Evaluation of cytologic and biochemical variables in blood, plasma, and peritoneal fluid from calves before and after umbilical herniorrhaphy

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Objective—To establish reference intervals for cytologic and biochemical variables in peritoneal fluid, whole blood, and plasma in calves with congenital umbilical hernias (CUHs) before and after herniorrhaphy and to assess whether those variables in calves with CUHs were altered, compared with findings in clinically normal calves.

Animals—20 Holstein calves with or without a CUH.

Procedures—10 calves with CUHs underwent herniorrhaphy. Blood and peritoneal fluid samples from all 20 calves were collected for cytologic and biochemical analyses on days 0 (before surgery), 1, 3, 5, 7, and 15. Data from the 2 groups were compared.

Results—Reference intervals for the variables of interest were established for each group. Before surgery, calves with CUHs had significantly greater plasma total protein concentration and creatine kinase (CK) and aspartate aminotransferase activities and peritoneal fluid specific gravity values, compared with values for calves without CUHs. At various time points after surgery, peritoneal fluid total protein concentration; fibrinogen concentration; nucleated cell, polymorphonuclear cell, and lymphocyte counts; specific gravity; and lactate dehydrogenase, aspartate aminotransferase, and CK activities in calves with CUHs were significantly different from values in calves without CUHs. Some plasma and blood variables (eg, total protein concentration, neutrophil count, and CK activity) were significantly different between the 2 groups.

Conclusions and Clinical Relevance—Values of certain cytologic and biochemical variables in peritoneal fluid, blood, and plasma were different between calves with and without CUHs. Thus, determination of reference intervals for these variables is important for interpreting diagnostic test results in calves with CUHs. (*Am J Vet Res* 2009;70:423–432)

Congenital umbilical hernias are relatively common defects in calves and have been observed in several breeds of cattle, including Holstein Friesians.[1–3] The overall incidence of umbilical hernias among cattle with congenital defects ranges from 8% to 30%.[4] It is generally accepted that a genetic component is involved in congenital umbilical hernias, but hypotheses regarding the mode of inheritance are conflicting.[3,5,6] Compared with males, female calves have a significantly higher risk for umbilical hernias.[7] The occurrence and economic importance of congenital umbilical hernias were investigated in German Fleckvieh calves.[8] In that study,[8] the mean difference in market price between calves with and without congenital umbilical hernias was determined; for males, the market price differed by 50%, whereas for females, the market price differed by only 10%.

Surgical procedures involving the abdomen typically require entry into the peritoneal cavity. During healing, changes in the visceral and parietal peritoneum

Abbreviations

AST	Aspartate aminotransferase
CK	Creatine kinase
LDH	Lactate dehydrogenase
NCC	Nucleated cell count

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may affect the permeability of the peritoneal membrane to body fluids. Surgical trauma to the abdominal wall and the peritoneal and visceral surfaces during celiotomy is expected to cause postoperative inflammation.[9] Subsequently, peritoneal fluid volume increases and leukocytes migrate into the abdominal cavity. These changes make interpretation of the results of peritoneal fluid analysis after surgery more challenging.[10,11]

Changes in the constituents of peritoneal fluid in adult cattle associated with left-sided displacement of the abomasum, traumatic reticuloperitonitis, septic peritonitis, and intra-abdominal neoplasia have been described, as have changes following laparoscopic surgery, exploratory celiotomy, and omentopexy.[10,12,13] The concentration of electrolytes in peritoneal fluid of goats following rumenotomy[14] and the evaluation of biochemical and cytologic variables in blood and peritoneal fluid after enterectomy and enteroanastomosis[11] have also been reported.

Serial peritoneal fluid evaluation appears to be a useful indicator for assessing peritoneal response to injury.[11,15] Early identification of postoperative complications decreases the morbidity and mortality rates among animals undergoing surgery. However, interpretation of values of peritoneal fluid variables following extensive abdominal surgery has not been adequately defined.[11]

Values of peritoneal fluid variables in calves differ from values in adult cows.[16] Calves typically have higher neutrophil and lymphocyte counts and lower total protein concentration in peritoneal fluid, compared with findings in adult cows.[16] Other previously described differences are a greater mean absolute eosinophil cell count and a lower fibrinogen concentration, whereas values for mean absolute lymphocyte and mononuclear cell counts are similar to those in adults.[17]

In cattle that develop abdominal lesions, the leukogram may not change.[12,13] Because additional lesions or complications may develop during the postoperative period,[18] prior determination of postoperative peritoneal fluid variable values is important for subsequent analysis and interpretation of peritoneal fluid findings.[10] However, peritoneal fluid data from young calves with abdominal lesions are not available.[17,19]

To our knowledge, serial evaluation of the constituents of the peritoneal fluid, whole blood, or plasma in calves with uncomplicated umbilical hernias has not been described. The purpose of the study reported here was to establish reference intervals for cytologic and biochemical variables in peritoneal fluid, blood, and plasma in calves with congenital umbilical hernias before and after herniorrhaphy; assess whether those variables in calves with an umbilical hernia were altered, compared with findings in clinically normal calves; and determine whether such alterations were detectable first in peritoneal fluid, blood, or plasma. All data were compared between calves with and without an umbilical hernia over a 15-day period to exclude a possible effect of repeated paracentesis on peritoneal fluid constituents.

Materials and Methods

Animals—Twenty clinically normal Holstein calves (11 females and 9 males) were used in the study. The calves' ages ranged from 2 to 7 months. Calves were assigned to 1 of 2 groups: a control group (calves without an umbilical hernia; n = 10) and an experimental group (calves with an umbilical hernia; 10). Mean ± SD age of the calves in the control group was 3.8 ± 1.22 months; mean age of the calves in the experimental group was 3.1 ± 0.74 months. All calves were dewormed with abamectin[a] and kept on pasture (coast-cross grass). Water and concentrate were available ad libitum. Sucklingage calves were bottle-fed whole milk twice daily. Only calves for which CBC findings were within reference intervals were included in the study. The experimental protocol was approved by the local animal care and use committee.

Physical examination—Daily physical examinations (including assessments of heart and respiratory rates, attitude, ruminal movements, and rectal temperature) were performed on every calf during the 15-day study period. These assessments were performed at the same times that blood and peritoneal fluid samples were collected. The umbilical stalk was evaluated as previously described.[17] All calves were considered clinically normal and had no detectable heat or firmness of the umbilical stalk before commencement of the study.

Abdominocentesis and peritoneal fluid sample collections—Each calf in both groups underwent peritoneal fluid collections at day 0 (ie, before surgery in the experimental group [baseline]) and at 1, 3, 5, 7, and 15 days after surgery. For the abdominocentesis,[17,19] each calf was sedated with diazepam[b] (0.05 mg/kg, IV) and xylazine[c] (0.05 mg/kg, IM) and positioned in left lateral recumbency with the right hind limb lifted dorsally and caudally. The hair was clipped over an area (approx 15 cm in diameter) slightly dorsal and caudal to the umbilicus. Hair was also clipped over an area (approx 10 to 15 cm in diameter) at a second site in the center of the right inguinal region (10 cm caudal to the umbilical scar and 10 cm lateral to the midline toward the right inguinal region). The skin at both sites was prepared aseptically.[d] The first site for abdominocentesis (4 to 5 cm dorsal from the umbilical scar toward the right abdominal wall) was injected SC with 1 mL of 2% lidocaine hydrochloride.[e] A 14-gauge, 5-cm needle[f] (assembled with a polyurethane catheter inside a sterile nylon sleeve) was inserted into the anesthetized site in a direction slightly caudal and toward the midline; the needle was kept parallel to the inner abdominal wall once the abdominal cavity was penetrated. A polyurethane catheter[f] (30.4 cm) was passed through the needle, and a 10-mL sterile syringe was attached to the catheter; gentle aspiration was used to obtain peritoneal fluid. While applying gentle aspiration on the syringe, the catheter was inserted gently to its full length dorsally, caudally, and ventrally within the abdominal cavity to maximize fluid retrieval. When the sample volume obtained from the first site was insufficient or if it was not possible to collect any fluid, aspiration was performed at the center of the previously prepared inguinal site (second site) by use of the same technique. Care was taken to maintain the catheter tip parallel to the inner abdominal wall from the time of insertion until removal of the needle.

Peritoneal fluid sample analysis—After collection, the peritoneal fluid was transferred into a tube containing EDTA[g] and immediately submitted to the laboratory for cytologic and biochemical analyses. Peritoneal fluid assessment included physical characteristics (color and turbidity) and laboratory measurements (total and differential cell counts, total protein concentration, fibrinogen concentration, specific gravity, and activities of CK, AST, and LDH).

To determine peritoneal fluid total protein concentration and specific gravity, the supernatant obtained after centrifugation at 200 × *g* for 5 minutes was assessed by use of a light refractometer.[h] Peritoneal fluid fibrinogen concentration was determined by use of the heat-precipitation-refractometry method.[20] Activities of enzymes (CK, AST, and LDH) were determined by use of an automated biochemistry analyzer[i]; the analyzer was calibrated before each enzyme assay run with an internal reaction calibrator,[j] and the reactions were monitored with a level I[k] control sample and a level II[l] control sample. Enzyme activity analyses were performed via a continuous kinetic method at 37°C involving commercial assay kits for CK,[m] AST,[n] and LDH.[o] Total counts of nucleated cells were determined by use of a Neubauer chamber. Cytologic evaluation and differential determination of cells were performed on slide preparations of samples following staining with panotic dye (Romanowsky staining).[p] Differential counting of 100 cells was performed, and nucleated cells were classified into 1 of 3 categories: polymorphonuclear cells (neutrophils, basophils, and eosinophils), mesothelial cells or macrophages, and lymphocytes.

Blood sample analysis—Each time that a sample of peritoneal fluid was collected, a blood sample was obtained via jugular venipuncture and placed into a sterile tube[g] containing EDTA. A CBC was performed by use of a veterinary automated blood counter[q] that was calibrated for bovids, according to the manufacturer's instructions. Cytologic and differential evaluations were performed and plasma total protein concentration, fibrinogen concentration, and activities of enzymes (CK, AST, and LDH) were determined as described for peritoneal fluid samples.

Surgical procedure for repair of umbilical hernias—Calves in the experimental group were sedated with diazepam[b] and xylazine[c] and then positioned in dorsal recumbency. Surgical intervention was performed as previously described.[21,22] Briefly, the ventral aspect of the abdomen was clipped over an area extending from sternum to pubis and 15 cm lateral to the midline on each side. Skin and abdominal wall surrounding the hernial sac were infiltrated with 2% lidocaine hydrochloride,[e] and skin was scrubbed with povidone-iodine solution.[d] An elliptical incision was made through the skin and centered around the hernial sac. Hemorrhage was controlled, and connective tissue around the base of the hernial ring was freed from the sac via blunt dissection with Metzenbaum scissors. After elevating the sac by use of Allis forceps, a 3-cm incision over the midline cranial to the hernia base was made into the abdominal wall and peritoneum. The umbilical area was explored digitally from the peritoneal side to determine involvement of additional structures. When no adhesions were present, the incision was extended around the base of the hernial sac. The hernia opening was closed with size-2 polyester suture[r] in an interrupted cruciate pattern. Subcutaneous tissue was closed with 2-0 polyglactin 910 suture[s] in a Cushing-type pattern. Finally, skin was closed with 2-0 nylon suture[t] in an interrupted horizontal mattress (Wolff) pattern.

Long-acting oxytetracycline[u] (20 mg/kg, IM, q 48 h) was administered for 10 days to each calf that underwent surgery to prevent infection. Each surgical wound was cleaned with povidone-iodine solution[e] twice daily. Sutures were removed 10 days after surgery.

Statistical analysis—A computer software program[v] was used for statistical calculations. An ANOVA was performed to determine whether there were any significant differences between groups, and mean values were compared by use of a Tukey test. Nonparametric data were analyzed by use of the Friedman test. Differences between median values for multiple comparisons between days within each group were compared by use of the Dunn test. A Mann-Whitney *U* test was performed to compare group values from the same day, and a Wilcoxon rank sum test[w] was used to evaluate whether there were any differences between values of blood or plasma and peritoneal fluid variables in both groups. Data are presented as mean ± SD or median (range). A value of $P \leq 0.05$ was considered significant for all tests.

Results

Physical evaluation—Calves remained alert, and feed, water, and milk intakes did not change during the study. Complications from the herniorrhaphy or abdominocentesis were not observed in any study calf.

During the study, calves developed mild alterations in heart rate, respiratory rate, and rectal temperature (**Table 1**). Values for heart rate were significantly increased in calves of the experimental group at all times, compared with heart rate in the control group calves. Heart rate was lower for each group on day 15, compared with the value on day 1. In calves of the experimental group, respiratory rate was significantly greater before and at 1 and 5 days after surgery, compared with findings in the control group calves. Compared with the respective baseline values, respiratory rate was significantly decreased on day 5 in the control group and at 3 and 7 days after surgery in the experimental group.

Mean ± SD rectal temperature was 38.92 ± 0.22°C and 39.01 ± 0.51°C for the control and experimental groups, respectively. On day 7, the value in experimental group calves was significantly increased, compared with the value in control group calves (39.99 ± 0.48°C and 38.50 ± 0.23°C, respectively). Over the 15-day study period, no significant change in rectal temperature was detected in the control group calves. However, rectal temperature was significantly lower 15 days after surgery in the experimental group (38.57 ± 0.31°C), compared with baseline values.

Surgical procedures—In 3 (1 male and 2 female twins) of 10 calves with umbilical hernias, omentum

Table 1—Mean ± SD heart and respiratory rates in 20 young Holstein calves without (control group; n = 10) and with congenital umbilical hernias (experimental group; 10) before (baseline) and at intervals after herniorrhaphy performed on day 0.

Variable	Day	Control group	Experimental group
Heart rate (beats/min)	Baseline	65.2 ± 12.4[a,b]	74.7 ± 6.0[a,b,*]
	1	68.2 ± 12.0[a]	77.8 ± 9.6[a,*]
	3	64.8 ± 14.5[a,b]	75.0 ± 8.9[a,b,*]
	5	65.6 ± 16.2[a,b]	77.6 ± 9.7[a,*]
	7	68.4 ± 11.7[a]	77.0 ± 11.7[a,*]
	15	62.0 ± 9.8[b]	70.8 ± 7.8[b,*]
Respiratory rate (breaths/min)	Baseline	23.6 ± 6.9[a]	26.9 ± 3.5[a,b,*]
	1	22.4 ± 2.8[a]	28.6 ± 4.4[a,*]
	3	23.2 ± 5.6[a]	23.2 ± 4.5[c]
	5	18.8 ± 3.8[b]	24.8 ± 4.1[b,c,*]
	7	22.0 ± 2.1[a]	22.4 ± 4.7[c]
	15	21.6 ± 2.1[a,b]	24.0 ± 2.7[b,c]

*Within a row for a given variable, values in the 2 groups differ significantly ($P \leq 0.05$; Tukey test).

[a–c]Within a column for a given variable, values with different superscript letters differ significantly ($P \leq 0.05$; Tukey test).

was adhered to the base of the hernia and had to be partially resected before the sac could be discarded. Only the male calf had an irreducible hernia before surgery.

Peritoneal fluid sample collection—Among the 10 calves with umbilical hernias, the combination of diazepam and xylazine was effective in providing sedation to allow collection of peritoneal fluid from the predetermined sites. There was no evidence that accidental puncture of abdominal viscera occurred. Peritoneal fluid samples were successfully obtained in 119 of 120 collection procedures. Of the 119 successful procedures, 103 (86.55%) samples were collected at the first site; 16 (13.45%) were collected at the second site because the volume recovered at the first site was not sufficient to perform the laboratory analyses. The volume of peritoneal fluid collected during each procedure varied from 1 to 5 mL.

Peritoneal fluid findings—Peritoneal fluid color varied from pale yellow to pink, and samples were slightly cloudy in control group calves throughout the study and in calves with umbilical hernias before surgery. After herniorrhaphy, peritoneal fluid in calves of the experimental group changed to orange and cloudy during the first 24 hours after surgery, then became reddish and turbid until day 7. On day 15, the color and turbidity of peritoneal fluid samples from calves in the experimental group were not different from the findings in the baseline samples of both groups.

Peritoneal fluid analysis revealed differences in total protein concentration, fibrinogen concentration, specific gravity, NCC, and polymorphonuclear cell and lymphocyte counts between calves with or without umbilical hernias at various time points in the study (**Tables 2 and 3**).

Total protein concentration in peritoneal fluid of calves in the experimental group was significantly increased at all time points after surgery, compared with values in the calves in the control group. Also, compared with baseline values, total protein concentration was increased at days 1 through 5 in both groups. Fibrinogen concentration in peritoneal fluid was higher at days 5 and 7 in the experimental group than it was in the control group. Total protein concentration and fibrinogen concentration in peritoneal fluid were significantly lower than the values in plasma in both groups.

Specific gravity of peritoneal fluid was significantly increased throughout the study in calves with umbilical hernias, compared with findings in unaffected calves. However, specific gravity of peritoneal fluid after surgery in the experimental group or after repeated paracentesis in the control group did not differ significantly from their respective baseline values. There was a significant (r^2 = 0.993; $P < 0.001$) linear correlation between mean total protein concentration and mean specific gravity in peritoneal fluid in both groups from days 1 through 15.

In the experimental group, total NCCs in peritoneal fluid were greater than the baseline value on days 1, 3, and 7 after surgery because of increased polymorphonuclear cell counts. No changes in peritoneal fluid lymphocyte and mesothelial or macrophage cell counts were observed in calves with umbilical hernias over time after surgery.

The peritoneal fluid NCCs in the experimental group calves were different from values in control group calves at days 1 through 5 after surgery; however, polymorphonuclear cell counts remained higher in the experimental group than in the control group from day 1 until day 15. Interestingly, the peritoneal fluid NCCs in calves with umbilical hernias were not different from their respective blood WBC counts at all time points after surgery. The lymphocyte counts in peritoneal fluid were significantly greater in the experimental group calves on days 3, 7, and 15 after surgery, compared with counts in the control group calves at the same time points. In both groups, these values were consistently lower in the peritoneal fluid samples than in blood samples.

In the control group, the peritoneal fluid NCC did not change from baseline over time, and these values were lower in peritoneal fluid than in blood on days 0, 1, 3, and 15. Similarly, no changes in polymorphonuclear, lymphocyte, and mesothelial or macrophage cell counts were observed in the control group during the 15-day study period. Median values for RBC counts in peritoneal fluid in the control or experimental groups

did not differ significantly over the 6 sampling times within a group or between groups during the study.

Biochemical variables in peritoneal fluid were assessed (**Table 4**). Compared with findings in the control group calves, median peritoneal fluid activities of AST and CK were greater at baseline in experimental group calves; at days 1, 3, 5, 7, and 15, activities of LDH, AST, and CK were greater in calves that had undergone surgery. Also, LDH activity in the peritoneal fluid was significantly increased at day 5 in calves in the experimental group, compared with baseline.

Blood and plasma findings—Compared with baseline values, significant increases in RBC count, Hct values, and hemoglobin concentration were detected in the experimental group at day 15 after surgery (Table 2). In control group calves, the RBC count and Hct values were decreased from baseline at days 5 through 15 and at days 5 and 7, respectively. At day 3, hemoglobin concentration was significantly increased from baseline in control group calves. Compared with control group findings, significant increases in RBC count, Hct, and hemoglobin concentration were evident in experimental group calves at days 3 and 5, at day 7, and at days 7 and 15, respectively.

Plasma total protein concentration in calves in the experimental group was significantly increased at baseline and at days 1, 7, and 15, compared with values in the calves in the control group. Plasma fibrinogen concentration was greater in experimental group calves than in control group calves at day 7.

No significant differences in total WBC counts in blood samples were detected between groups during the 15-day study period. Neutrophils were the predominant leukocyte; counts in the experimental group were increased at days 1, 5, and 15, compared with counts in the control group. There were no significant differences in counts of band cells, basophils,

Table 2—Variables assessed in samples of peritoneal fluid and whole blood obtained from 20 young Holstein calves without (control group; n = 10) and with congenital umbilical hernias (experimental group; 10) before (baseline) and at intervals after herniorrhaphy performed on day 0. Data are presented as mean ± SD or median (range).

		Peritoneal fluid		Blood	
Variable	**Day**	**Control group**	**Experimental group**	**Control group**	**Experimental group**
RBC count ($\times 10^6$ cells/µL)	Baseline	0.07 (0.00–0.19)	0.04 (0.00–0.31)	8.82 ± 1.27[a]	8.60 ± 2.24[b]
	1	0.12 (0.01–0.56)	0.19 (0.04–1.06)	8.36 ± 1.71[a,b]	8.70 ± 1.81[b]
	3	0.10 (0.01–0.34)	0.13 (0.05–1.32)	8.26 ± 1.23[a,b]	9.05 ± 1.59[b,*]
	5	0.08 (0.01–1.14)	0.12 (0.06–0.49)	7.89 ± 1.38[b]	9.10 ± 1.56[b,*]
	7	0.06 (0.04–0.79)	0.10 (0.02–0.20)	7.83 ± 1.09[b]	8.77 ± 1.54[b]
	15	0.05 (0.01–0.68)	0.06 (0.01–0.18)	7.74 ± 1.39[b]	9.85 ± 1.84[a]
Hct (%)	Baseline	—	—	31.2 ± 4.2[a]	30.3 ± 7.1[b]
	1	—	—	31.0 ± 4.4[a,b]	30.4 ± 6.1[b]
	3	—	—	31.1 ± 4.1[a]	30.9 ± 4.8[a,b]
	5	—	—	29.2 ± 2.8[b,c]	30.6 ± 4.1[a,b]
	7	—	—	28.9 ± 4.0[c]	31.1 ± 3.1[a,b,*]
	15	—	—	31.3 ± 3.9[a]	32.4 ± 4.2[a]
Hemoglobin (g/dL)	Baseline	—	—	9.36 ± 1.58[b]	10.01 ± 2.47[b,c]
	1	—	—	9.99 ± 1.45[a,b]	10.34 ± 1.94[b]
	3	—	—	10.25 ± 1.20[a]	10.37 ± 1.69[b]
	5	—	—	9.30 ± 0.95[b]	9.64 ± 1.67[c]
	7	—	—	9.41 ± 1.29[b]	10.38 ± 0.91[b,*]
	15	—	—	9.31 ± 1.35[b]	11.09 ± 1.40[a,*]
Total protein (g/dL)	Baseline	1.51 ± 0.45[b,†]	1.93 ± 0.61[b,‡]	6.12 ± 0.67	6.48 ± 0.52[a,b,*]
	1	2.35 ± 0.74[a,†]	3.31 ± 1.10[a,*,‡]	6.14 ± 0.73	6.53 ± 0.73[a,b,*]
	3	2.30 ± 0.76[a,†]	3.43 ± 0.81[a,*,‡]	6.24 ± 0.65	6.39 ± 0.60[a,b]
	5	2.31 ± 0.71[a,†]	3.63 ± 1.09[a,*,‡]	6.04 ± 0.54	6.33 ± 0.58[b]
	7	1.94 ± 0.54[a,b,†]	2.82 ± 1.11[a,b,*,‡]	6.12 ± 0.61	6.69 ± 0.45[a,*]
	15	2.15 ± 0.65[a,b,†]	2.83 ± 0.70[a,b,*,‡]	6.20 ± 0.66	6.59 ± 0.46[a,b,*]
Fibrinogen (g/dL)	Baseline	0.10† (0.05–0.30)	0.15‡ (0.05–0.40)	0.40 (0.20–0.80)	0.40 (0.10–0.60)
	1	0.15† (0.05–0.30)	0.10‡ (0.05–0.50)	0.40 (0.05–0.70)	0.60 (0.10–0.80)
	3	0.15† (0.05–0.20)	0.30‡ (0.05–0.60)	0.40 (0.10–0.90)	0.45 (0.30–1.00)
	5	0.10† (0.05–0.10)	0.20*,‡ (0.05–0.60)	0.40 (0.20–0.60)	0.45 (0.30–0.80)
	7	0.10† (0.05–0.20)	0.20*,‡ (0.05–0.60)	0.30 (0.20–0.60)	0.65* (0.40–0.80)
	15	0.10† (0.01–0.20)	0.10‡ (0.05–0.20)	0.50 (0.10–0.60)	0.40 (0.10–0.70)
Specific gravity	Baseline	1.016 ± 0.002	1.024 ± 0.010*	—	—
	1	1.020 ± 0.003	1.026 ± 0.007*	—	—
	3	1.020 ± 0.004	1.026 ± 0.005*	—	—
	5	1.020 ± 0.004	1.028 ± 0.006*	—	—
	7	1.019 ± 0.003	1.023 ± 0.007*	—	—
	15	1.020 ± 0.004	1.024 ± 0.005*	—	—

*Within a row for a given variable in peritoneal fluid or blood, values in the 2 groups differ significantly ($P \leq 0.05$; Mann-Whitney *U* test for comparisons between calves in the control and experimental groups). †Within the control group, the value for this variable in peritoneal fluid is significantly ($P \leq 0.05$) different from the value in blood at the same time point (Wilcoxon rank sum test). ‡Within the experimental group, the value for this variable in peritoneal fluid is significantly ($P \leq 0.05$) different from the value in blood at the same time point (Wilcoxon rank sum test).

— = Not done.

[a–c]Within a column for a given variable in peritoneal fluid or blood, values with different superscript letters differ significantly ($P \leq 0.05$; Dunn test for comparisons among days); values with no superscript letters do not differ significantly.

eosinophils, and lymphocytes between the control and experimental groups throughout the study period. However, calves in the experimental group had an increase in monocyte count in blood samples on day 3 after surgery, whereas mean monocyte count did not differ significantly over time in control group calves.

Although plasma AST and LDH activities varied moderately, a significant difference in either activity was not identified between groups at baseline or days

Table 3—White blood cell counts assessed in samples of peritoneal fluid and whole blood obtained from 20 young Holstein calves without (control group; n = 10) and with congenital umbilical hernias (experimental group; 10) before (baseline) and at intervals after herniorrhaphy performed on day 0. Data are reported as mean ± SD or median (range).

		Peritoneal fluid		Blood	
Variable	**Day**	**Control group**	**Experimental group**	**Control group**	**Experimental group**
NCC (peritoneal fluid) or WBC count (blood [× 10^3 cells/μL])	Baseline	3.75† (1.20–8.70)	4.55[c,‡] (0.24–12.90)	9.00[a,b] (5.60–21.10)	11.40[a,b] (4.20–20.40)
	1	5.50† (1.40–20.90)	15.25[a,*] (0.55–84.40)	8.23[a,b] (4.60–18.10)	11.78[a,b] (3.30–21.70)
	3	3.78† (1.05–16.50)	12.80[a,b,*] (4.60–31.50)	9.73[a] (5.60–19.90)	13.30[a] (5.65–22.80)
	5	5.53 (1.00–27.50)	9.38[a,b,c,*] (6.90–15.00)	8.00[a,b] (4.15–16.10)	11.98[a,b] (7.05–18.30)
	7	7.40 (0.70–14.70)	10.95[a,b] (4.90–16.50)	8.10[a,b] (4.35–17.00)	10.95[a,b] (5.90–22.40)
	15	4.88† (0.91–17.60)	6.70[b,c] (4.38–11.70)	7.47[b] (5.10–16.50)	9.80[b] (5.60–14.70)
Neutrophil count (× 10^3 cells/μL)	Baseline	—	—	2.54 (1.03–4.85)	3.47 (0.59–9.59)
	1	—	—	2.49 (0.60–4.32)	4.52* (0.79–9.94)
	3	—	—	2.42 (1.19–9.09)	4.16 (1.24–12.08)
	5	—	—	2.48 (0.62–3.57)	4.72* (2.26–10.74)
	7	—	—	1.79 (0.81–6.97)	3.29 (1.15–9.30)
	15	—	—	1.64 (0.74–6.54)	2.45* (1.74–6.45)
Band cell count (× 10^3 cells/μL)	Baseline	—	—	0.00 (0.00–0.17)	0.00 (0.00–0.00)
	1	—	—	0.00 (0.00–0.10)	0.00 (0.00–0.11)
	3	—	—	0.00 (0.00–0.06)	0.00 (0.00–0.46)
	5	—	—	0.00 (0.00–0.09)	0.00 (0.00–0.41)
	7	—	—	0.00 (0.00–0.17)	0.00 (0.00–0.22)
	15	—	—	0.00 (0.00–0.10)	0.00 (0.00–0.10)
Basophil count (× 10^3 cells/μL)	Baseline	—	—	0.00 (0.00–0.00)	0.00 (0.00–0.09)
	1	—	—	0.04 (0.00–0.11)	0.00 (0.00–0.18)
	3	—	—	0.00 (0.00–0.00)	0.00 (0.00–0.10)
	5	—	—	0.00 (0.00–0.00)	0.00 (0.00–0.12)
	7	—	—	0.00 (0.00–0.6)	0.00 (0.00–0.17)
	15	—	—	0.00 (0.00–0.00)	0.00 (0.00–0.10)
Eosinophil count (× 10^3 cells/μL)	Baseline	—	—	0.00 (0.00–0.27)	0.00 (0.00–0.20)
	1	—	—	0.08 (0.00–0.32)	0.00 (0.00–0.22)
	3	—	—	0.12 (0.00–0.23)	0.13 (0.00–0.62)
	5	—	—	0.02 (0.00–0.32)	0.10 (0.00–0.20)
	7	—	—	0.05 (0.00–0.33)	0.09 (0.00–0.21)
	15	—	—	0.04 (0.00–0.28)	0.04 (0.00–0.33)
Lymphocyte count (× 10^3 cells/μL)	Baseline	0.30† (0.05–1.15)	0.46‡ (0.02–1.91)	6.62 (3.53–14.98)	6.78 (3.28–10.60)
	1	0.73† (0.14–14.17)	0.73‡ (0.05–5.60)	5.15 (3.00–14.84)	7.10 (2.01–11.94)
	3	0.34† (0.03–2.32)	2.11*,‡ (0–4.75)	6.44 (3.02–15.52)	7.64 (3.84–11.52)
	5	0.77† (0.20–3.85)	1.10‡ (0.35–4.40)	4.96 (2.08–11.11)	6.83 (2.83–9.88)
	7	0.59† (0.09–1.78)	1.64*,‡ (0.35–6.10)	5.60 (2.48–13.86)	7.11 (3.14–11.76)
	15	0.46† (0.20–4.22)	1.17*,‡ (0.42–3.89)	5.40 (3.12–13.37)	6.46 (3.06–9.94)
Monocyte count (× 10^3 cells/μL)	Baseline	—	—	0.56 ± 0.38	0.59 ± 0.22[b]
	1	—	—	0.55 ± 0.25	0.55 ± 0.23[b]
	3	—	—	0.60 ± 0.31	0.84 ± 0.50[a]
	5	—	—	0.62 ± 0.18	0.77 ± 0.24[a,b]
	7	—	—	0.53 ± 0.30	0.64 ± 0.26[a,b]
	15	—	—	0.55 ± 0.29	0.58 ± 0.28[b]
Polymorphonuclear cell count (× 10^3 cells/μL)	Baseline	1.87 (0.29–5.57)	2.22[b] (0.03–6.79)	—	—
	1	2.36 (0.55–8.64)	8.27[a,*] (0.35–70.90)	—	—
	3	1.85 (0.14–4.15)	7.92[a,*] (1.90–24.57)	—	—
	5	2.85 (0.47–13.75)	5.21[a,*] (3.19–10.80)	—	—
	7	3.39 (0.20–9.85)	6.56[a,*] (2.11–10.40)	—	—
	15	2.27 (0.30–9.68)	4.00[a,b,*] (2.07–8.89)	—	—
Mesothelial or mononuclear cell count (× 10^3 cells/μL)	Baseline	1.00 (0.63–2.07)	1.73 (0.02–6.32)	—	—
	1	3.08 (0.41–14.21)	3.99 (0.15–9.28)	—	—
	3	1.41 (0.43–12.38)	1.90 (0.00–7.15)	—	—
	5	1.15 (0.34–9.90)	2.26 (0.90–8.59)	—	—
	7	1.80 (0.34–5.44)	1.82 (0.00–4.26)	—	—
	15	1.98 (0.36–3.92)	1.52 (0.83–2.41)	—	—

For peritoneal fluid analysis, numbers of neutrophils were combined with numbers of basophils and eosinophils and reported as polymorphonuclear cell counts.

See Table 2 for key.

Table 4—Biochemical variables assessed in samples of peritoneal fluid and plasma obtained from 20 young Holstein calves without (control group; n = 10) and with congenital umbilical hernias (experimental group; 10) before (baseline) and at intervals after herniorrhaphy performed on day 0. Data are presented as median (range).

		Peritoneal fluid		Blood	
Variable	**Day**	**Control group**	**Experimental group**	**Control group**	**Experimental group**
CK (U/L)	Baseline	21.32 (1.08–1,378.10)	325.14* (31.84–1,022.70)	142.97 (0.55–334.93)	167.56[a,b] (55.25–619.10)
	1	134.61 (16.06–2,141.90)	499.66 (69.35–5,755.40)	164.19 (89.95–484.45)	298.00[a,*] (136.17–1,167.20)
	3	107.64 (37.46–498.74)	387.67* (232.08–8,151.60)	148.62 (6.67–907.83)	195.18[a] (97.30–1,148.70)
	5	55.03 (8.88–594.88)	492.71*,‡ (30.57–1,109.70)	114.12 (0.23–490.29)	157.03[a,b] (52.15–530.58)
	7	31.92 (1.96–207.37)	193.33* (28.09–2,656.30)	109.89 (5.05–405.54)	115.96[a,b] (44.31–269.20)
	15	21.55 (2.96–821.36)	221.71 (14.68–748.76)	93.53 (5.05–405.54)	97.82[b] (44.31–204.38)
AST (U/L)	Baseline	37.20 (27.60–141.80)	55.20* (45.40–74.70)	44.75 (0.90–69.60)	54.05 (18.10–72.30)
	1	72.35 (25.80–244.0)	90.80 (47.90–195.20)	53.45 (11.30–60.50)	57.45 (19.20–80.80)
	3	51.60 (35.40–133.20)	68.40‡ (49.00–357.20)	50.05 (11.50–112.83)	49.90 (23.00–130.20)
	5	39.90 (27.60–78.20)	104.00*,‡ (18.40–371.20)	47.80 (3.00–70.80)	44.85 (16.70–66.70)
	7	29.30 (25.00–118.20)	65.25‡ (28.60–121.60)	44.65 (18.90–72.40)	36.25 (24.90–61.50)
	15	36.20 (25.00–114.40)	58.70‡ (23.40–121.60)	42.45 (18.90–59.50)	47.65 (24.90–57.60)
LDH (U/L)	Baseline	299.5† (163.0–1,900.0)	699.0[b,‡] (286.0–1,033.0)	1,375.5 (194.0–1,900.0)	1,445.5 (1,156.0–2,590.0)
	1	343.5† (12.0–1,659.0)	1,552.5[a,b,*] (447.0–5,238.0)	1,440.5 (638.0–2,314.0)	1,546.5 (1,357.0–2,019.0)
	3	634.0† (456.0–1,612.0)	1,079.0[a,b] (649.0–6,820.0)	1,379.0 (776.0–1,967.0)	1,592.0 (1,238.0–2,134.0)
	5	598.0† (344.0–1,330.0)	2,048.00[a,*] (194.0–6,268.0)	1,474.5 (912.0–2,202.0)	1,376.0 (1,040.0–2,045.0)
	7	284.5† (134.0–1,864.0)	1,490.0[a,b,*] (475.0–2,452.0)	1,376.0 (851.0–2,153.0)	1,442.0 (868.0–2,446.0)
	15	398.0† (130.0–1,864.0)	979.0[a,b,*] (229.0–2,160.0)	1,403.5 (715.0–2,153.0)	1,426.0 (71.0–2,446.0)

See Table 2 for key.

1, 3, 5, 7, or 15 of the study. Plasma CK activity was increased in calves with umbilical hernias at days 1 and 3 after surgery.

Plasma versus peritoneal fluid biochemical findings—At baseline, LDH activity in peritoneal fluid was significantly lower than the activity in plasma in the experimental group. Activities of CK and AST in peritoneal fluid were significantly greater than those in plasma in the experimental group on day 5 and from day 3 through day 15, respectively. In the control group, significant differences between peritoneal fluid and plasma AST activity and between peritoneal fluid and plasma CK activity were not identified over time; however, at baseline and days 1, 3, 5, 7, and 15, LDH activity in peritoneal fluid was significantly lower than that in plasma in this group.

Interestingly, compared with findings in control group calves, plasma fibrinogen concentration in experimental group calves was significantly increased at day 7; however, peritoneal fluid fibrinogen concentration in experimental group calves was significantly increased at day 5 (ie, 48 hours earlier).

Discussion

On the basis of the results of the present study, it appears that the presence of an umbilical hernia has an effect on biochemical values in blood and peritoneal fluid of calves. Likewise, corrective surgery evoked changes in cellular and biochemical variables in both plasma and peritoneal fluid.

At baseline (ie, before surgery), calves with congenital umbilical hernias had greater plasma total protein concentration and CK and AST activities than calves without umbilical hernias. At this time point, the specific gravity of peritoneal fluid samples obtained from affected calves was also greater than the value in peritoneal fluid samples obtained from unaffected calves. However, although the baseline plasma total protein concentrations in calves with hernias were increased, compared with the values in calves in the control group, protein concentrations were within the published reference interval.[17] Plasma total protein concentrations in the experimental group at days 7 and 15 after surgery were greater than those in the control group and were likely attributable to inflammatory processes associated with the surgical procedure.

Because muscle damage may result in marked increases in both serum AST and LDH activities, these activities should be interpreted in conjunction with that of a muscle specific enzyme, such as CK, to determine the source of the tissue insult.[23] Peritoneal fluid AST activity at baseline was higher in experimental group calves than it was in control group calves, which could reflect an increased intestinal permeability.[24] Likewise, the comparatively higher baseline CK activity in peritoneal fluid from the experimental calves may also indicate an alteration in the muscular fibers of the abdominal wall or intestinal loops. Because it was subsequently determined that the intestinal loops were not incarcerated in the hernial sac in any calf and were apparently normal on inspection during surgery, we believe that the baseline peritoneal fluid CK activity was increased in affected calves as a result of an inflammatory process associated with the hernia.

Specific gravity values in peritoneal fluid were increased throughout the study in the experimental group, compared with findings in the control group, which supports a direct effect of the umbilical hernia. The high values detected after surgery were evidence of a local inflammatory response and were greater than those reported for older cattle after laparotomy.[10] Total protein concentrations in peritoneal fluid samples from calves with hernias were also increased (compared with unaffected calves) in concert with the specific gravity values throughout the study, as previously described.[24]

At various time points after surgery, the peritoneal fluid total protein and fibrinogen concentrations; nucleated cell, polymorphonuclear cell, and lymphocyte counts; specific gravity; and LDH, AST, and CK activities were significantly increased in calves with umbilical hernias, compared with values in unaffected calves. Some of these variables (total protein concentration, neutrophil count, and CK activity) were significantly increased in plasma samples from calves with umbilical hernias, compared with the values in plasma samples from calves without umbilical hernias. These findings in both peritoneal fluid and plasma are consistent with a mild inflammatory response to the surgery,[25] mainly during the first week of the study. Oxytetracycline injections can contribute to increases in serum CK or AST activity,[26–28] and may have contributed to the minor elevations in CK and AST activities in the calves with umbilical hernias after surgery.

Total protein concentration in peritoneal fluid of calves after surgery (days 1 through 15) was greater than values previously reported for calves in the same age range,[19] parturient cows,[13] 8-week-old calves,[16] and cows after exploratory laparotomy.[10] Total protein concentrations and fibrinogen concentrations in peritoneal fluid in calves after herniorrhaphy in the present study (days 0 through 15) were similar to values in healthy horses[24] but lower than values in foals[29] after abdominal surgery. Fibrinogen concentrations in peritoneal fluid samples obtained from calves with umbilical hernias were significantly higher at days 5 and 7 after surgery than those detected in unaffected calves; even though these values were within reference ranges,[17] measurement of fibrinogen concentrations in peritoneal fluid can be used to evaluate the inflammatory process during a normal surgical reaction, and the magnitude of the response is typically directly related to the invasiveness and duration of the procedure.[24]

Another diagnostic measure of an inflammatory response to disease is plasma fibrinogen concentration.[30] Assessment of this variable may be used as an adjunct to leukocyte count determination and is sometimes included in hematologic analysis of bovine blood samples.[31] In the present study, plasma fibrinogen concentration in calves in the experimental group was significantly increased, compared with the value in calves in the control group, at day 7; however, peritoneal fluid fibrinogen concentration in calves in the experimental group was significantly increased, compared with the value in calves in the control group, at day 5 (ie, 48 hours earlier). Thus, fibrinogen concentration in peritoneal fluid appears to be an important indicator of the inflammatory response during the postoperative period in calves undergoing abdominal surgery. It is possible that peritoneal fluid analysis would be a better diagnostic test for detection of peritonitis than plasma fibrinogen determination in cattle.[13]

Compared with control group data, neutrophil counts in blood samples obtained from the experimental group were significantly higher at days 1, 3, 5, and 15; nevertheless, these values remained within the reference intervals reported for calves and adult cattle,[16] young calves,[17,19] and Holstein bulls.[30,32] On the other hand, band cell, lymphocyte, monocyte, eosinophil, and basophil counts did not differ between the 2 groups during the study period and remained within reference intervals.[16,19,30,32] These facts reinforce the idea that surgical stimulus in calves with umbilical hernias caused only a mild alteration in hematologic variables during the inflammatory process in the abdominal cavity.

In cattle, peritoneal fluid with an NCC > 6,000 cells/μL in addition to a protein content > 3 g/dL is associated with peritonitis.[12] Cattle with peritonitis usually do not attain the extremely high peritoneal fluid NCCs detected in horses with peritonitis (ie, 50,000 to 100,000 cells/μL); cattle with peritonitis typically have a mean NCC of only 14,700 cells/μL.[12,13] In our study, NCC in peritoneal fluid in calves with umbilical hernias peaked 24 hours after surgery, then slowly decreased until day 15 (at which time the value was not significantly different from that in control group calves), indicating a decrease in the local inflammatory response. At 24 hours after surgery, NCC in the peritoneal fluid samples obtained from calves with umbilical hernias was increased, compared with the value in samples obtained from unaffected calves; the polymorphonuclear cell count and total protein concentration at day 1 were also significantly greater for calves with hernias than values for unaffected calves. These data support other reports[12,29] that surgical intervention causes a rapid, peritoneal inflammatory reaction. Values of NCC in peritoneal fluid in control group calves were similar to those reported previously for healthy calves or cows,[10,16,17,19] whereas the values in calves after abdominal surgery were more similar to NCCs in pregnant cows but lower than values in cattle with peritonitis,[13] cattle with nonseptic peritonitis,[12] and cattle at 24 to 48 hours after laparotomy.[10]

Peritoneal phagocytes such as macrophages and emigrated neutrophils are vital in the local host defense against peritoneal infection.[25] During the present study, counts of polymorphonuclear cells, mesothelial cells or macrophages, and lymphocytes were similar to values in cows before and after celiotomy and omentopexy.[10] In addition, lymphocyte counts were greater than those reported for young clinically normal calves.[17,19] This rapid movement of neutrophils and later macrophages is an important mechanism of control against infection.[33]

The increased LDH activity in peritoneal fluid at day 1 in the experimental group calves, compared with the value in the control group calves, was presumably a result of the release of this enzyme from polymorphonuclear cells in the abdominal cavity or its production by the peritoneum[34] after surgical trauma. Alternatively, release of these enzymes from RBCs could contribute to the increases in both peritoneal fluid AST (at days 0 and 5) and LDH activities.[23]

At the time points at which LDH activity in peritoneal fluid from calves after herniorrhaphy was increased (compared with control group values), so were counts of polymorphonuclear cells. It is believed that both polymorphonuclear cells and peritoneal injury after surgery[35] may have contributed to increased peritoneal fluid LDH activity observed in these calves. Thus, polymorphonuclear cells, rather than RBCs, were considered the source for the in-

creased peritoneal fluid LDH activity because there were no significant differences in RBC counts in the control and experimental groups, the samples were centrifuged with rapid recovery of supernatant, and there was no hemolysis observed in the samples. Furthermore, we had previously determined that serial paracentesis did not induce tissue injury[17] and consequently did not alter peritoneal fluid LDH activity in these calves, as others[35] have hypothesized.

In healthy horses, activity of AST in peritoneal fluid is significantly less than that of serum activity, and high AST peritoneal fluid activity has been suggested to potentially reflect damage to the liver and intestinal mucosa.[24] When evaluated in conjunction with peritoneal fluid LDH and CK[23] activities, the increases (compared with control group findings) in these enzyme activities in calves with umbilical hernias are indicative of a mild inflammatory response associated with manipulation of the abdominal wall and subsequent injury caused by surgery. Similar to other variables described previously, it is the authors' opinion that measurement of these enzymes in samples of peritoneal fluid is more reliable than their measurement in samples of plasma when evaluating potential abdominal cavity disease or assessing postoperative recovery in calves.

During the physical examination, heart rate was increased at baseline and throughout the study and respiratory rate was increased at baseline and early in the study period in the experimental group calves, compared with findings in control group calves; however, these values were within the reference range for calves.[36,37] These slight differences could be a result of higher levels of metabolism in the experimental group calves (because they were slightly younger than those in the control group) or a result of the stress caused by the surgery.[33]

In contrast to goats after enterectomy,[11] fever was not detected in the calves that underwent surgery at any time point throughout the study. A febrile response is initiated by the introduction of an exogenous pyrogen to the body.[36] Because the calves were administered an antimicrobial (in contrast to treatment of goats in a previous study[11]) and hemostasis was performed during surgery, the release of these pyrogens was minimal and not sufficient to increase body temperature. Also, a normal daily variation in body temperature is expected and body temperature typically decreases as animals grow older.[37]

No hemoparasites or intestinal parasites were detected in any of the calves, and the few erythrocytes detected in peritoneal fluid samples did not characterize hemorrhage. Changes in hemoglobin concentration with a decrease in RBC counts in the circulation are characteristics of maturity.[32,38] The changes may have been altered or masked in those calves that underwent surgery and developed inflammation. Nevertheless, these small variations were within reference limits for calves in this age range.[17,32]

Blood WBC counts in the experimental group calves were within reference range throughout the study period,[16,17,19,30,31] and peritoneal fluid analysis did not reveal significant changes (from baseline) in NCC and polymorphonuclear cell and lymphocyte counts in the control group calves. Nonetheless, because significant increases in absolute numbers of leukocytes in peritoneal fluid were not detected over time in control group calves and differences in AST and CK activities between plasma and peritoneal fluid samples were not evident in the same group, we concluded that serial paracentesis per se was not a sufficiently strong stimulus to cause significant changes in peritoneal fluid composition, as previously determined from serial samples obtained with longer intervals between collections.[17]

Total protein concentration of the peritoneal fluid was increased slightly from baseline at days 1, 3, and 5 in control group calves, and this could be a possible sequela of repeated abdominal paracentesis in these calves because of the interval of sample collection (every other day). When paracenteses were performed at an extended interval, the peritoneal fluid total protein concentration returned to baseline.

In clinically normal cattle, peritoneal fluid is colorless to yellow; the fluid changes to light pink during late pregnancy[39–41] and is slightly cloudy and yellow in young calves.[17] The changes in color and turbidity in peritoneal fluid of calves after surgery indicate the presence of blood or free hemoglobin and increased cellularity,[39,40] respectively. When contamination with blood occurs, the color of the peritoneal fluid turns slightly reddish and a low RBC count on cytologic slides is evident, but neither interferes with the interpretation of the peritoneal fluid data, as previously reported.[17] Fifteen days after surgery, the peritoneal fluid recovered its previous characteristics.

The results of the study reported here provide reference intervals for peritoneal fluid variables in calves with umbilical hernias before and 15 days after surgery and in similar-aged healthy control calves for comparison. To evaluate the presence of an inflammatory process in the abdominal cavity of calves, assessments of peritoneal fluid total protein and fibrinogen concentrations, total NCCs (mainly polymorphonuclear cells), and LDH and AST activities are preferable to blood or plasma measurements because peritoneal fluid alterations are more likely to be discernible earlier and to be of a greater magnitude. Also, as this study revealed, serial paracentesis in calves does not appear to cause significant changes in peritoneal fluid variables.

a. Albendathor 10%, Tortuga, Santo Amaro, SP, Brazil.
b. Compaz, Pharmacon, Itapira, SP, Brazil.
c. Coopazine, Coopers, Cotia, SP, Brazil.
d. Riodeíne tópico p.v.p.i. 10%, Rioquímica, São José do Rio Preto, SP, Brazil.
e. Lidovet, Bravet Ltda, Rio de Janeiro, RJ, Brazil.
f. I-Cath, Becton-Dickinson Ltda, Juiz de Fora, MG, Brazil.
g. Vacutainer, Becton Dickinson Vacutainer Systems, Plymouth, England.
h. Refratômetro manual Uridens, Inlab, São Paulo, SP, Brazil.
i. Biosystems model BTS-370 plus, Biosystems, Barcelona, Spain.
j. Calibrator serum, BioSystems, Barcelona, Spain.
k. Assayed control level I, BioSystems, Barcelona, Spain.
l. Assayed control level II, BioSystems, Barcelona, Spain.
m. BioSystems, Barcelona, Spain.
n. Aspartate aminotransferase (AST/GOT), BioSystems, Barcelona, Spain.
o. Lactate dehydrogenase (LDH)-IFCC (γ-GT), BioSystems, Barcelona, Spain.
p. Instant Prov, NEWPROV, Pinhais, PR, Brazil.
q. Abc Vet Animal blood counter, HoribaABx Diagnostics, Montpellier, France.

r. Ethibond Excel, Johnson & Johnson Ltda, São José dos Campos, SP, Brazil.
s. Vycril II, Johnson & Johnson Ltda, São José dos Campos, SP, Brazil.
t. Mononylon, Johnson & Johnson Ltda, São José dos Campos, SP, Brazil.
u. Cyamicina LA 20%, Fort Dodge Ltda, Campinas, SP, Brazil.
v. SAS, version 8, SAS Institute Inc, Cary, NC.
w. ProcNPAR1WAY, SAS Institute Inc, Cary, NC.

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