groups in intestinal segments obtained from 6 horses. Values for bursting strength and bursting wall tension are rounded to correct for instrumentation setup.

Groups	Bursting strength (mm Hg)	Bursting wall tension (dynes/cm)
Duodenum C	210 ± 65[a]	840,000 ± 315,000[b]
Duodenum HS	170 ± 80[a]	540,000 ± 265,000[b]
Duodenum ELS	80 ± 40	250,000 ± 145,000
Jejunum C	415 ± 65	2,000,000 ± 415,000
Jejunum HS	200 ± 35	780,000 ± 140,000
Jejunum ELS	40 ± 10	150,000 ± 60,000

C = Control. HS = Hand sewn.
[a,b]Values with the same superscript were not significantly different.

Table 2—Mean and 95% confidence interval values of WBC counts in peritoneal fluid obtained before surgery and on days 2, 4, 6, and 8 after surgery for the first and second laparoscopies in 7 healthy experiemental horses that underwent 3 or 4 laparoscopic small intestinal biopsy procedures in which an ELS was used to obtain the specimen and construct the site.

	Nucleated cell count X 10^9/L	
Peritoneal fluid sample	**Laparoscopy 1**	**Laparoscopy 2**
Before surgery	0.99 (0.45–2.20)	6.34 (2.88–13.95)
Day 2	23.54 (8–69.25)	24.07 (8.09–71.60)
Day 4	13. 76 (5.98–31.67)	18.21 (6.12–54.18)
Day 6	52.73 (20.10–138.35)	25.58 (9.62–67.98)
Day 8	21.92 (9.55–50. 34)	27.82 (10.49–73.76)

segments in the ELS group failed by leakage along the staple line. In all hand-sewn group intestinal segments, the serosa was consistently observed to tear first, followed by tearing in the muscular and submucosal layers. The mucosa was observed to bulge through the defect until failure by bursting. Failures in the ELS group occurred in the duodenum at the V junction of the 2 crossed staple lines in 3 tested segments and on the staple line but at a site distant from the V junction in the other 3 segments. In the distal portion of the jejunum, 2 segments failed at the V junction of the 2 crossed staple lines, whereas the 4 other segments failed on the staple line but at a site distant from the V junction.

Evaluation of luminal reduction

Intestinal segments in both biopsy groups had reduced luminal diameter at the biopsy site, compared with control luminal diameter. The double-layer hand-sewn closure technique resulted in significantly ($P = 0.001$) greater luminal reduction at the biopsy site than the stapling technique. Mean percentage in luminal diameter reduction was 21.51 ± 2.13% for the stapling technique and 32.23 ± 2.13% for the double-layer hand-sewn closure technique, relative to control luminal size.

Part 2—Of 26 laparoscopic procedures performed overall, 5 horses underwent 4 procedures and 2 horses underwent 3 procedures. Overall, 52 full-thickness biopsy specimens were collected with no substantial intraoperative complications. In 1 horse, the jaws of the ELS failed to engage correctly and activate the staples through the full thickness of the intestinal wall during jejunal biopsy. An endoscopic suturing device[p] was used to oversew the biopsy site with 2-0 lactomer 9-1[q] under laparoscopic guidance without further complications. Overall surgical time ranged from 50 to 130 minutes (mean, 94 minutes) and decreased as surgeons gained experience with the procedure. Examining the small intestine by use of laparoscopic Babcock forceps was performed from the most oral aspect of the ascending duodenum to the point at which the ileum was identified. No serosal damage was detected in the small intestine of any horse in association with this maneuver.

No substantial postoperative complications were detected in any horses. All variables assessed during the postoperative period remained within anticipated limits, and all horses passed normal feces within 24 hours after the laparoscopic procedure. None of the 7 horses developed incisional problems at any portal sites throughout 26 laparoscopic procedures. No abdominal ultrasonographic abnormalities were detected in any horses. Peritoneal fluid analysis revealed a significant ($P < 0.001$) increase in WBC count in all horses on postoperative day 2, with a predominance of nondegenerative neutrophils. Peritoneal fluid WBC count remained high in all horses up to postoperative day 8 ($P < 0.001$; **Table 2**). The peritoneal fluid WBC count obtained preoperatively before the second laparoscopy (1 month after the first laparoscopy) was significantly higher in all horses, compared with preoperative peritoneal fluid WBC counts obtained before the first laparoscopy ($P < 0.001$). However, peritoneal fluid WBC count was within the reference range, and all horses were deemed to be healthy and able to undergo the second laparoscopy.

Characteristics of biopsy specimens

Biopsy specimens had a mean weight of 1.66 ± 0.07 g, a mean length of 31.61 ± 0.95 mm, and a mean width of 14.12 ± 0.43 mm. All specimens contained all layers of the small intestinal wall and were judged to be of good quality and adequate for histologic examination on the basis of size, depth, and absence of distortion artifacts from the biopsy technique. All specimens met the criteria for diagnostic adequacy. None of the biopsy sites evaluated during the first 2 laparoscopic procedures yielded bacterial growth.

Healing at biopsy sites

Thirty-eight healing biopsy sites were observed during 19 laparoscopic procedures. The only abnormalities observed were 4 focal omental abdominal adhesions at 4 biopsy sites in 3 horses. None of the abdominal adhesions observed resulted in distortion or stricture of the intestinal lumen, and no clinical problems were detected. One horse developed 2 focal omental abdominal adhesions at 2 biopsy sites, 1 in the distal portion of the jejunum and 1 in the duodenum. These abdominal adhesions were diagnosed and reduced during laparoscopic procedures 2 and 4, respectively. One horse developed a focal omental abdominal adhesion at a distal jejunum biopsy site, and 1 horse developed the same type of adhesion at a duodenal biopsy site. These abdominal adhesions were diagnosed and reduced during laparoscopic procedures 2 and 3, respectively. Three of the 4 diagnosed postoperative omental abdominal adhesions had not reformed when observed during subsequent laparoscopies. Follow-up information on 1 abdominal adhesion that was diagnosed and treated during laparoscopic procedure 4 was not available because the horse did not undergo a fifth laparoscopy.

Follow-up information

Five of the 7 horses underwent an additional laparoscopy 1 year after the last laparoscopic intestinal biopsy collection. Of those 5 horses, 1 had undergone 3 laparoscopic biopsy collections, and 4 had undergone 4 laparoscopic biopsy collections. Three of the 5 horses developed omental adhesions after biopsy. One year after the last laparoscopic biopsy, no abnormalities were observed in any of the 5 horses, and biopsy sites were difficult to differentiate grossly from normal intestine during follow-up laparoscopic abdominal exploration.

Discussion

Until recently, full-thickness intestinal biopsy specimens could only be obtained in horses via laparotomy or celiotomy.[5,7-9] These techniques could not be used for experimental studies requiring serial full-thickness biopsy specimens because they are invasive and can compromise experimental animals. In the past, serial sampling of intestinal tissue necessitated general anesthesia and collection of specimens during a terminal procedure. This allowed for collection of intestinal tissue only over a period of a few hours.[3,28] Laparoscopic surgery is a minimally invasive method for obtaining full-thickness intestinal biopsy specimens in humans.[12,13] To the authors' knowledge, a laparoscopic technique for collection of serial full-thickness small intestinal biopsy specimens in horses has not been reported. Our results indicated that laparoscopic collection of serial biopsy specimens with an ELS is safe in healthy experimental horses.

With the advent of a laparoscopic approach for collecting full-thickness biopsy specimens, determination of bursting strength to test the biopsy site becomes important. Bursting strength measures resistance of the intestinal wall to increasing intraluminal pressure until leakage or disruption occurs and can be expressed as bursting pressure or bursting wall tension.[29] Bursting strength and bursting wall tension were significantly lower in the ELS group than in the hand-sewn group at the time of failure for both the duodenum and distal portion of the jejunum. All tested intestinal segments from the ELS group failed at pressures from 40 to 80 mm Hg, values that are higher than the intraluminal pressure (21 cm H_2O) reported[30] in horses with obstructive small intestinal disease during exploratory celiotomy. In an experimental study[31] in which motility patterns during extraluminal intestinal obstruction were investigated, intraluminal pressures ranged from 8 to 24 mm Hg in conscious ponies. However, cyclic pressure peaks up to 60 mm Hg were frequently measured during the obstruction trials, values that are higher than many of the bursting pressures measured in the present study. These alterations in intestinal motility patterns could be a concern if intestinal biopsy specimens were to be collected with an ELS in clinical horses because diseased intestine may undergo forceful tonic contractions or spasms and reach intraluminal pressures higher than pressures that sites biopsied with an ELS can withstand and may therefore be at risk of failure at the constructed intestinal biopsy site.

Although bursting pressure and bursting wall tension values from our study were low, our results are similar to those reported in association with 2 stapling anastomosis techniques that are used clinically by many equine surgeons at present.[22] Use of stapling devices has become more prevalent in equine abdominal surgery because of the reduction in surgery time and minimized risk of abdominal contamination that are possible when the instruments are used appropriately.[14-16] Furthermore, stapled anastomoses are as strong as or stronger than sutured anastomoses during the early fibrinolytic period.[16,32-34] In most experimental studies,[25,29,33,35] anastomotic bursting strength has been lowest 3 to 7 days after surgery. It is during this crucial period when anastomotic holding strength appears to be most dependent on suture strength.[25,33,36] However, staple lines heal with an abbreviated or absent lag period, during which bursting strength increases linearly from the first postoperative day, and have superior bursting strength during the first week after surgery, compared with hand-sewn sutured lines.[32] On the basis of these results, the lower bursting strength of sites at which an ELS was used may be clinically relevant only in the first few postoperative days, when mechanical strength is lowest. Thereafter, sites at which endoscopic stapling devices were used would be strong enough to withstand the intraluminal pressures that could develop during postoperative ileus. Further research is needed to determine the mechanical strength and healing pattern of intestinal biopsy sites when endoscopic stapling devices were used before they can be recommended for clinical use.

Intracorporeal suturing is technically challenging. Even when the technique is limited to use by surgeons with advanced laparoscopic training, an associated risk of complications remains. Endoscopic stapling devices have gained widespread clinical use. In humans, use of endoscopic staplers for obtaining laparoscopic full-thickness biopsy specimens is safe and effective.[12,13] Intestinal biopsy specimens are collected by applying traction on the antimesenteric border of the small intestine with a laparoscopic forceps and firing the ELS across the tented antimesenteric border of the intestine. This technique decreases the risk of postoperative stenosis.[12,13] In the present study, specimens were collected by applying 2 crossing staple lines to obtain a V-shaped biopsy specimen from the antimesenteric border. Placement of the endoscopic stapler transverse to the antimesenteric surface was not performed because, in the authors' opinion, the shorter mesentery of the duodenum limits the handling of that portion of the intestine needed to obtain a biopsy specimen suitable for full histologic assessment. Moreover, we found that if the ELS was placed transversely, 2 overlapping longitudinal cuts would be needed to obtain a suitable specimen. The point at which the 2 staple lines overlap may be at high risk for leakage. However, applying 2 crossing staple lines to obtain the biopsy specimen reduces the chances of leakage at the junction of the 2 crossed staple lines. The junction of the 2 crossed staple lines was not a consistent site of leakage during the bursting trials, and tested intestinal segments failed at different points along the staple line. Bickers et al[22] reported stapled functional end-to-end jejunojejunostomy segments to consistently fail at the crotch of the V where the 2 intestinal segments merged.[22] The junction of the crossed staple line may warrant further investigation to determine whether this region is the weakest point of the constructed intestinal biopsy site.

Maintenance of adequate luminal diameter after collection of full-thickness small intestinal biopsy specimens is an important surgical goal with regard to minimizing stricture formation. Reduction of luminal diameter by > 33% at an intestinal anastomotic site is potentially dangerous in horses.[37] Excessive luminal diameter reduction at the biopsy site may cause chronic intestinal obstruction and necessitate additional surgery. In small animals, removal of full-thickness intes-

tinal biopsy specimens larger than 20% of the luminal diameter may cause stricture.[38] However, intestinal biopsy specimens must still be of adequate size to enable a pathologist to make a correct interpretation and yield an accurate diagnosis.[38] In the present study, the degree of luminal diameter reduction (21.5%) that resulted when biopsy specimens were collected with an endoscopic stapling technique proved to be safe because intestinal obstruction was not associated with biopsy sites during follow-up evaluations. Open laparotomy techniques have been associated with greater risk of adhesion formation than laparoscopic procedures in humans and other animals.[39,40] In the present study, adhesion formation was detected at 4 of 38 healing biopsy sites. These adhesions caused no clinical problems and were easily reduced laparoscopically. Peritoneal lavage reduces the incidence of postoperative abdominal adhesion formation.[41] Peritoneal lavage was not performed in the present study because the chief objective of this study was to report abnormalities that developed in the abdominal cavity in association with the laparoscopic technique. It is possible that the incidence of adhesion formation could have been decreased by implementing other strategies to minimize adhesion formation. For instance, instillation of carboxymethylcellulose in the peritoneal cavity is a commonly used method of preventing adhesion formation[42] that could have been performed at the conclusion of each laparoscopic procedure.

The ELS cartridges used for collection of biopsy specimens contained 4 staggered rows of titanium staples with a 4.1-mm leg length. This results in placement of 2 staggered rows of staples on each side of the transected tissue. The 4.1-mm staple compresses tissue to a maximum thickness of 2.0 mm. The usefulness of an ELS may be limited when the device is applied to excessively thickened or edematous tissue. In such instances, the security of the closure may be inadequate and leakage at the biopsy site may occur. Reinforcement of the staple line by placement of inverting suture patterns has been recommended in situations in which the linear stapler does not discharge easily or difficulty is encountered during application of the stapling instrument.[16] In such instances, the staple line primarily serves to reduce contamination and the inverting suture pattern serves as the primary closure. During collection of a biopsy specimen at the distal portion of the jejunum in 1 horse, the jaws of the endoscopic stapling device failed to engage correctly and activate the staples through the entire thickness of the intestinal wall; therefore, the staple line was oversewn laparoscopically for reinforcement. Use of the endoscopic stapling device beyond the number of times recommended by the manufacturer may have led to this problem. In clinical horses, excessively thickened and edematous small intestine may limit collection of a full-thickness biopsy specimen by use of automatic endoscopic stapling devices and there is risk of leakage at the biopsy site. However, the described laparoscopic technique can be adapted to clinical situations in which collection of small intestinal biopsy specimens can be performed in 2 stages, with staples first placed with the ELS and primarily functioning to reduce the risk of peritoneal contamination, followed by oversewing with an endoscopic suturing device.[p] Staging collection of the full-thickness biopsy specimen may make the technique more suitable for surgeons with basic laparoscopic training. In addition, the versatility of the articulating head of the ELS enables the surgeon to adapt the size of the biopsy specimen to the luminal diameter in a given horse and avoid excessive luminal reduction. Although this laparoscopic technique is likely to be adaptable for collection of full-thickness intestinal biopsy specimens, further research is required before the technique can be recommended for clinical use in horses.

An inflammatory response was observed in the abdominal fluid of all horses up to postoperative day 8. Signs of systemic inflammation or infection were not detected in any horses after collection of the biopsy specimens, and no other factors were detected in association with the laparoscopic technique that could explain the inflammatory response. In earlier studies in horses, an increase in WBC count and total protein concentration in abdominal fluid was detected 24 hours after exploratory laparoscopy[18] and 6 days prior to a second-look laparoscopic procedure.[21] In both studies, it was postulated that the inflammatory response may have been caused by peritoneal irritation from carbon dioxide insufflation. To the authors' knowledge, long-term studies of postlaparoscopy peritoneal fluid changes have not been performed in horses. In the present study, the WBC count in abdominocentesis fluid collected preoperatively before the first laparoscopy was significantly higher, compared with values before the second laparoscopy (after a 1-month washout period). Although the preoperative abdominal fluid WBC count for the second laparoscopic procedure was within the reference range, our results suggest that peritoneal fluid changes evident after laparoscopy may take longer than 1 month to decrease to baseline values. These peritoneal fluid changes should be taken into consideration if abdominocentesis is performed in horses that have undergone exploratory laparoscopy.

Results of the present study indicated that laparoscopic collection of serial full-thickness small intestinal biopsy specimens by use of a 45-mm ELS is a safe technique in healthy experimental horses. However, the low bursting pressures and bursting wall tensions observed with use of endoscopic staplers are a potential source of concern because intraluminal pressures reached in clinical situations may be higher than pressures that staples placed with an ELS will withstand. Although results indicated that laparoscopic collection of serial full-thickness small intestinal biopsy specimens is safe and effective, this technique was only performed in healthy experimental horses. Oversewing the staple line laparoscopically with an auto-suturing device may be required in clinical horses. Further investigation is needed before this technique can be recommended for clinical use.

a. Biosyn, Auto Suture Co, Ville St-Laurent, QC, Canada.
b. Long 45A Endocutter ETS-Flex45, ENDOPATH, Ethicon Endo-Surgery Inc, Cincinnati, Ohio.
c. Reloads 4.1-mm ETS45, ENDOPATH, Ethicon Endo-Surgery Inc, Cincinnati, Ohio.
d. Magic Wrap, Glasgow Manufacturing, London, ON, Canada.

e. SENSOTEC BP211BN Model LM, 50 PSIG, SENSOTEC Inc, Columbus, Ohio.
f. A-Tech S7DC, A-Tech instruments Ltd, Scarborough, ON, Canada.
g. National Instruments PCI-6023E, National Instruments, Austin, Tex.
h. LabVIEW, version 7.1, National Instruments, Austin, Tex.
i. Model No. Sw-1K, Transducer technologies, Rio Nedo, Temecula, Calif.
j. Image J, version 1.37, Research Services Branch, National Institutes of Health, Bethesda, Md.
k. Electronic syringe infusion pump, Model AS40A, Baxter Healthcare Corp, Deerfield, Ill.
l. 512X Endopath disposable surgical trocar, Ethicon Endo-Surgery Inc, Cincinnati, Ohio.
m. Endopath Disposable 10-mm Babcock Forceps, Ethicon Endo-Surgery Inc, Cincinnati, Ohio.
n. Prolene, Ethicon Inc, Somerville, NJ.
o. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.
p. Endo Stitch suturing device, Auto Suture Co, Ville St-Laurent, QC, Canada.
q. Polysorb Endo Stitch disposable loading unit, Auto Suture Co, Ville St-Laurent, QC, Canada.

References

1. Koenig JB, Sawhney S, Cote N, et al. Effect of intraluminal distention or ischemic strangulation obstruction of the equine jejunum on jejunum motilin receptors and binding of erythromycin lactobionate. *Am J Vet Res* 2006;67:815–820.
2. Little D, Tomlinson JE, Blikslager AT. Post operative neutrophilic inflammation in equine small intestine after manipulation and ischaemia. *Equine Vet J* 2005;37:329–335.
3. Prichard M, Ducharme NG, Wilkins PA, et al. Xanthine oxidase formation during experimental ischemia of the equine small intestine. *Can J Vet Res* 1991;55:310–314.
4. Vatistas NJ, Snyder JR, Nieto J, et al. Morphologic changes and xanthine oxidase activity in the equine jejunum during low flow ischemia and reperfusion. *Am J Vet Res* 1998;59:772–776.
5. Schramme M. Investigation and management of recurrent colic in the horse. *Equine Pract* 1995;17:303–313.
6. Schumacher J, Edwards JF, Cohen ND. Chronic idiopathic inflammatory bowel diseases of the horse. *J Vet Intern Med* 2000;14:258–265.
7. Murray MJ, Smith BP. Diseases of the alimentary tract. In: Smith BP, ed. *Large animal internal medicine*. 3rd ed. St Louis: Mosby, 2002;593–789.
8. Richardson D. Intestinal surgery: a review. *Compend Contin Educ Pract Vet* 1981;3:259–271.
9. Traub-Dargatz JL. Laparoscopy, endoscopy, and surgical biopsy. In: Anderson NV, ed. *Veterinary gastroenterology*. Philadelphia: Lea & Febiger, 1992;61–85.
10. Kobluk CN, Ducharme NG, Lumsden JH, et al. Factors affecting incisional complication rates associated with colic surgery in horses: 78 cases (1983–1985). *J Am Vet Med Assoc* 1989;195:639–642.
11. Mair TS, Smith LJ. Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 2: short-term complications. *Equine Vet J* 2005;37:303–309.
12. Greig JD, Miles WFA, Nixon SJ. Laparoscopic technique for small bowel biopsy. *Br J Surg* 1995;82:360–364.
13. Mazziotti MV, Langer JC. Laparoscopic full-thickness intestinal biopsies in children. *J Pediatr Gastroenterol Nutr* 2001;33:54–57.
14. Baxter GM, Hunt RJ, Tyler DE, et al. Sutured end-to-end and stapled side-to-side jejunal anastomoses in the horse. *Vet Surg* 1992;21:47–55.
15. Doran RE, Allen D. The use of stapling devices in equine gastrointestinal surgery. *Compend Contin Educ Pract Vet* 1987;9:854–860.
16. Mueller POE, Allen D. Instrumentation and techniques in equine gastrointestinal surgery. *Vet Clin North Am Equine Pract* 1996;12:207–233.
17. Walmsley JP. Review of equine laparoscopy and an analysis of 158 laparoscopies in the horse. *Equine Vet J* 1999;31:456–464.
18. Fisher AT, Lloyd KC, Carlson GP, et al. Diagnostic laparoscopy in the horse. *J Am Vet Med Assoc* 1986;189:289–292.
19. Fisher AT. Standing laparoscopic surgery. *Vet Clin North Am Equine Pract* 1991;7:641–647.
20. Schambourg MA, Marcoux M. Laparoscopic intestinal biopsies in the standing horse, in *Proceedings*. 14th Annu Sci Meet Eur Coll Vet Surg 2005;179–181.
21. Schambourg MM, Marcoux M. Laparoscopic intestinal exploration and full-thickness intestinal biopsy in standing horses: a pilot study. *Vet Surg* 2006;35:689–696.
22. Bickers RJ, Blackford JT, Eiler H, et al. A comparison of the mechanical strength of two stapled anastomosis techniques for equine small intestine. *Vet Surg* 2002;31:104–110.
23. Kokudo Y, Furuya I, Takeyoshi K, et al. Comparison of University of Wisconsin, Euro-Collins, and lactated Ringer's solutions in rat small bowel preservation for orthotopic small bowel transplantation. *Transplant Proc* 1994;26:1492–1493.
24. Zang S, Kokudo Y, Nemoto EM, et al. Biochemical evidence of mucosal damage of intestinal grafts during cold preservation in University of Wisconsin, Euro-Collins, and lactated Ringer's solutions. *Transplant Proc* 1994;26:1494–1495.
25. Eggleston RB, Mueller EPO, Parviainen AK, et al. Effect of carboxymethylcellulose and hyaluronate solutions on jejunal healing in horses. *Am J Vet Res* 2004;65:637–643.
26. Christensen H, Langfelt S, Laurberg S. Bursting strength of experimental colonic anastomoses: a methodological study. *Eur Surg Res* 1993;25:38–45.
27. 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.
28. Dabareiner RM, White NA, Donaldson LL. Effects of intraluminal distention and decompresion on microvascular permeability and hemodynamics of the equine jejunum. *Am J Vet Res* 2001;62:225–236.
29. Hendriks T, Mastboom WJB. Healing of experimental intestinal anastomoses. *Dis Colon Rectum* 1990;33:891–901.
30. Allen D, White NA, Tyler DE. Factors for prognostic use in equine obstructive small intestinal disease. *J Am Vet Med Assoc* 1986;189:777–780.
31. MacHarg MA, Adams SB, Lamar CH, et al. Electromyographic, myomechanical, and intraluminal pressure changes associated with acute extraluminal obstruction of the jejunum in conscious ponies. *Am J Vet Res* 1986;47:7–11.
32. Ballantyne GH, Burke JB, Rogers G, et al. Accelerated wound healing with stapled enteric suture lines. *Ann Surg* 1985;201:360–364.
33. Pascoe JR, Peterson PR. Intestinal healing and methods of anastomosis. *Vet Clin North Am Equine Pract* 1989;5:309–333.
34. Sullins K. Surgical stapling devices and their applications. In: White NA, Moore JN, eds. *Current practice of equine surgery*. Philadelphia: JB Lippincott, 1990;6–12.
35. Ikeuchi D, Onodera H, Aung T, et al. Correlation of tensile strength with bursting pressure in the evaluation of intestinal anastomosis. *Dig Surg* 1998;16:478–485.
36. Fellows NM, Burge J, Hatch CS, et al. Suture strength and healing strength of end-to-end intestinal anastomoses. *Surg Forum* 1951;111–117.
37. Reinertson E. Comparison of three techniques for intestinal anastomosis in Equidae. *J Am Vet Med Assoc* 1976;169:208–212.
38. Mansell J, Willard MD. Biopsy of the gastrointestinal tract. *Vet Clin North Am Small Anim Pract* 2003;33:1099–1116.
39. Moore RG, Kavoussi LR, Bloom DA, et al. Postoperative adhesion formation after urological laparoscopy in the pediatric population. *J Urol* 1995;153:792–795.
40. Schippers E, Tittel A, Ottinger A, et al. Laparoscopy versus laparotomy: comparison of adhesion-formation after bowel resection in a canine model. *Dig Surg* 1998;15:145–147.
41. Hague BA, Honnas CM, Berridge BR, et al. Evaluation of postoperative peritoneal lavage in standing horses for prevention of experimentally induced abdominal adhesions. *Vet Surg* 1998;27:122–126.
42. Murphy DJ, Peck LS, Detrisac CJ, et al. Use of a high-molecular-weight carboxymethylcellulose in a tissue protective solution for prevention of postoperative abdominal adhesions in ponies. *Am J Vet Res* 2002;63:1448–1454.