Comparison of histomorphometric characteristics of dorsal colon and pelvic flexure biopsy specimens obtained from horses with large colon volvulus that underwent resection

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OBJECTIVE
To determine the degree of histomorphometric damage in dorsal colon and pelvic flexure biopsy specimens (DCBSs and PFBSs, respectively) obtained from horses with large colon volvulus (LCV) and assess the accuracy of predicting short-term outcome for those horses on the basis of DCBS or PFBS characteristics.

ANIMALS
18 horses with ≥ 360° LCV that underwent large colon resection.

PROCEDURES
During surgery, biopsy specimens from the dorsal colon resection site and the pelvic flexure (when available) were collected from each horse. Interstitial-to-crypt (I:C) ratio (ratio of the lamina propria space occupied by the interstitium to that occupied by crypts), hemorrhage within the lamina propria (mucosal hemorrhage score [MHS] from 0 to 4), and percentage losses of glandular and luminal epithelium were determined in paired biopsy specimens and compared to determine optimal cutoff values for calculating the accuracy of DCBS and PFBS characteristics to predict short-term outcome (survival or nonsurvival after recovery from surgery).

RESULTS
Paired biopsy specimens were obtained from 17 of the 18 horses. The I:C ratio and percentage glandular epithelial loss differed between DCBSs and PFBSs. For DCBSs, an I:C ratio ≥ 0.9 and MHS ≥ 3 each predicted patient nonsurvival with 77.8% accuracy. For PFBSs, an I:C ratio ≥ 1 and MHS ≥ 3 predicted patient nonsurvival with 70.6% and 82.4% accuracy, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Although different, histomorphometric measurements for either DCBSs or PFBSs could be used to accurately predict short-term outcome for horses with LCV that underwent large colon resection, and arguably PFBSs are easier to collect. (*Am J Vet Res* 2020;81:899–903)

Large colon volvulus is a severe form of intestinal strangulation that has been associated with poor survival rate in horses.[1–4] Death is inevitable without surgical intervention. Injury to the colonic tissue is the result of vascular occlusion associated with the twisting of the colon around its mesenteric axis that commonly occurs near the base of the cecum. This causes obstruction of blood flow immediately distal to the site of strangulation. Delivery of oxygenated blood to the ascending colon of horses is derived from the arterial loop created by the colic branch of the ileocolic artery and the right colic artery. Within the ascending colon, the PF is anatomically farther away from its principal blood supply than is the DC. Therefore, it may be surmised that a gradient of increased injury would be present at sites more distant to the site of strangulation, particularly the PF. Although histomorphometric evaluations of PFBSs used to predict short-term outcome have indicated that PFBSs are representative of the entire affected portion of the colon,[5–7] the optimal biopsy location continues to be debated.[6] Therefore, the objective of the study reported here was to determine whether the degree of histomorphometric damage in DCBSs and PFBSs from horses with LCV differed and whether assessment of measurements of a DCBS from adjacent to the site of colonic resection more accurately predicts short-term outcome, compared with assessment of measurements of a PFBS, for those horses.

ABBREVIATIONS

DC	Dorsal colon
DCBS	Dorsal colon biopsy specimen
I:C	Interstitial-to-crypt
LCV	Large colon volvulus
MHS	Mucosal hemorrhage score
PF	Pelvic flexure
PFBS	Pelvic flexure biopsy specimen
ROC	Receiver operating characteristic

Materials and Methods

Study population and inclusion criteria

Medical records for 2006 through 2009 were obtained from Peterson and Smith Equine Hospital in Ocala, Fla. Horses were selected for inclusion in the study if they had an LCV of ≥ 360°, if an intraoperative biopsy specimen of the DC at the site of colonic resection had been obtained, and if the horse had recovered from surgery. In most cases, a paired PFBS was also collected during surgery. Mucosal biopsies were performed with the consent of the owners to provide clinical evaluation of resected tissue.

Surgical and perioperative management

On admission to the hospital, physical examination findings and preoperative hematologic values (PCV, serum total protein concentration, and WBC count) were recorded for each horse. Each horse was anesthetized and underwent routine ventral midline exploratory celiotomy. After the LCV was manually corrected, serosal color, wall thickness, friability, and motility of the colon; appearance of the mesentery; and presence of a detectable colonic arterial pulse were used to make a clinical determination as to the viability of the large colon.[2] A PF enterotomy was variably performed on the basis of surgeon preference. For horses in which the colon was clinically deemed nonviable, the client was consulted and given the option of a large colon resection. When performed, the large colon was resected at the most proximal and most readily exteriorizable extent with an end-to-end technique described by Hughes et al.[8] Intraoperative abdominal drain placement and postoperative abdominal lavage after large colon resection were performed in all cases. After surgery, all horses were administered gentamicin (6.6 mg/kg, IV, q 24 h) and penicillin G potassium (22,000 U/kg, IV, q 6 h) for a minimum of 5 days and flunixin meglumine (1.1 mg/kg, IV, q 12 h, or 0.5 mg/kg, IV, q 8 h) for a minimum of 3 days. Additional antimicrobial and anti-inflammatory treatments were administered on the basis of clinical assessment and hematologic analysis results. Depending on the degree of endotoxemia or hypoproteinemia, some horses received polymyxin B (6,000 U/kg, IV, q 8 to 12 h), plasma,[a] or both. Other medications used on a case-by-case basis included metronidazole (15 mg/kg, PO, q 8 h) and lidocaine hydrochloride (loading dose [1.3 mg/kg, IV], followed by a constant rate infusion [0.05 mg/kg/min, IV]).

Specimen preparation

All mucosal biopsies were performed at the time of resection and anastomosis. The DCBSs were obtained from the edge of the resected colon. All specimens were immediately placed in neutral-buffered 10% formalin. The tissues were routinely processed and sectioned, stained with H&E stain, archived, and then independently evaluated by 2 investigators (LMG and CAF) who were blinded to individual case details (horse identity and outcome) and type of biopsy specimen.

Histomorphometric measurements

Light microscopy (with 10X and 40X objective lenses) was used to evaluate sections of the DCBSs and PFBSs; at least 4 fields were examined for each specimen. The I:C ratio was defined as the ratio of the measured lamina propria space occupied by the interstitium to the measured lamina propria space occupied by the crypts. An MHS for the lamina propria of the specimen was assigned a score from 0 to 4 (0, no hemorrhage; 1, few individual RBCs within the lamina propria; 2, large number of individual RBCs within the lamina propria; 3, appearance of clumps of RBCs within the lamina propria; and 4, confluent RBCs obscuring the demarcation of the lamina propria).[7] In PFBSs, histomorphometric measurements associated with short-term outcome were based on established criteria (ie, I:C ratio ≥ 1 and hemorrhage score ≥ 3) but were recalculated for this population of animals to confirm reproducibility and comparison with values obtained from the DCBSs.[7,9,10] The percentage loss of luminal epithelium was based on visual estimation of the percentage of luminal epithelium that was separated from the basement membrane.[9] The percentage of glandular epithelial loss was calculated by measurement of the distance from the crypt base to the point of glandular epithelial loss or separation from the basement membrane divided by the total length of the crypt.[9,10]

Data analysis

All statistical analyses were performed with statistical software.[b] A Wilcoxon matched-pairs signed rank test was used to compare paired DCBS and PFBS histomorphometric data by horse. Short-term outcome was classified as survival (ie, the horse recovered from surgery and survived to discharge from the hospital) and nonsurvival (ie, the horse recovered from surgery but died before discharge from the hospital). An ROC curve analysis was used to determine the optimal cutoff value for short-term outcome for the selected test variables. The optimal cutoff value was determined by use of an equation as follows:

$$d = (1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$$

and the cutoff value that corresponded to the smallest value of d was chosen. The optimal cutoff value was then used to determine the accuracy of that variable with regard to outcome prediction. The accuracy of each histomorphometric variable was determined by the sum of the number of true-positive and true-negative results divided by the sum of the true-positive, false-positive, false-negative, and true-negative results. A value of $P \leq 0.05$ was considered significant.

Results

Eighteen horses met the inclusion criteria. The age of 1 horse was unknown, and the ages of the other 17 horses ranged from 4 months to 16 years (median age, 8 years). There were 15 mares and 3 geldings. Three breeds were represented (13 Thoroughbreds, 4 warmbloods, and 1 Paso Fino). Paired PFBSs and DCBSs were available for 17 of the 18 horses. All 18 horses underwent colonic resection and anastomosis, and all recovered from surgery. Fourteen of 18 horses survived until discharge from the hospital.

Histomorphometric variables from 17 paired DCBSs and PFBSs were compared. The I:C ratio for DCBSs (median, 0.835; minimum, 0.510; maximum, 1.67) and PFBSs (median, 0.998; minimum, 0.693; maximum, 1.91) differed significantly ($P = 0.006$). The percentage glandular epithelial loss for DCBSs (median, 34.2%; minimum, 19.9%; maximum, 67.3) and PFBSs (median, 13.29%; minimum, 0.00%; maximum, 52.46%) also differed significantly ($P < 0.001$). The MHS for DCBSs (median, 2.38; minimum, 0.25; maximum, 4.00) and PFBSs (median, 1.88; minimum, 0.25; maximum, 3.88) did not differ significantly ($P = 0.8$). There was no significant ($P = 0.3$) difference with regard to percentage luminal epithelial loss between DCBSs (median, 3.63; minimum, 1.00; maximum, 4.00) and PFBSs (median, 3.38; minimum, 1.00; maximum, 4.00).

The ROC curve analysis yielded a cutoff measurement for DCBS I:C ratio of 0.9; values ≥ predicted nonsurvival with a sensitivity and specificity of 75% and 71%, respectively ($P = 0.09$; **Table 1**). Mucosal hemorrhage score for DCBSs was determined to have a cutoff measurement of 3; values ≥ 3 predicted nonsurvival with a sensitivity and specificity of 100% and 86%, respectively ($P = 0.03$). The cutoff measurements determined for DCBS percentage glandular epithelial loss and percentage luminal epithelial loss were not significant and not associated with prediction of short-term outcome. Of the variables evaluated in PFBSs, the only cutoff measurements that could be used to predict short-term outcome were I:C ratio and MHS.

On the basis of the DCBS I:C ratio, 6 horses were predicted to not survive; among those 6 horses, nonsurvival was accurately predicted for 3 horses. Among the 12 horses predicted to survive on the basis of the DCBS I:C ratio, survival to hospital discharge was ac-

Table 1—Results of ROC curve analysis of histomorphometric measurements of DCBSs and PFBSs obtained from horses with LCV that underwent resection.

Variable	Biopsy specimen	AUC (SE)	95% CI	*P* value*	Short-term outcome: Survival (No. of horses)	Short-term outcome: Nonsurvival (No. of horses)	Cutoff value	Sensitivity (% [95% CI])	Specificity (% [95% CI])
I:C ratio	DC	0.8 (0.1)	0.5–1.0	0.09	14	4	0.9	75 (19.4–99.4)	71 (41.9–91.6)
	PF	0.8 (0.1)	0.6–1.0	0.05*	13	4	1.0	75 (9.4–99.4)	77 (46.2–95.0)
MHS	DC	0.9 (0.09)	0.7–1.0	0.03*	14	4	3.0	100 (39.8–100.0)	86 (57.2–98.2)
	PF	0.9 (0.07)	0.8–1.0	0.01*	13	4	3.1	100 (39.8–100.0)	84 54.6–98.1)
Percentage glandular epithelial loss	DC	0.7 (0.1)	0.4–0.9	0.3	14	4	35.4	75 (19.4–99.4)	57 (28.9–82.3)
	PF	0.5 (0.2)	0.2–0.8	0.9	13	4	26.0	100 (39.8–100.0)	31 (9.1–61.4)
Percentage luminal epithelial loss	DC	0.7 (0.1)	0.5–09	0.2	14	4	3.2	100 (39.8–100.0)	57 (28.9–82.3)
	PF	0.8 (0.1)	0.5–0.9	0.1	13	4	3.3	100 (39.8–100.0)	62 (31.6–86.1)

AUC = Area under the curve.

Eighteen horses with LCV underwent resection; during surgery, biopsy specimens from the DC resection site (18 horses) and the PF (17 horses) were collected. Interstitial-to-crypt ratio (ratio of the lamina propria space occupied by the interstitium to that occupied by crypts), hemorrhage within the lamina propria (MHS from 0 to 4), and percentage losses of glandular and luminal epithelium were determined in paired biopsy specimens and compared. An ROC curve analysis was performed to determine the optimal cutoff values of those DCBS and PFBS characteristics for prediction of the horses' short-term outcome (survival or nonsurvival after recovery from surgery). For each variable, values greater than or equal to the cutoff value were used to establish a prediction of nonsurvival.

*A *P* value ≤ 0.05 indicates that a given cutoff value can be used to correctly distinguish between horses that will or will not survive following resection.

Table 2—Accuracy of ROC curve–determined cutoff values of MHS and I:C ratio in Table 1 for prediction of short-term outcome (survival or nonsurvival after recovery from surgery) among horses with LCV that underwent resection and recovered from surgery.

Histomorphometric measurement	Biopsy location	PPV	NPV	Accuracy (%)
MHS	PF	57.1	100.0	82.4
	DC	50.0	100.0	77.8
I:C ratio	PF	42.9	90.0	70.6
	DC	50.0	91.7	77.8

NPV = Negative predictive value. PPV = Positive predictive value.

For each variable, values greater than or equal to the cutoff value were used to establish a prediction of nonsurvival.

curately predicted for 11 horses. The accuracy of the DCBS I:C ratio to predict short-term outcome was 77.8% **(Table 2)**. On the basis of the PFBS I:C ratio, 7 horses were predicted to not survive; among those 7 horses, nonsurvival was accurately predicted for 3 horses. Among the 10 horses predicted to survive on the basis of the PFBS I:C ratio, survival to hospital discharge was accurately predicted for 9 horses. The accuracy of the PFBS I:C ratio to predict short-term outcome was 70.6%.

On the basis of the DCBS MHS, 8 horses were predicted to not survive; among those 8 horses, nonsurvival was accurately predicted for 4 horses. Among the 10 horses predicted to survive on the basis of DCBS MHS, survival to hospital discharge was accurately predicted for all 10 horses. The accuracy of the DCBS MHS to predict nonsurvival was 77.8% (Table 2). On the basis of the PFBS MHS, 7 horses were predicted to not survive; among those 7 horses, nonsurvival was accurately predicted for 4 horses. Among the 10 horses predicted to survive on the basis of the PFBS MHS, survival to hospital discharge was accurately predicted for all 10 horses. The accuracy of the PFBS MHS to predict nonsurvival was 82.4%.

Discussion

To the authors' knowledge, this was the first study to evaluate paired DCBSs and PFBSs obtained from horses with LCV that underwent resection. The findings from the present study indicated that there are histomorphometric differences between biopsy specimens obtained from the 2 sites. However, these differences did not appear to impact the predictive accuracy of use of some histomorphometric variables to predict short-term outcome. Short-term outcome for horses with LCV that underwent resection was predicted with similar accuracy from PFBS and DCBS histomorphometric data.

Histopathologic and histomorphometric changes in PFBSs have been successfully used to predict outcome in equine cases of LCV.[7,10] Research has previously indicated that injury to the colon associated with LCV is uniformly distributed along the entire length of the involved intestine.[5] However, this finding appears counterintuitive given the spatial relationship between the sources of blood supply to the colon, the colonic site most commonly twisted during LCV, and the PF biopsy location. The most common location for the colonic twist in horses with LCV is near the base of the cecum, relatively near the origins of the colic branch of the ileocolic artery and the right colic artery.[c] Anatomically, the PF is the site most distant from these arterial sources and therefore would be expected to be the colonic location most susceptible to decreases in oxygenated blood supply, assuming there is not a complete occlusion of arterial blood flow at the onset of strangulation. Microscopically, a similar process occurs in the small intestine following an ischemic event, wherein a gradient of injury severity develops owing to a gradient of oxygen availability that exists between areas farthest from and areas closest to the oxygenated blood supply.[11] As a result, epithelial loss begins nearest the villus tip and extends toward the crypt base as the duration of ischemia increases.[11] The findings of the present report indicated that there may be differences in the degree of injury between DCBSs and PFBSs. However, in the LCV cases evaluated, histomorphometric data from specimens collected from both biopsy sites were similarly useful for prediction of the horses' short-term outcome.

A limitation of the present study was an absence of data regarding the interval between onset of LCV and referral of the horses. It could be argued that the findings may differ depending on the duration of colic and that in the most severe cases, one would expect little histopathologic difference between the PF and DC biopsy sites as the colon becomes irreversibly devitalized. Nonetheless, with regard to the present study, the referral practice where the biopsies were performed was generally considered to have a referral time that was representative of most referral practices, unlike a particular practice that has a very rapid referral time.[12] An additional limitation of the present study was that all horses underwent large colon resection and anastomosis. Therefore, the histomorphometric findings for these horses may not represent findings for horses in which colonic resection was not deemed necessary by the surgeon. Currently, the decision to perform large colon resection is subjective; therefore, some horses that underwent resection may have survived with manual correction of the LCV alone. This study was limited to a subset of horses with LCV that underwent DC resection owing to the accessibility of the site; for horses in which the colon was not resected, an additional enterotomy site would have had to be created for collection of a biopsy specimen, thereby increasing the potential for complications.

The PFBS MHS is a known predictor of nonsurvival in horses with LCV.[7] Owing to a difference in respective vessel wall thickness, venous return is commonly obstructed prior to obstruction of the arterial supply of blood. This results in the pooling of RBCs within the intestinal wall that is detectable during surgery as gross discoloration (red to purple) and thickening of the colon wall. This form of LCV, involving hemorrhagic strangulating obstruction, is more common than ischemic strangulating obstruction. All horses in the present study had characteristics of the hemorrhagic form of LCV. In support of previous findings,[7] the results of the present study indicated that an MHS ≥ 3 for either a DCBS or PFBS was associated with nonsurvival. However, the sensitivity and calculated accuracy of MHS determined from the ROC curve analysis data have suggested that this variable can be used with more confidence to predict which horses with LCV that undergo resection will survive to discharge from the hospital. Given the evidence obtained in the present study, the usefulness of certain histomorphometric data from PFBSs or DCBSs to predict short-term outcome in cases of LCV appears comparable. However, the small sample size of the population evaluated did influence the power of statistical analyses and may have affected detection of statistical differences. Despite this limitation, PF biopsies can be readily performed in horses with LCV regardless of whether resection is elected.

The usefulness of histologic evaluation of biopsy specimens is undermined by the delay between acquisition of biopsy specimens and provision of assessment results. Although histologic evaluation of frozen sections of biopsy specimens can be accomplished at the time of surgery, this is uncommonly performed, especially within a private practice setting. More commonly, fixed and embedded colonic tissue sections are examined, and results are provided within 48 hours after biopsy specimen submission. Although delayed, the information provided by histologic evaluation of biopsy specimens can aid in case management. The additional information obtained from biopsy specimens can help direct continued postoperative treatment. Furthermore, in cases with a high likelihood of nonsurvival, the decision by an owner to euthanize a horse would be supported with additional evidence, thereby decreasing hospitalization time and the associated expense.

Presently, intraoperative decision-making during the treatment of horses with LCV is largely based on a surgeon's assessment of gross estimates of colonic viability. Further research should aim to refine the use of the MHS through development of a table-side test that can more accurately quantify discoloration of colonic tissue, thereby providing guidance for intraoperative decisions, such as the necessity of resection, and potentially improving the likelihood of survival among horses with LCV.

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Footnotes

a. Immuno-glo, MG Biologics, Ames, Iowa.
b. GraphPad Prism Software, version 8.4.3, La Jolla, Calif.
c. Emberson RM, Cook G, Hance SR, et al. Large colon volvulus: surgical treatment of 204 horses (1986–1995)(abstr), in *Proceedings*. 42nd Annu Meet Am Assoc Equine Pract 1996;254–255.

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