Evaluation of peritoneal fluid following intestinal resection and anastomosis in horses

R. Reid Hanson, DVM; Alan J. Nixon, BVSc, MS; R. Gronwall, DVM, PhD; D. Meyer, DVM; J. Pendergast, PhD

SUMMARY

Postoperative abdominal fluid changes were compared in 2 groups of horses; those undergoing double small-colon resection and anastomosis (n = 10) and those undergoing exploratory celiotomy alone (n = 5). Peritoneal fluid was collected before surgery and on postoperative days 1, 3, 5, and 7. Total and differential nucleated cell counts, RBC numbers, and total protein and fibrinogen concentrations were evaluated. In both groups, all values were significantly higher than normal on the first postoperative day (after small-colon resection and anastomoses, WBC = 130,350 ± 23,310 cells/μl, RBC = 7,389,000 ± 6,234,000 cells/μl, total protein = 3.63 ± 0.16 g/dl; after exploratory celiotomy alone, WBC = 166,620 ± 34,340 cells/μl, RBC = 295,000 ± 86,070 cells/μl, total protein 4.38 ± 0.54 g/dl). The number of total peritoneal nucleated cells and RBC significantly decreased after the first postoperative day, whereas total protein and fibrinogen concentrations, percent neutrophils, and percent mononuclear cells remained unchanged. None of the values had returned to normal by postoperative day 7 (after small-colon resection and anastomoses, WBC = 45,600 ± 8,765 cells/μl, RBC = 95,390 ± 53,380 cells/μl, total protein = 4.39 ± 0.23 g/dl; after exploratory celiotomy alone, WBC = 43,340 ± 7,746 cells/μl, RBC = 12,860 ± 11,790 cells/μl, total protein = 3.92 ± 2.20 g/dl.) The resection and anastomosis group had a significantly lower total protein concentration on the first postoperative day and a significantly higher mean total RBC count over the entire 7-day postoperative evaluation than did horses that underwent celiotomy alone. Other values in the 2 groups of horses did not differ significantly. As a result, there was insufficient evidence to conclude that resection and anastomosis of the small colon in healthy horses causes a different inflammatory response than does manipulation of the intestine alone.

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From the Departments of Surgical Sciencs (Hanson, Nixon), Medical Sciences (Gronwall, Meyer), College of Veterinary Medicine, and Biostatistics Division, Department of Statistics (Pendergast), College of Liberal Arts and Sciences, University of Florida, Gainesville, FL 32610. Dr. Hanson's present address is Georgetown Equine Hospital, 175 Georgetown Road, Charlottesville, VA 22901. Dr. Nixon's present address is Department of Clinical Sciences, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853. Dr. Meyer's present address is Smithkline Beecham Pharmaceuticals, Department of Experimental Pathology, PO Box 1539, L-60, King of Prussia, PA 19406.

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Abdominal paracentesis has become an important aid in the diagnosis of abdominal diseases in horses. Techniques for obtaining samples and the normal values are well described.[1–4] Abnormal values have been reported with abdominal wall penetration, splenic abscess, parasite migration, systemic abscessation, neoplasia, gastrointestinal rupture, cranial mesenteric thrombosis, surgical contamination, and idiopathic phenomena.[5–12] The primary use of abdominal paracentesis in horses, however, has been in establishing a differential diagnosis for acute abdominal crisis. Various disease states have been correlated with trends in color, turbidity, WBC count, RBC count, plasma protein concentration, enzyme changes, and shifts in the cytologic distribution of peritoneal fluid. These changes have been used as an aid to determine the need for surgery and to provide a prognosis for life.[13–16] However, interpretation of abdominal fluid findings have not always been as diagnostically or prognostically consistent and accurate as reported.[17,18]

Serial peritoneal fluid evaluation is a useful indicator for assessing the response of peritonitis and abdominal trauma to medical treatment.[5,10,11,19] Early identification of postoperative complications decreases the morbidity and mortality of these animals. However, unless obvious cytologic abnormalities exist, interpretation of peritoneal fluid values following extensive abdominal surgery has not been adequately defined.

In experimental work, it has been shown that a notable postoperative inflammatory response occurs in the peritoneal fluid following simple manipulation of the abdominal viscera and this response is greater than that following opening and closing of the abdomen without manipulation of the viscera.[20,21] However, it is not known how major intestine resection at the time of exploratory celiotomy affects peritoneal fluid constituents.

Percutaneous peritoneal lavage with saline solution or saline solution containing antibiotics induced a transient inflammatory response with little or no change in the peritoneal surfaces 96 hours later at postmortem examination.[22] Peritoneal lavage with povidone iodine solutions as dilute as 3%, however, caused severe inflammation of peritoneal surfaces, which was evident in peritoneal fluid.[22] Extracellular bacteria and a severe degenerative inflammatory response within the peritoneal fluid was seen over a 72-hour period in ponies that underwent experimental infarction of 2 feet of jejunum.[23] Although some neutrophils and eosinophils are phagocytosed by monocyte/macrophage cells in the peritoneal cavity, most of these cells migrate through diaphragmatic stoma to the mediastinal lymphatics.[24]

The small colon was selected for resection and anasto-

mosis because the descending colon has a high intraluminal bacteria count and high collagenase activity, compared with other intestinal segments.[25] Because of these inherent properties, it should provide maximal stimulus for peritoneal fluid changes.

To our knowledge, serial evaluation of the peritoneal fluid following uncomplicated intestinal resection and anastomosis in horses has not been described. The purpose of the study reported here was to document the cellular fluid and protein characteristics of peritoneal fluid in horses following noncomplicated, but extensive, intestinal resection and anastomosis and to compare these data with values in sham controls over a 7-day postoperative course.

Materials and Methods

Fifteen healthy horses of various breeds, ages (mean, 10 ± 2.3 years), weights (mean, 453 ± 15.8 kg), and both genders were used in this study. All horses were evaluated by physical examination and CBC prior to being included in this study. They were then vaccinated, dewormed, and allowed to acclimatize to the surrounding conditions for 2 weeks prior to the study. The day preceding surgery, an abdominocentesis was performed, using a needle technique.[3]

After the initial centesis had been performed, 10 horses (group 1) were randomly selected for exploratory celiotomy and double small-colon resection and anastomosis. An exploratory celiotomy with manual exploration of the viscera without resection and anastomosis was performed on the remaining 5 horses (group 2). The techniques for small-colon resection and anastomosis have been described elsewhere.[26,27]

Manual exploration, including retrieval of the small colon from the abdomen was performed in all horses. Normal physiologic Ringer's solution (22 ml/kg) containing 1 IU of sodium heparin/ml and 1,000 IU of potassium penicillin/ml was instilled into the abdominal cavity after completion of the double small-colon resection and anastomosis or manual exploration of the abdomen. As much of the instilled fluid as possible was retrieved via continuous intraoperative suction. A perforated polyvinyl abdominal drain[a] (3.2 mm ID) was placed in the abdomen with a further unperforated 10-cm portion exteriorized 5 cm rostral to the ventral midline skin incision. The drain was heparinized and capped until further abdominal fluid drainage after surgery.

Rectal temperature, pulse, respiratory rate, and clinical observations were recorded every 2 hours during the initial 12-hour postoperative period and every 4 hours thereafter. Procaine penicillin G, gentamicin sulfate, sodium heparin, and flunixin meglumine were administered for 72 hours after surgery (22,000 IU/kg, IM, q 12 h; 2.2 mg/kg, IV, q 8 h; 40 IU/kg, IV, q 8 h, and 0.5 mg/kg, IV, q 8 h, respectively). Twenty liters of lactated Ringer's solution was given IV for 12 hours after surgery. Alfalfa hay was provided following the IV fluid therapy. The abdominal drain was opened when the horse stood after anesthesia and the drain was capped when no further drainage was evident.

The abdominal catheter was cleared 4 times daily by allowing it to drain until all fibrinous and exudative material had been flushed from the tube. On days 1, 3, 5, and 7 following surgery, peritoneal fluid was collected after clearing of the catheter. Following sampling on day 7, the catheter was withdrawn from the abdominal cavity. At the end of the experimental period, horses were euthanatized and necropsied.

Peritoneal fluid was evaluated for color, turbidity, nucleated cell count, RBC count, and fibrinogen and plasma protein concentration.[b] Samples were stained (Wright-Giemsa) for evaluation of morphologic features (percentage of neutrophils, percentage of monocytes, percentage of small mononuclear cells, cell toxicity, and bacteria). Cell counts in the peritoneal fluid were performed with an electronic cell counter.[c]

Statistical analysis was performed by use of a repeated-measures analysis of variance on each of the 7 objective response measures (total peritoneal nucleated cell counts, RBC numbers, total protein, fibrinogen, percentage of neutrophils, large mononuclear cells, and small mononuclear cells). Peritoneal fluid total nucleated cell counts, and RBC counts were transformed by use of the natural log transformation to stabilize the subgroup variance. Measurements were taken before surgery and on postoperative days 1, 3, 5, and 7. Treatment and time factors were crossed and the horses were nested within treatment. Significant treatment with time interactions were further evaluated with post-hoc tests as described by Winer.[28] If no significant interaction existed, any significant main effect of treatment or time was further evaluated by use of similar post-hoc tests.[28] Statistical significance was considered if $P \leq 0.05$.

Results

Clinical response—All horses in both experimental groups had no signs of abdominal discomfort requiring further analgesic treatment after the 72-hour postoperative period. Because the resection and anastomosis is a more complicated surgery than exploratory celiotomy, group-1 horses were not returned to full feed (3 flakes of hay 2 times/d) until 48 hours after surgery, whereas group-2 horses were on full feed 24 hours after surgery.

Peritoneal fluid—The abdominal catheter provided 71 of 75 samples (95%). Forceable manual restraint or sedation was not necessary to collect the peritoneal fluid (Tables 1 and 2). Other more painful methods of abdominocentesis (ie, needle or teat cannulas) were not necessary in any of the horses. Enterocentesis was not a complicating factor.

Nucleated cells—Evaluation of the natural log of the peritoneal fluid total nucleated cell count (Table 1) revealed no significant difference between group 1, compared with control group 2 over the postoperative course of the evaluation. A significant increase in the total nucleated cell count was evident for both groups the first day following surgery. By postoperative day 3, the total nucleated cell count for both groups had decreased significantly, although the count for both groups was still

[a] Redi-Vacette, Orthopedic Equipment Co, Bourbon, Ind.

[b] Plasma Protein Meter, American Optical Co, Buffalo, NY.

[c] Model ZBI 3634, Coulter Electronics Inc, Hialeah, Fla.

Table 1—Peritoneal fluid values in horses undergoing small colon resection and anastomosis (n = 10) and manual exploration (n = 5)

	Preoperative day 0	Postoperative days 1	3	5	7
White blood cells (/μl)					
Group 1	1,464±381[9] (500 to 4,200)	130,350±23,310*,† (42,500 to 260,000)	64,720±10,760*,† (22,700 to 130,600)	47,530±5,367[9]* (14,700 to 64,200)	45,600±8,765[8]* (19,100 to 92,000)
Group 2	1,420±229 (600 to 2,000)	166,620±34,340*,† (86,500 to 254,000)	51,520±15,480*,† (13,000 to 105,600)	44,180±7,862* (22,200 to 60,000)	43,340±7,746* (21,000 to 62,000)
% Neutrophils					
Group 1	46±6.38[6] (25 to 65)	77.2±4.32*,† (52 to 92)	87.3±2.52* (73 to 97)	80.25±3.72[8]* (65 to 94)	79.88±2.34[8]* (68 to 91)
Group 2	51.25±18.37[4] (16 to 90)	89.2±1.6*,† (85 to 93)	83.4±2.54* (75 to 90)	79.2±3.54* (70 to 88)	73.8±6.89* (57 to 91)
% Large mononuclear cells					
Group 1	47.67±6.28[6] (33 to 70)	23.5±4.49* (8 to 48)	11.2±2.15* (3 to 24)	17.63±3.49[8]* (5 to 30)	17.5±2.14[8]* (8 to 28)
Group 2	45.25±17.33[4] (10 to 76)	9.2±1.72* (6 to 15)	11.4±2.52* (6 to 20)	17.2±2.52* (10 to 25)	21.8±6.14* (8 to 39)
% Small mononuclear cells					
Group 1	4.83±2.6[6] (0 to 17)	0.3±0.15 (0 to 1)	1.4±0.54 (0 to 5)	2.13±0.95[8] (0 to 7)	2.5±0.80[8] (0 to 6)
Group 2	2.25±2.25[4] (0 to 9)	1.6±0.51 (0 to 3)	4.6±1.36 (3 to 10)	2.6±1.08 (0 to 6)	4.0±1.64 (1 to 10)

* Significantly different ($P \leq 0.05$) than preoperative values. † Significantly different ($P \leq 0.05$) from the preceding period data.
Group 1—Small-colon resection and anastomosis. Group 2—Manual exploration. Data are expressed as mean ± SEM (range). Values in brackets indicate N = value when less than indicated.

Table 2—Peritoneal fluid values in horses undergoing small-colon resection and anastomosis (n = 10) and sham (n = 5) surgery

	Preoperative day 0	Postoperative days 1	3	5	7
Red blood cells (/μl)					
Group 1	3,000±1,546[7] (200 to 1,730,000)	7,389,000±6,234,000*,† (20,000 to 63,330,000)	212,000±43,390*,† (50,300 to 450,000)	77,600±19,750[8]* (1,000 to 150,000)	95,390±53,380[8]*,‡ (1,000 to 460,000)
Group 2	1,700±462 (1,000 to 3,300)	295,000±86,070[4]*,† (140,000 to 540,000)	43,280±23,570*,† (1,000 to 130,000)	11,480±6,922* (1,000 to 35,400)	12,860±11,790*,‡ (1,000 to 60,000)
Total protein (g/dl)					
Group 1	< 2.5[9]	3.63±0.16§ (3 to 4.5)	4.32±0.12 (3.6 to 4.8)	4.52±0.28[9] (3.5 to 6.2)	4.39±0.23[8] (3.3 to 5.2)
Group 2	< 2.5	4.38±0.54§ (2.5 to 5.8)	4.02±0.17 (3.5 to 4.5)	4.0±0.21 (3.6 to 4.8)	3.92±2.20 (3.4 to 4.5)
Fibrinogen (mg/dl)					
Group 1	0[9]	193.80±27.45[8]*,† (100 to 300)	266.70±28.87[9]* (100 to 400)	212.50±22.66[8]* (100 to 300)	158.3±41.67[6]* (0 to 300)
Group 2	0	200.00±40.82[4]*,† (100 to 300)	180.0±37.42* (100 to 300)	200±31.62* (100 to 300)	130±20.0* (100 to 200)

* Significantly different ($P \leq 0.05$) than preoperative values. † Significantly different ($P \leq 0.05$) from the previous period data. ‡ Significantly different ($P \leq 0.05$) mean cell count between group 1 and group 2 over the 7-day postoperative course. § Significantly different ($P \leq 0.05$) between group 1 and group 2.
Group 1 — Small colon resection and anastomosis. Group 2 — Manual exploration. Data are expressed as mean ± SEM (range.). Values in brackets indicate N = value when less than indicated.

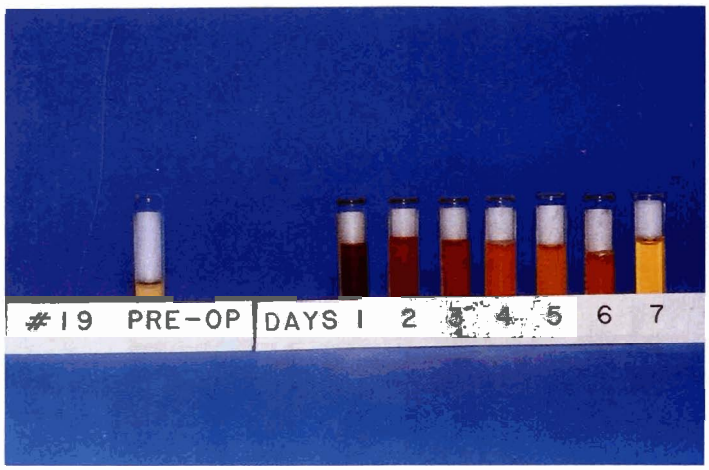
significantly higher than normal values on postoperative day 7.

Red blood cells—A difference was not detected in RBC count between group 1 and group 2 at each time period over the postoperative course of the evaluation (Table 2). For both groups, the RBC count increased the first postoperative day, decreased significantly at day 3, but was still significantly higher on postoperative day 7, compared with preoperative values. However, statistical evaluation of the mean RBC count over the entire 7-day postoperative course revealed a significant increase of RBC within group 1 (208,000 cells/μl), compared with group 2 (52,000 cells/μl).

Total protein—There was a significant time-by-group interaction, implying that the average total protein concentrations followed different patterns over time in the 2 groups (Table 2). On postoperative day 1, group-2 horses had an increased concentration of 4.4 g/dl, which was significantly higher than the average value of 3.7 g/dl in group 1 (Table 2). Total protein values for both groups did not decrease significantly after the increase on the first postoperative day, but were no longer significantly different from each other by postoperative day 3, and remained high over the course of the evaluation.

Fibrinogen—A difference in fibrinogen concentration was not detected between the 2 groups (Table 2). Fibrinogen concentrations were significantly higher than normal on postoperative day 1 and did not decrease significantly over the course of the 7-day evaluation.

Color and clarity—For both groups, peritoneal fluid was red and turbid the first 3 postoperative days. A transition period or change in color and opacity was evident on day 4, with a similar clearing of the fluid for both groups over the last 3 days (Fig 1).



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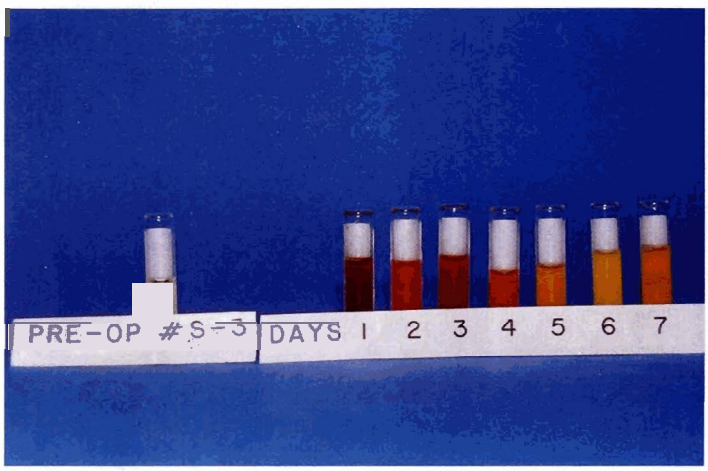

B

Figure 1—Color and clarity trends of abdominal fluid before and after surgery.
A—Fluid from a horse that underwent small-colon resection and anastomosis.
B—Fluid from a horse that underwent manual exploration of the abdomen alone.

Neutrophils—A sharp and similar increase in the percentage of neutrophils was evident on postoperative day 1 for both groups (Table 1). A significant decrease was not evident during the postoperative course of the evaluation.

Large mononuclear cells—Before surgery, typical large mononuclear cells were reactive mesothelial cells characterized by deep blue cytoplasm with multiple round to oval nuclei (Table 1). A sharp and similar decrease in the percentage of these cells was evident on postoperative day 1 for both groups (Table 1). The macrophage, with a lesser distribution of reactive mesothelial cells, was the predominant mononuclear cell type after surgery through day 7 for both groups. These large cells had vacuolated cytoplasm, contained various size granules, and only a few had evidence of phagocytosis. A trend toward increasing percentage of these large mononuclear cells for both groups was suggested following the first postoperative day; however, these changes were not significant. The percentage of large mononuclear cells remained decreased at postoperative day 7, compared with preoperative values.

Small mononuclear cells—Numbers of small mononuclear cells did not change significantly on postoperative day 1 or over the entire course of the evaluation for both groups (Table 1).

Cellular morphologic features—For both groups, the nondegenerate neutrophil was the predominant cell in the postoperative response. Rare karyolysis of the neutrophil was evident in 2 horses from both groups at day 1. In group 1, rare intracellular bacteria were seen in 1 horse on day 1 and in another horse on postoperative day 3.

Peritoneal cavity—Gross morphologic reactions did not develop within the peritoneal cavity by the presence of the abdominal drain. Isolation of the drain by walling off an area through adhesions and fibroplasia of the ventral body wall and large colon into a separate effusion pool did not occur in any of the horses evaluated.

Discussion

Peritoneal fluid reflects the pathophysiologic state of the parietal and visceral mesothelial surfaces. Our findings indicate that additional manipulation of the peritoneum associated with resection and anastomosis of 2 isolated segments of intestine may be of little consequence, compared with the trauma induced during a simple exploratory celiotomy. Group-1 (resection and anastomosis) horses had a postoperative peritoneal fluid inflammatory response that was similar to group-2 (manual exploration control) horses during the 7-day course of the evaluation. All values (except the percentage of small mononuclear cells) were significantly changed the day following surgery. The total peritoneal nucleated and RBC counts significantly decreased after the first postoperative day, whereas the total protein and fibrinogen concentrations, and percentage of neutrophils and large mononuclear cells remained unchanged. Values had not returned to normal limits by postoperative day 7.

These findings support those of other studies that indicate surgical manipulation creates a significant and rapid postoperative peritoneal inflammatory reaction[20,21] The critical mediator of this reaction seemed to be manipulation of the abdominal viscera and damage to other mesothelial surfaces (ie, celiotomy). A significant difference was noticed in the total peritoneal nucleated cell counts and total protein values for horses undergoing exploratory celiotomy,[20,21] compared with horses undergoing laparoscopy or celiotomy without exploratory surgery.[20,29] This supports the suggestion that the size of the celiotomy wound as well as the extent of manipulation of viscera strongly effect the postoperative inflammatory response.

Horses in this study had a similar postoperative total peritoneal nucleated cell response, compared with those reported previously.[20,21] The magnitude of inflammation and relative trends for the cell count to decrease over a 7-day postoperative course were also similar to those in other studies.[20–22] However, total peritoneal nucleated cell values in this and other studies remained significantly higher than preoperative values.[20–22] Nonetheless, a significant downward trend in total nucleated peritoneal cell count was not evident until postoperative day 5 in 2

studies,[20,21] whereas the cell count in this study and another report decreased after the first postoperative day.[22]

Percentage of neutrophils increased sharply on day 1 and stayed high in both groups. However, in both groups, percentage of neutrophils decreased nonsignificantly over the postoperative course, similar to that in other studies.[20,21] Neutrophils are the most common and important cell type in peritoneal effusions. They are attracted to the peritoneal cavity by chemotactic stimuli, and act in the primary cellular defense mechanisms against invading microorganisms.[24] Mononuclear cells are part of the normal surveillance cell population and are proportionally overrun in the inflammatory process.[24] Transformation of the large mononuclear cells into a distribution of mostly macrophages with a lesser degree of reactive mesothelial cells after surgery is directly related to increased irritation within the abdominal cavity.[30] Mesothelial cells in peritoneal fluid may be seen in various stages of a continuum from the recently sloughed cuboidal serosal lining cell to a mature, active macrophage. Along this progression, the cells assume a variety of sizes and transitional forms, which are termed reactive mesothelial cells.[31] The cells may be alone or in clusters and generally have intense cytoplasmic basophilia and an eosinophilic brush border or pseudopods. Mitotic figures and binucleate cells are common. Reactive mesothelial cells and macrophages commonly increase in any peritoneal fluid transudate or exudate. The prevalence, distribution, or activity of these cells has not been a useful indicator, however, in predicting various types of acute abdominal disease.[17]

The use of intra-abdominal lavage did not mask a difference in the inflammatory response between the 2 groups because postoperative peritoneal fluid changes were similar to those in other reports in which no postoperative lavage was used.[20,21] Addition of penicillin to the lavage solution does not worsen the inflammatory response.[22]

A perforated polyvinyl drain was used after surgery to collect peritoneal fluid samples. We chose this clinically proven method over the more traditional needle or teat cannula techniques because it consistently provided a sample of peritoneal fluid and was evidently painless to the horse for collection. Collection of peritoneal fluid with needles or cannulae is frequently unsuccessful in the early postoperative period. Enterocentesis also occurs with more traditional methods and this adversely affects the data.[22,32] The polyvinyl drain apparently did not affect peritoneal fluid data because our exploratory celiotomy controls had similar responses and trends to those in other studies in which needle and cannula centeses were used.[20,21] Drain-related problems result in a decreased total nucleated cell count and increased total protein concentration as would be evident with a peritoneal effusion from a chemical or mechanical peritonitis.[22] Reaction to, or an attempt to isolate the drain by serosal or abdominal wall adhesions were not seen in any of the horses at necropsy. Placement of abdominal drains as a sole procedure was not possible in this experiment because the abdomen has to be opened to properly locate them in the abdominal cavity.

An important finding between the 2 groups was the high mean peritoneal fluid RBC count for group 1, compared with that of group 2. There was a marked trend for increased RBC numbers in group 1, compared with group-2 horses at each time interval. However, this difference was only significant when data from the 4 postoperative sampling times were pooled for each group and compared. Actual hemorrhage or hemorrhage as a result of thrombi along the resection or anastomotic lines was a potential source of RBC. The RBC count in peritoneal fluid decreased with time, indicating that hemorrhage has diminished or ceased. Significantly lower peritoneal fluid total protein values on postoperative day 1 for the resection and anastomosis group may be a dilutional result of associated hemorrhage of this postoperative time period.

Interpretation of peritoneal nucleated cell morphologic features (ie, karyolysis, intracellular bacteria) is an important postoperative indication of the state of the abdominal cavity. The finding of karyolysis or rare intracellular bacteria within the first 24 to 72 hours of both groups after surgery created no special complications in horses evaluated in this study. It was expected that increased karyolysis and intracellular bacterial numbers would be prominent features 1 day after intestine resection and anastomosis. Moderate increases in these values are important findings and suggest that in the normal postoperative response bacteria are cleared from the intra-abdominal environment sooner than 24 hours after surgery and leave little karyolytic change to suggest their earlier numbers. Furthermore, even on day 1, prominent karyolysis and several bacteria would suggest ongoing peritoneal contamination or established peritonitis.

Resection of intestine did not induce a significant change in the peritoneal fluid inflammatory response, compared with exploratory celiotomy alone. Further studies are needed to determine when the abdominal fluid values return to normal.

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