Flowmetry and spectrophotometry can detect reduced intestinal microperfusion in nonsurvivors during equine colic surgery for large intestinal strangulation

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OBJECTIVE
To evaluate the use of laser Doppler flowmetry and spectrophotometry (LDFS) for large intestinal viability assessment in horses with naturally occurring large intestinal strangulations.

METHODS
By use of LDFS, intestinal microperfusion was quantified as tissue oxygen saturation (tSO₂), hemoglobin (tHB), and blood flow (tBF) in cases with large colon volvulus and small colon strangulations undergoing colic surgery (n = 17). Intestinal biopsies were taken from the pelvic flexure in all large colon cases and in small colon cases that underwent intraoperative euthanasia. Measurements were compared between survivors and nonsurvivors, and the correlation between LDFS and (immuno)histology was tested (P < .05).

RESULTS
The tSO₂ and tBF were clearly lower and tHB was higher than previously reported in healthy horses. Following correction of the lesion, pelvic flexure tBF was significantly lower than that of the left ventral colon. Prior to correction of the lesion, microperfusion did not differ between survivors and nonsurvivors, but following release of the strangulation the survivors had a significantly higher tSO₂ and tBF compared to the nonsurvivors. There was a negative correlation between tBF and interstitium-to-crypt ratio and a positive correlation between tHB and the histological hemorrhage score. There were no significant correlations between LDFS measurements and inflammatory cell counts or hypoxia-inducible factor-1α immunoreactivity.

CONCLUSIONS
Large intestinal microperfusion was decreased in nonsurvivors compared to survivors and was correlated with histological injury, suggesting that LDFS has the potential to predict tissue injury and postoperative survival.

CLINICAL RELEVANCE
The use of LDFS as an ancillary diagnostic aid may improve intraoperative viability assessment during colic surgery.

Keywords: horse, intestine, viability, volvulus, ischemia
on demand. Diagnostic methods quantifying blood flow such as surface oximetry and fluorescein fluorescence have been applied in the equine intestine yet were found to have a low sensitivity to identify nonviable intestine.\textsuperscript{1,6} In a more recent study,\textsuperscript{1,8} the use of laser Doppler flowmetry combined with white light spectrophotometry (LDFS) has been investigated in naturally occurring small intestinal strangulations, showing that lower microperfusion values coincided with more severe histological injury and a higher occurrence of postoperative ileus in horses.

The aim of the current study was to determine if LDFS can be applied as an ancillary diagnostic aid for the assessment of large intestinal viability. The first objective was to document the microperfusion in naturally occurring strangulating lesions of the large intestine during colic surgery. We hypothesized that this would yield lower values than reported in healthy horses and that the microperfusion would be homogenous throughout the large colon. The second objective was to compare the microperfusion between survivors and nonsurvivors, hypothesizing that survivors would have better microperfusion values than nonsurvivors. The last objective was to evaluate the correlation between LDFS and histology, and we hypothesized that microperfusion measured by LDFS would correlate with tissue injury.

**Methods**

**Animals**

An a priori power analysis (G-Power Version 3.1.9.6; Heinrich Heine Universität) yielded a sample size of 5 horses per group to detect a difference in tissue oxygen saturation (tSO\textsubscript{2}) of 30% between groups with an SD of 10% with a power of 0.8 and an α of 0.05. The ethical approval for this prospective clinical trial was obtained from the animal welfare officer of the University of Veterinary Medicine Hannover, Foundation, Germany, and the ethics committee of the responsible German federal state authority in accordance with the German Animal Welfare Law (Lower Saxony State Office for Consumer Protection and Food Safety [LAVES]; reference No. 33.8-42502-05-19A452). Client-owned horses undergoing a laparotomy for acute colic were enrolled in the study if a strangulating large intestinal lesion was diagnosed during colic surgery and if there was an opportunity to conduct the LDFS measurements. Cases were not included if any delay by performing measurements was considered unacceptable due to the situation in surgery or the anesthetic stability of the horse. Other reasons for exclusion were personnel constraints, technical issues, or that the strangulation had already been released before the measurement could be performed. Owner consent was obtained for all cases included in the study.

**Laparotomy and intraoperative measurements**

A routine ventral midline laparotomy was performed in dorsal recumbency under general anesthesia. Anesthesia was performed in accordance with the standardized hospital protocol. In brief, horses received xylazine to effect (0.5 to 0.8 mg/kg) and 50 μm/kg ketamine to induce general anesthesia. Following orotracheal intubation, anesthesia was maintained with isoflurane in 100% oxygen and a continuous rate infusion (CRI) of xylazine at 0.6 mg/kg/h. Lactated Ringer’s solution was started at 5 mL/kg/h and increased in increments of 5 mL/kg/h to maintain a mean arterial blood pressure (MAP) above 60 mm Hg. This was supplemented by a CRI of dobutamine at a rate of 0.3 μg/kg/min titrated to effect with increments of 0.3 μg/kg/min. If hypotension persisted despite these measures, a noradrenaline CRI of 0.1 mcg/kg/h was initiated and titrated to effect. Cardiovascular and respiratory variables including direct blood pressure measurement in the facial artery and arterial blood gases were monitored. The LDFS measurements (O2C with LF-2 probe; Lea Medizintechnik GmbH) were performed prior to reduction of the lesion (ischemia measurement) and following release of the strangulation (reperfusion measurement), on the antimesenteric side of the PF and left ventral colon (LVC) in the large colon volvulus cases. In the small colon cases, the measurement was performed in the most severely affected strangulated small colon segment. The measurements were recorded for a minimum of 30 seconds with 50 Hz, and the output summary with averaged values for each 2 seconds was used for further analysis. To exclude motion artifacts, only the lower 50% of the values of each measurement were used for the final analysis. The operating surgeons could not be blinded to the results but were instructed not to base any intraoperative decisions on the LDFS measurements. Intraoperative findings including diagnosis, intestinal color, manual estimation of wall thickness, location, and timing of measurement and treatment were documented in a standardized surgery report and by use of an additional short questionnaire. None of the horses included in the study underwent intestinal resection and anastomosis. Intraoperative euthanasia was elected in some cases based on the severity of the findings and the wishes of the horse owners and was not influenced by the results of the measurements. Intraoperative euthanasia was performed by IV injection of 90 mg/kg pentobarbital while the horse remained under general anesthesia. Any complications, survival to hospital discharge, and findings at postmortem examination were documented. Postoperative treatment included IV Ringer lactate, lidocaine continuous rate infusion (0.05 mg/kg/min), flunixin-meglumine (routinely 1.1 mg/kg, q 12 h, altered to 0.25 mg/kg, q 6 h, in colitis cases) tinzaparin (50 or 100 anti-Xa IU/kg), polymyxin (6,000 IU/kg, q 12 h to q 8 h, in cases with clinical signs of endotoxemia), dto-smectite (0.1 kg/100 kg bodyweight, q 24 h, per nasogastric tube), and systemic antibiotics (3 days of amoxicillin 10 mg/kg, q 12 h, and gentamicin 6.6 mg/kg, q 24 h). In cases with colitis, 1 fecal sample was collected at the onset
of clinical signs and tested for salmonellosis by bacterial culture and PCR.

**Histology**

In the large colon volvulus cases, a full-thickness biopsy was taken from the site of the PF enterotomy immediately following the enterotomy incision. In the cases with a strangulating lesion of the small colon, biopsies were only taken if the horse was euthanized intraoperatively. The biopsy was carefully rinsed in isotonic saline solution, fixed in 4% neutral-buffered formaldehyde solution for 24 to 36 hours, and then embedded in paraffin. Following routine processing, the sections were stained with hematoxylin and eosin. Two sections per sample were assessed using light microscopy (40X objective) by 1 observer who was blinded for the identity of the slides.

The mucosal damage was quantified by visually estimating the percentage of luminal epithelium separated from the basement membrane as previously described\(^5\): grade 0, epithelium intact; grade 1, 1 to 25%; grade 2, 25 to 50%; grade 3, 50 to 75%; and grade 4, 75 to 100%. The interstitium-to-crypt ratio (I/C) was measured in minimum of 5 correctly oriented crypts and averaged per sample. Furthermore, a hemorrhage score (HS) was used to grade the extent of hemorrhage in all intestinal layers individually: grade 0, no hemorrhage; grade 1, few extravascular individual erythrocytes; grade 2, increased number of individual extravascular erythrocytes; grade 3, moderate hemorrhage and erythrocyte clumping at a few sites; grade 4, multifocal severe clumping of erythrocytes, tissue structure may be affected but is still recognizable; and grade 5, massive hemorrhage obscuring the majority of normal tissue architecture.\(^3\) For the correlation analysis, the HS grades of the intestinal layers were added up as total HS.

To assess the number of inflammatory cells, immunohistochemical staining for cytosolic calprotectin was performed as described previously (monoclonal mouse anti-human myeloid/histiocyte antigen; clone MAC 387; cat. no. M0747; IgG concentration, 375 mg/L; Dako Deutschland GmbH).\(^9\) Positive cells were counted in 5 adjacent hpf (40X objective) in each intestinal layer. If more than 50 positive cells were present in each hpf, a count of 250 cells/5 hpf was noted for that intestinal layer. Furthermore, slides were stained for hypoxia-inducible factor-1α (HIF-1α) using a polyclonal rabbit anti-human HIF-1α antibody (cat. no. NB100-134; IgG concentration, 1.0 mg/mL; Novus Biologicals LLC).\(^10\) Enterocyte immunoreactivity was graded for staining intensity of the cytoplasm and the nucleus using the following score: grade 0, no staining; grade 1, weak staining (staining hardly visible); grade 2, mild staining (light brown); grade 3, moderate staining (medium brown); and grade 4, intense staining (dark brown).\(^10\) For each sample, the nucleus-to-cytoplasm ratio was calculated from the immunoreactivity score for further analysis.

**Data analysis**

Commercially available software was used for graph design and statistical analysis (Graphpad Prism 10.2.2; Graphpad Software Inc). \(P\) values lower than 0.05 were considered significant. To test for (log)normal distribution, Shapiro-Wilk’s test was performed. Normal distributed variables were expressed as mean ± SD, and the other variables as median (minimum – maximum). The histology scores were displayed as frequency distribution. The t\(\text{SO}_2\) showed a normal distribution, whereas tissue hemoglobin (tHB) and tissue blood flow (tBF) were not (log)normally distributed. The PF measurements of the LCV cases and measurements of the strangulated segment of the small colon cases were combined for comparison of the results between survivors and nonsurvivors. For this, the horses that were euthanized during surgery and postoperatively were combined into 1 group, and results were compared using a 2-sample \(\bar{t}\) test for the t\(\text{SO}_2\) and a Mann-Whitney \(U\) test for the tHB and tBF. The same statistical tests were used to compare the PF with the LVC measurements of the large colon volvulus cases. To test for correlation between different variables, the nonparametric Spearman correlation coefficient was calculated.

**Results**

**Case characteristics**

During the study period, 13 horses with a large colon volvulus and 4 horses with small colon strangulations due to a pedunculated lipoma were included (Figure 1). The mean age of the horses was 14 (± 8) years, and there were 9 mares and 8 geldings. The study included 10 warmbloods, 2 ponies, 2 Arabians, 36 horses undergoing abdominal surgery during study period (April 2020 – October 2022)

356 horses

165 cases with large intestinal disease

163 cases with small intestinal disease

28 cases with other diseases (ie, stomach rupture, peritonitis, ...)

4 cases with strangulation of descending colon by pedunculated lipoma

34 cases with large colon volvulus

127 nonstrangulating lesions

All 4 cases included

13 cases included

21 cases not included

3 horses euthanized intraoperatively

1 horse survived until discharge

5 horses euthanized postoperatively

4 horses euthanized postoperatively

4 horses euthanized

Figure 1—Flow diagram depicting the cases that were included in this prospective study investigating intestinal microperfusion in large intestinal strangulating lesions during colic surgery.
1 Friesian, 1 Quarter Horse, and 1 Thoroughbred. The duration of the colic signs could not be reliably determined. Of the horses with large colon volvulus, 5 horses were euthanized intraoperatively because of clinically apparent severe ischemic injury of the colon in 3 cases and intestinal rupture in 1, and 1 case was euthanized at the owner’s request despite the operating clinician judging the intestinal viability and prognosis to be good. This horse was excluded from the comparison between survivors and nonsurvivors. In the 8 horses with large colon volvulus where treatment was continued, the colon was repositioned and its contents emptied through a PF enterotomy. Four of 8 horses survived to discharge without major complications. The other 4 all developed severe colitis with endotoxemia refractory to intensive medical treatment and had to be euthanized between 2 and 14 days following surgery. Fecal microbiological examinations were negative for salmonellosis. Necropsy was not performed in any of the cases.

Three of the horses with strangulation of the small colon were euthanized during surgery due to severe compromise of the intestinal wall and the inability to resect all affected tissue. One horse with strangulation of the small colon underwent repositioning without resection and was discharged without any complications.

During anesthesia, all horses required dobutamine with a mean dose of 0.9 ± 0.4 μm/kg/min. Noradrenaline was administered to 5/8 horses that were euthanized intraoperatively, 2/4 horses euthanized postoperatively, and 2/5 survivors, with a mean dose of 0.2 ± 0.2 μm/kg/min.

**LDFS measurements**

Prior to and following correction of the lesion, tS\(\text{O}_2\) and tBF were lower than previously reported in healthy anesthetized horses (Table 1). The tHB was determined. Of the horses with large colon volvulus, 5 horses were euthanized intraoperatively because of clinically apparent severe ischemic injury of the colon in 3 cases and intestinal rupture in 1, and 1 case was excluded from the comparison between survivors and nonsurvivors. In the 8 horses with large colon volvulus where treatment was continued, the colon was repositioned and its contents emptied through a PF enterotomy. Four of 8 horses survived to discharge without major complications. The other 4 all developed severe colitis with endotoxemia refractory to intensive medical treatment and had to be euthanized between 2 and 14 days following surgery. Fecal microbiological examinations were negative for salmonellosis. Necropsy was not performed in any of the cases.

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**Table 1**—Intestinal microperfusion measured in the pelvic flexure in horses with large colon volvulus or central in the strangulated segment of small colon.

<table>
<thead>
<tr>
<th></th>
<th>tS(\text{O}_2) (%)</th>
<th>tHB (AU)</th>
<th>tBF (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n = 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>27 ± 18</td>
<td>76 (16 to 131)</td>
<td>36 (13 to 96)</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>57 ± 29</td>
<td>90 (39 to 196)</td>
<td>87 (34 to 323)</td>
</tr>
<tr>
<td>Delta t-R</td>
<td>28 ± 24</td>
<td>12 (~42 to 110)</td>
<td>36 (~35 to 296)</td>
</tr>
<tr>
<td>Large colon volvulus (n = 13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>28 ± 21</td>
<td>76 (31 to 108)</td>
<td>41 (13 to 109)</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>61 ± 30</td>
<td>93 (56 to 196)</td>
<td>87 (38 to 145)</td>
</tr>
<tr>
<td>Delta t-R</td>
<td>43 ± 34</td>
<td>22 (~17 to 110)</td>
<td>36 (~35 to 79)</td>
</tr>
<tr>
<td>Small colon strangulation by pedunculated lipoma (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>27 ± 10</td>
<td>81 (16 to 131)</td>
<td>27 (20 to 28)</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>46 ± 25</td>
<td>71 (39 to 99)</td>
<td>88 (34 to 323)</td>
</tr>
<tr>
<td>Delta t-R</td>
<td>19 ± 25</td>
<td>~5 (~42 to 23)</td>
<td>64 (6 to 296)</td>
</tr>
<tr>
<td>Pelvic flexure reference value*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>83 ± 12</td>
<td>66 ± 7</td>
<td>216 ± 67</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (minimum – maximum).

AU = Arbitrary units. I = Ischemia. R = Reperfusion. tBF = Tissue blood flow. tHB = Tissue hemoglobin; tS\(\text{O}_2\) = Tissue oxygen saturation.

*In anesthetized horses without gastrointestinal disease.11
in the muscularis and serosa. The total HS showed a significant strong positive correlation with tHB measurements during reperfusion ($r = 0.67$; CI, 0.22 to 0.88; $P = .008$).

The total median calprotectin cell count was 49 (range, 8 to 701) cells/5 hpf in cases euthanized intraoperatively, 143 (range, 4 to 144) cells/5 hpf in cases euthanized postoperatively, and 43 (range, 34 to 121) cells/5 hpf in horses that survived to discharge. There were no significant correlations between the cell count and $tS_0^2$ or $tBF$. All samples showed weak to moderate cytoplasmic and nuclear HIF-1α immunoreactivity (Table 3). There was no significant correlation between the HIF-1α nucleus-to-cytoplasm ratio and $tS_0^2$.

### Table 2—Histology score frequency distribution of cases with naturally occurring large intestinal ischemia.

<table>
<thead>
<tr>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal epithelial score$^5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthanized intraoperatively</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Euthanized postoperatively</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage score$^3$ (all cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Submucosa</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Muscularis</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Serosa</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3—Frequency distribution of hypoxia-inducible factor-1α immunoreactivity score in cases with naturally occurring large intestinal ischemia.

<table>
<thead>
<tr>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2—Individual value plot of the tissue oxygen saturation (A), tissue hemoglobin (B), tissue blood flow (C) of the large intestine during strangulation and following reperfusion. The horse that was excluded from analysis was euthanized at the owner’s request despite the operating clinician judging the prognosis to be good. AU = Arbitrary units. **$P < .01$; ****$P < .0001$.

Figure 3—Individual value plots of the tissue oxygen saturation (A) and tissue blood flow (B) of the large intestine during strangulation and following reperfusion plotted against the oxygen pressure in arterial blood ($P_{aO_2}$) taken from the facial artery and the mean arterial pressure (MAP), respectively. There were no significant correlations between tissue oxygen saturation and $P_{aO_2}$ or between tissue blood flow and MAP. AU = Arbitrary units.
Discussion

This study investigated the application of LDFS for the assessment of colon viability during colic surgery for large intestinal strangulations. Prior to and following correction of the lesion, tS_\text{O}_2 and tBF were clearly lower in nonsurvivors. However, following correction of the lesion, PF and LVC showed similar microperfusion variables, but tBF was significantly lower in the PF following correction of the lesion. Therefore, we could partially accept the first hypothesis that LDFS would yield lower values than reported in healthy horses but would not be homogenous throughout the large colon. The degree of ischemia as determined by LDFS did not differ between survivors and nonsurvivors. However, following correction of the lesion, tS_\text{O}_2 and tBF were significantly higher in the survivors, thereby accepting the second hypothesis that survivors would have better microperfusion values than nonsurvivors. Hemoglobin measured by LDFS was strongly correlated with hemorrhage detected histologically, and lower tBF values coincided with higher I/C (ie, more severe mucosal damage). However, the other tested histological variables were not correlated with the LDFS measurements. Therefore, we could only partially accept the third hypothesis that microperfusion measured by LDFS would correlate with histological injury.

The main finding of the study is that both tS_\text{O}_2 and tBF differed significantly between survivors and nonsurvivors, possibly indicating that LDFS could be used as an additional diagnostic aid to determine large intestinal viability. The low number of cases in the current study preclude any conclusions on a set cut-off value, but the results suggest that colonic tS_\text{O}_2 values of more than 90% following reperfusion may be associated with survival. There are no studies available for direct comparison of the tS_\text{O}_2, but this value of oxygen saturation is in line with an investigation in human patients assessing microperfusion by use of pulse oximetry during colorectal surgery, showing that tS_\text{O}_2 of less than or equal to 90% in the area of the anastomoses was associated with anastomotic leakage.\(^\text{12}\) There has been 1 equine report\(^\text{2}\) evaluating surface oximetry, finding that horses with Po_2 of less than 20 mm Hg were less likely to survive. However, the sensitivity for identifying nonviable intestines was only 53%. This technique of oximetry using a Clark electrode only measures the superficial oxygen tension, in contrast with the white light spectrophotometry device used in the current study, which measures the S_\text{O}_2 up to the penetration depth of 2.5 mm. Therefore, the technique used in the current study may be more representative of tissue injury throughout different layers of the intestinal wall. Nevertheless, it should be noted that the measurement will most likely reach (part of) the submucosa but not the mucosa.

The tBF, measured by the use of LDF, also showed a significant correlation with mucosal injury detected with histology. As with the white light spectrophotometry, there are no reports on the use of LDF in the large intestine, limiting the comparison with existing literature. Doppler ultrasound has been reported for the assessment of intestinal blood flow and employs a technique similar to LDF yet was found to be less sensitive than LDF in experimental intestinal ischemia in dogs.\(^\text{11,14}\) In horses, Doppler ultrasound was shown to accurately predict viability in hemorrhagic strangulating obstructions but was less accurate in ischemic strangulating obstructions of the small intestine.\(^\text{1}\) This raises the question of whether the type of strangulation could also affect the accuracy of LDF measurements. Judging from the clinical appearance of the intestine, the tHB, and the HS in histology, the cases included in the present report suffered from hemorrhagic strangulating obstructions. Therefore, one cannot conclude anything about the accuracy of LDF in ischemic strangulating obstructions based on the current study.

Another important result was that tBF correlated with histological hemorrhage, suggesting that this modality could be used as an indicator for hemorrhage in the intestinal wall. This is of clinical significance because of the association between mucosal hemorrhage and survival.\(^\text{3}\) As stated previously, the probe used in the current study would not be able to include the mucosa when placed on the serosal side; hence, a probe with a larger depth range would be required for this indication. Alternatively, the probe could be placed on the mucosal side of the intestine through a PF enterotomy. The LDFS results did not correlate with all tested histological variables, yielding no correlations with the inflammatory cell count or HIF-1α. This could indicate that the degree of ischemia may not be decisive for the extent of cell infiltration into the colon or HIF-1α expression during ischemia.

An interesting finding was that microperfusion differed between the PF and LVC following reperfusion but not during ischemia. Histological investigations have yielded conflicting results on whether intestinal injury is homogenous throughout the entire large colon.\(^\text{15,16}\) With the PF located farthest away from the arterial colonic blood supply, it could be more susceptible to decreasing perfusion pressure with partial ischemia. A possible explanation for the disparity between the colon segments only being found during reperfusion may be a varying occurrence of local thrombosis of the vessels limiting complete reperfusion, which could be more pronounced in the more peripherally located PF.\(^\text{17,18}\) Intraluminal pressure may also affect intestinal perfusion;\(^\text{19,20}\) although this is unlikely to explain the difference in perfusion between the PF and LVC, considering the continuous lumen and proximity of both segments.

The present study has several limitations, including the low number of cases, measuring just 2 sites in the colon, and not including the mucosa in the measurement. Furthermore, the inclusion of the small colon cases may have caused a less homogenous population due to differences in anatomy and strangulating lesions. On the other hand, the blood supply to the intestinal layers is very similar in the large and small colon,\(^\text{21,22}\) and histology of the
intestinal samples showed comparable pathology. It is a limitation that no biopsy was taken in the case that was recovered following small colon strangulation. However, the intestine was judged to be viable by visual assessment, and taking a biopsy without a clear indication of intestinal resection and anastomosis was considered unacceptable. In the small colon cases that were euthanized during surgery, resection was not considered a viable option because the ischemic injury was extensive and precluded resection of all affected tissue. Another important limitation is that the surgeon could not be blinded to the LDFS results during surgery. Therefore, one cannot completely exclude the surgeon from being influenced by the measurement in judging the prognosis for intestinal viability. Nevertheless, we believe that intraoperative decisions were made independent of the LDFS results, because at the time of the study, it was unclear what values to expect, and nearly all contributing surgeons were new to the device and were not familiar with the published values in healthy horses. Another limitation is the use of a historical control group for comparison of the current results with the intestinal microperfusion of healthy horses. Necropsy was not performed in any of the cases; hence, there is no complete certainty about the postischemic intestine causing the postoperative deterioration. However, the clinical signs of colitis were very indicative, and generally the clinic refrains from routinely performing necropsies on horses suffering from colitis due to hygiene concerns. Another limitation in the interpretation of the results is that reduced intestinal perfusion is not only caused by strangulation but can also reflect the systemic cardiovascular state of the horse.23,24 The low microperfusion values could be the result of the systemic cardiovascular disturbances instead of the degree of ischemia or reperfusion of the colon itself. However, this is unlikely considering the correlation between LDFS and histological injury and the lack of correlation between LDFS and anesthetic monitoring parameters. Nevertheless, it must be noted that these values were obtained within 10 minutes yet not exactly at the same time as the respective LDFS measurements. All horses had an MAP greater than 60 mm Hg except for 1 reperfusion measurement indicating a relatively homogenous group in blood pressure. However, an effect of dobutamine dosage or the use of noradrenaline on the intestinal microperfusion measurements cannot be ruled out.25 Furthermore, breathing of the horse and positive pressure ventilation may affect intestinal perfusion. Subjectively, the breathing pattern was not clearly visible in the obtained LDFS data, the duration of the measurement was more than 30 seconds, and the use of several breathing cycles may have corrected some of this variation.

In conclusion, this study reports microperfusion characteristics of naturally occurring large intestinal strangulations, showing differences in reperfusion between the PF and LVC. Furthermore, the data suggest that the degree of ischemia is not the main indicator of postoperative survival. Most importantly, the results indicate that colonic blood flow measurements may aid in predicting postoperative survival and intestinal tissue injury. Therefore, LDFS could be of use as an ancillary diagnostic aid during colic surgery, measuring the microperfusion of the colon after release of a strangulation in addition to routine visual assessment. Very low tBF and tSO2 values are suggestive of nonsurvival, yet this must be seen as an exploratory study. Larger case numbers are needed to determine cut-off values and exact accuracy prior to a more widespread clinical implementation of this technique.

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References


