Peritoneal and Plasma D-lactate Concentrations in Horses with Colic

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Objective: To evaluate the association between peritoneal fluid and plasma D-lactate concentration with variables used in the diagnosis and prognosis of horses with colic.
Animals: Clinically healthy horses (n = 6) and 90 horses with colic.
Study Design: Prospective cross-sectional study.
Methods: D-lactate concentration was determined in peritoneal fluid and plasma of all horses. Information on other blood and peritoneal fluid variables, signalment, results from the physical examination, outcome, need for surgery, lesion location, and type was retrieved from medical records.
Results: Peritoneal D-lactate concentration was strongly correlated with plasma D-lactate concentration (r = 0.71; $P < .001$). Peritoneal and plasma D-lactate concentrations were positively correlated with peritoneal (r = 0.8; $P < .001$) and plasma L-lactate (r = 0.33; $P = .001$) concentrations, respectively. Peritoneal D-lactate concentration was negatively correlated with survival to discharge (U = 430.5; $P < .001$). Median peritoneal D-lactate concentration of horses with septic peritonitis (455.2 μmol/L) and horses with gastrointestinal rupture (599.5 μmol/L) were higher compared with horses with nonstrangulating obstructions (77.7 μmol/L). A cut-off concentration of peritoneal D-lactate of 116.6 μmol/L had a sensitivity of 0.813 and a specificity of 0.651 to differentiate between nonstrangulating and strangulating obstructions.
Conclusions: Peritoneal D-lactate concentration may be more useful for identifying horses with strangulating obstructions (high sensitivity, low probability of a false negative) than to ruling out strangulating obstruction (moderate specificity, high probability of a false positive).

Colic continues to be an important cause of morbidity and mortality in horses. Early and accurate recognition of an ischemic segment of bowel is essential to decrease postoperative complications and increase survival. Diagnosis of ischemic bowel before irreversible damage occurs is difficult because of the nonspecificity of signs of the disease. Elevated L-lactate concentration in peritoneal fluid and plasma has been associated with intestinal ischemia and decreased survival in horses[1–4]; however, elevated L-lactate concentration is not specific to bowel ischemia.[4] Recently, novel biomarkers have been studied in people to assist in the early diagnosis of ischemic intestine.[5,6] Acute intestinal ischemia in laboratory animals has been characterized by bacterial overgrowth and disruption of vascular and mucosal permeability.[7,8] Changes in mucosal permeability and bacterial overgrowth may consequently allow either bacteria or bacterial products to move into the systemic circulation. Therefore, the ability to assay bacterial products in blood or peritoneal fluid may be useful as an early marker of acute mesenteric ischemia.[7,8]

D-lactate is the stereoisomer of mammalian L-lactate and is a product of bacterial fermentation and is not produced by mammalian tissue.[9] Many bacteria found in the gastrointestinal tract including *Klebsiella*, *Escherichia coli*, *Lactobacillus* sp. and *Bacteroides* sp. produce D-lactate.[10,11] Conditions in which bacterial proliferation or translocation occurs (acute intestinal ischemia, intestinal perforation, or septic peritonitis) might cause elevations in D-lactate concentrations.[12] Mammals do not possess the enzyme systems to rapidly metabolize D-lactate and the D-lactate released by bacteria within the gastrointestinal tract will pass through the liver unchanged and appear in the peripheral blood early in those disease processes.[7] Recently, increased plasma D-lactate concentrations have

been reported in people with intestinal ischemia,[5–7,13–15] perforations of the appendix, and septic shock.[16,17] Reported D-lactate concentrations in blood of healthy people or those subjected to nonabdominal elective surgeries are low ranged from means of 55 to 230 μmol/L.[7,14,18]

Increase in plasma D-lactate concentrations have been associated with neonatal calf diarrhea,[19–21] failure of the reticular groove reflex in calves,[22] and grain overload in adult cattle.[23] Plasma D-lactate concentrations in healthy calves is ≤ 2 mmol/L and will increase to > 3 mmol/L in the above conditions to include reported means of 13 mmol/L.[24,25] Plasma D-lactate concentration has been measured in the plasma of horses admitted for colic. No significant difference in plasma D-lactate concentrations between controls and horses admitted for colic was identified.[26] Peritoneal fluid D-lactate concentrations have been reported in people as a useful marker for septic peritonitis.[27,28] We are unaware of studies investigating measurement of peritoneal D-lactate concentration in horses and association of peritoneal and plasma D-lactate with variables used in the diagnosis and prognosis of various causes of equine colic.

Our purpose was to measure peritoneal and plasma D-lactate concentrations in horses admitted with signs of colic, and to determine whether these variables are useful in predicting specific gastrointestinal lesions and outcome. We hypothesized that peritoneal D-lactate concentration is strongly correlated to plasma D-lactate concentration and that D-lactate concentrations in peritoneal fluid and plasma are higher for horses with intestinal ischemic lesions (strangulating obstructions) compared with horses with nonstrangulating gastrointestinal lesions. Finally, we hypothesized that peritoneal fluid and plasma D-lactate would have a strong correlation to peritoneal fluid and plasma L-lactate and that D- and L-lactate would be useful markers for determining presence of strangulating obstructions.

MATERIALS AND METHODS

The study was performed on 6 clinically healthy (control) horses and 90 horses admitted for signs of colic of intestinal origin from August 2007 to November 2009.

All control horses were considered healthy based on physical examination, complete blood count, and serum biochemical profile. Horses determined to have concomitant primary kidney, lung, or liver disease were excluded. All clinical cases had an initial physical examination that included abdominal auscultation and percussion, *per rectum* examination, nasogastric intubation, abdominal radiographs, and abdominal ultrasonographic examination. Venous blood was collected aseptically and submitted for hematology and serum biochemistry. Peritoneal fluid was obtained aseptically in the standing horse by using a stainless-steel teat cannula inserted through a small stab incision on or immediately to the right of the *linea alba* and collected by gravity flow.

Blood and peritoneal fluid for the determination of D- and L-lactate concentrations were collected into Vacutainer tubes containing lithium heparin (BD Vacutainer, Franklin Lakes, NJ). Samples were analyzed for L-lactate concentration within 10 minutes of harvesting by using bench top gas and biochemistry analyzer ABL 700 (Radiometer America Inc., Westlake, OH). Other variables recorded for peritoneal fluid included gross appearance (normal or abnormal), total protein concentration, nucleated cell count, glucose concentration, and pH.

The probability of the horse having a strangulating obstruction (prediction of ischemia) was calculated as previously described by using variables from the peritoneal fluid analysis (gross appearance, lactate concentration, pH, and chloride concentration).[1] Individual horse data collected from the medical record included age, sex and breed, duration of colic signs before admission, respiratory and heart rates, capillary refill time (seconds), peripheral packed cell volume (PCV), serum total protein concentration, and total nucleated cell count at admission to the clinic. Necessity of surgical intervention for resolution of the problem and outcome (survival to discharge) were also recorded.

Horses were grouped according to location of lesion (stomach or small intestine, large colon and cecum, small colon or peritoneum) based on rectal examination, radiographs, surgery, or necropsy findings. Horses where the location of primary problem could not be identified were excluded from the location of lesion analysis. All clinical horses in this study were also grouped based on type of primary lesion: nonstrangulating obstruction, strangulating obstruction, septic peritonitis, and gastrointestinal ruptures. Nonstrangulating obstructions included horses with a nonstrangulating lesion diagnosed at surgery or necropsy, and horses diagnosed with impaction, gas, or spasmodic colic. Horses with an unidentified cause for colic that responded to medical treatment were also included in the nonstrangulating group. Strangulating obstructions included horses with intestinal strangulating obstructions producing intestinal necrosis diagnosed at surgery or necropsy. Septic peritonitis included horses with a positive growth on bacterial culture of peritoneal fluid, or horses with high total nucleated cell count ($> 100{,}000$ cells/μL) in the peritoneal fluid and 2 of the following peritoneal fluid variables (differential count of $> 90\%$ neutrophils, glucose < 30 mg/dL, pH < 7.3) indicative of septic peritonitis. Gastrointestinal ruptures included horses diagnosed at necropsy or when gastrointestinal contents were obtained with an ultrasound guided abdominocentesis. Horses that did not fit the above lesion type group were excluded. The group of 6 healthy horses was introduced in this study to compare the peritoneal and plasma D-lactate of this control group with the various lesion types in the clinical cases described above.

Sample Preparation and Quantification of D-Lactate

Peritoneal fluid and blood samples collected in Vacutainer tubes containing lithium heparin were centrifuged

($1000 \times g$) for 5 minutes within 10 minutes of collection. Supernatants were kept frozen at -80°C until further analysis (up to 30 days). Before D-lactate measurements, protein was removed from samples (300 μL plasma) by ultrafiltration through a cellulose membrane (Millipore Corp. Billerica, MA) by centrifugation ($12{,}000 \times g$) for 90 minutes as recommended by the manufacturer.

A commercial D-lactate analysis kit (Megazyme International, Wicklow, Ireland) was used according to the manufacturer's recommendations.[29] All calculations were made automatically by the analyzer's software (MegaCalc. Megazyme International). The limit of detection of the tests is 2.38 μmol/L; a value of "0" was used for measurements below the limit of detection.

Statistical Analysis

According to skewness and kurtosis statistics, the distribution of peritoneal and plasma concentrations of D-lactate depart from normality, for which transformation $(x+1)^{0.1}$ was required. To measure the strength of the association between peritoneal and plasma D-lactate concentrations, the Spearman rank correlation coefficient was performed for horses with colic and controls. For horses with colic, the correlation between peritoneal and plasma concentrations of D-lactate and the independent variables was calculated using the appropriate statistical tests. A Spearman's test was used to analyze all continuous variables. A 1 way ANOVA test followed by Tukey's *post hoc* test was used to analyze lesion location and lesion type. A Mann–Whitney test was used to analyze the remaining categorical variables.

A receiver–operator curve was constructed to facilitate the identification of a cut-off value that maximized sensitivity and specificity when using peritoneal fluid and plasma D-lactate concentrations to differentiate between horses with strangulating and nonstrangulating obstructions. The concentration that gives the best cut-off value was selected based on the highest value for κ. Using the same clinical data, sensitivity and specificity for plasma and peritoneal L-lactate were calculated to compare with the D-lactate values. Statistical analysis was performed by using commercial software (SAS, Cary, NC; SPSS, version 10.0, SPSS Inc., Chicago, IL). A P-value $< .05$ was considered to be significant. Data for D-lactate is presented as median (range), other data is presented as mean ± SD unless specified.

RESULTS

Control Horses

Of the 6 control horses, 3 were female and 3 were geldings. Breed distribution was Quarter Horse (n = 3), Thoroughbred (n = 1), Arabian (n = 1), and grade horse (n = 1). Mean ± SD age of control horses was.12.4 ± 3.3 years. Median (range) D-lactate concentrations for the control horses were 49.9 μmol/L (0–111 μmol/L) and 38.9 μmol/L (11.1–88.8 μmol/L) for peritoneal fluid and plasma, respectively. One control horse had a peritoneal fluid concentration below the limit of detection of the test.

Clinical Cases

Of the horses referred with signs of colic 90 meet the inclusion criteria. There were 32 mares and 58 males (6 intact, 52 geldings). Breed distribution was Quarter Horse or Appaloosa (n = 27; 30%), Thoroughbreds (n = 15; 17%), Arabians (n = 13; 14%), ponies or miniature horses (n = 4; 4%), Warmbloods (n = 7; 8%), and of other breeds (n = 24; 27%). Mean age was 14.2 ± 7.2 years. Peritoneal and plasma D-lactate was not associated with gender, breed, and age in clinical cases. The average period for which horses had first been observed with clinical signs of colic before admission was 19 ± 23 hours. Peritoneal fluid D-lactate concentration, but not plasma D-lactate concentration, was correlated with hours of observed colic before presentation. Median (range) D-lactate concentrations for the clinical cases were 138.8 μmol/L (11–6549.7 μmol/L) and 83.26 μmol/L (0–932.5 μmol/L) for peritoneal fluid and plasma, respectively. D-lactate concentrations were above the limit of detection of the test for all peritoneal samples; 8 plasma samples had D-lactate concentrations below the limit of detection of the test. Peritoneal concentrations for D-lactate were strongly correlated to plasma concentrations in clinical cases ($r = 0.7$, $P < .001$); however, the correlation was not linear, as the quadratic term for peritoneal D-lactate was highly significant and with a negative sign ($P < .0001$; linear model = plasma D-lactate∼peritoneal D-lactate+peritoneal D-lactate^2). At low concentration values, increments in abdominal D-lactate are accompanied by increases in D-lactate plasma concentrations; but this association becomes increasingly weaker as peritoneal D-lactate concentrations reach high concentrations.

Independent Variables

Categorical Variables. Peritoneal fluid from the clinical cases was abnormal in appearance (turbid or serosanguinous) in 25 horses (27.5%), or normal (clear) in 65 horses (72.5%). Peritoneal fluid D-lactate concentrations were significantly ($P < .001$) higher in horses with abnormal appearance of peritoneal fluid (median, 399.6 μmol/L; range, 22–6550 μmol/L) than in horses with normal appearance of peritoneal fluid (median, 66.6 μmol/L; range, 11–421 μmol/L).

Abdominal surgery was necessary for resolution of the primary problem in 50/90 (55.6%) of horses with colic. Ischemic strangulating obstruction was diagnosed in 32/50 (64%) of these horses. Peritoneal fluid D-lactate concentrations were significantly ($P = .014$) higher in horses that required surgery (median 216 μmol/L, range 0–6549 μmol/L) than in horses that did not (median 94 μmol/L, range 11–988 μmol/L).

Of the clinical cases, 48 horses were discharged from the clinic and 42 were euthanatized. Peritoneal fluid D-lactate concentrations were significantly ($P < .001$) lower in horses that were discharged (77 μmol/L, range 11–988 μmol/L) than

in horses that were not (median 248 μmol/L, range 0–6549 μmol/L).

The location of the lesion was reported in 69 horses. Out of the localized lesions, the primary problem was located to the stomach or small intestine (n = 32, 46.5%), cecum or large colon (n = 16, 23%), small colon (n = 15, 21.8%), and peritoneum (n = 6, 8.7%). No significant association between peritoneal fluid D-lactate concentration and lesion location was identified.

The type of lesion identified included horses with ischemic strangulating obstruction (n = 32, 35.5%), horses with nonstrangulating obstruction (n = 43, 48%), horses with septic peritonitis (n = 5, 5.5%), or horses with rupture of the gastrointestinal tract (n = 10, 11%). The concentrations of peritoneal and plasma D-lactate in various groups according to lesion types and the significant differences in D-lactate concentrations within groups is presented in Table 1. A significant difference ($P < .05$) between peritoneal fluid D-lactate concentration in controls and each of the different types of lesions was identified. A significant difference was also observed in peritoneal fluid D-lactate concentrations between horses in the nonstrangulating obstruction group and the septic peritonitis ($P = .03$) and gastrointestinal rupture groups ($P = .002$). No significant differences were observed between horses in the strangulating obstruction, gastrointestinal rupture and septic peritonitis groups or between the nonstrangulating and strangulating obstructions groups. No significant difference was present between plasma D-lactate concentration of the control group and nonstrangulating obstruction, strangulating obstruction and septic peritonitis groups. A significant difference was observed in plasma D-lactate concentrations between horses in the control and the gastrointestinal rupture group ($P = .031$).

There was no significant association of plasma D-lactate concentration and the following categorical variables: gross appearance of peritoneal fluid, need for surgery, survival to discharge, and lesion location.

The receiver–operator curve used to determine the cut-off values for plasma and peritoneal D-lactate concentrations to differentiate horses with nonstrangulating obstructions to strangulating obstructions is presented in Figure 1. A concentration of peritoneal D-lactate of 116.6 μmol/L showed an optimal prediction with a sensitivity of 81% and a specificity of 65%, to differentiate between horses with nonstrangulating from strangulating obstructions. Calculated values (area under the curve, cut-off value, sensitivity, specificity, and 95% confidence intervals) for plasma and peritoneal D- and L-lactate concentrations in horses with colic are shown in Table 2.

Table 1 Peritoneal and Plasma D-Lactate Concentrations in Control Horses and Clinical Cases Grouped According to the Type of Gastrointestinal Lesion

	Peritoneal Fluid D-lactate (μmol/L)	Plasma D-lactate (μmol/L)
Controls	49.9 (0–111)[a] n = 6	38.9 (11.1–88.8)[a] n = 6
Nonstrangulating obstruction	77.7 (11.1–444)[b] n = 43	66.6 (0–477.4)[a,b] n = 43
Strangulating obstruction	216.5 (22.2–1090)[b,c] n = 32	88.8 (0–754.9)[a,b] n = 32
Peritonitis	455.2 (421.8–1010.2)[c] n = 5	199.8 (44–621.7)[a,b] n = 5
Rupture	599.5 (44.4–6549.7)[c] n = 10	244.2 (0–932.5)[b] n = 10

Values are reported as median (range), n = number of horses. Values were compared within groups and a *P*-value $< .05$ was considered to be significant. Values with different superscripts indicate statistical significance.

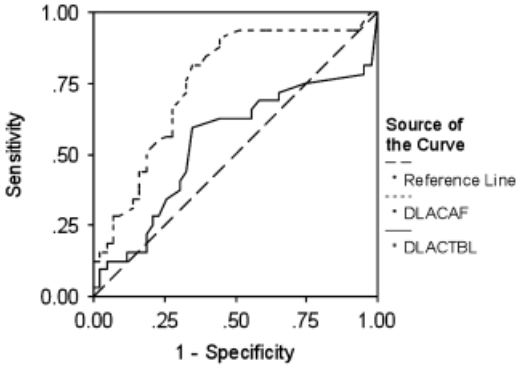

Figure 1 Receiver operating characteristic curves for peritoneal and plasma D-lactate used for the determination cut-off values to differentiate horses with nonstrangulating obstructions (n = 43) from horses with a strangulating obstructions (n = 32). DLACTBL, plasma D-lactate; DLACAF, peritoneal D-lactate.

Continuous Variables. Peritoneal fluid D-lactate concentration was correlated with heart rate, capillary refill time, hematocrit, peritoneal total protein, peritoneal L-lactate, pH, and the calculated prediction of ischemia. Plasma D-lactate concentration was correlated with capillary refill time, hematocrit, plasma L-lactate concentration, peritoneal total protein, and peritoneal pH (Tables 3–5).

DISCUSSION

We evaluated the clinical use of peritoneal fluid and plasma D-lactate concentrations in horses admitted for various causes of colic. Overall the performance of plasma D-lactate as a marker for diagnosis of intestinal ischemia in our population of horses admitted for colic, was not particularly helpful. In addition, plasma D-lactate concentration was weakly correlated with plasma L-lactate concentration. Peritoneal D-lactate concentration, however, had a high sensitivity and moderate specificity in differentiation of horses with nonstrangulating from strangulating obstructions. Furthermore, peritoneal D-lactate concentration had a high correlation with peritoneal L-lactate concentration. Contrary to plasma D-lactate, peritoneal D-lactate may have further use particularly, when considering its high sensitivity, to accurately diagnose intestinal ischemia.

In agreement with our 1st hypothesis, the peritoneal and plasma D-lactate concentrations were strongly correlated with each other. This correlation was, however, not

Table 2 Calculated Predictive Values to Differentiate Horses with Colic Because of Nonstrangulating Obstructions from Strangulating Obstructions

	AUC	Cut-off	Sensitivity	Specificity	95% CI
Plasma D-lactate	0.542	83.3 μmol/L	0.594	0.651	0.403–0.681
Peritoneal D-lactate	0.753	116.6 μmol/L	0.813	0.651	0.640–0.866
Plasma L-lactate	0.727	3.05 mmol/L	0.594	0.81	0.607–0.848
Peritoneal L-lactate	0.813	2.6 mmol/L	0.875	0.667	0.715–0.912

Data was obtained from 75 clinical cases of colic; horses were grouped as nonstrangulating obstructions (n = 43) or strangulating obstructions (n = 32). AUC, area under the curve; cut-off, cut-off value; 95% CI, 95% confidence interval.

linear and became weak as peritoneal D-lactate values were high. A possible explanation for the nonlinear correlation may be that very sick horses with high peritoneal D-lactate concentrations are taken to surgery or euthanatized before plasma lactate has enough time to increase.

Contrary to our 2nd hypothesis plasma D-lactate concentration in horses with strangulating obstruction, gastrointestinal rupture and septic peritonitis was not significantly different from horses with nonstrangulating obstruction. Plasma D-lactate concentration has been reported to be useful in cases of acute intestinal ischemia in people.[6,8,14] An experimental study evaluating plasma D-lactate concentration as a marker for intestinal ischemia, in laboratory animals, compared 2 models of ischemia: mesenteric ischemia (arterial obstruction) and strangulating mesenteric ischemia (arterial and venous obstruction) to 1 model of simple nonstrangulating obstruction and a sham surgery.[7] No difference in plasma D-lactate concentrations between the strangulating mesenteric ischemic model, the nonstrangulating obstruction model or sham operated controls was identified. However, the mesenteric ischemic group had significant elevation of plasma D-lactate concentration compared with the other groups. The most common causes of ischemia in studies assessing D-lactate as a plasma marker for intestinal ischemia are because of arterial embolism or a state of low arterial blood flow[5,6,13,30] leaving the venous system intact and allowing D-lactate to reach the veins and consequently the systemic circulation. Intestinal ischemia in our study was mostly because of strangulations similar to the strangulating mesenteric ischemia model. Therefore obstruction of the venous return may have restricted intraluminal D-lactate from entering the portal system and subsequently the systemic circulation. It is also possible that severe distention in the nonstrangulating group may have caused increases on microvascular and/or mucosal permeability[31,32] allowing the movement of D-lactate into the systemic circulation contributing to these negative results.

A concentration of peritoneal D-lactate of 116.6 μmol/L showed a high sensitivity (81%) but moderate specificity (65%) for differentiation of horses with nonstrangulating from strangulating obstructions. Therefore determination of peritoneal D-lactate concentration may be more useful to determine the presence of strangulating obstructions in horses with colic than to rule out the presence of strangulating obstruction. Plasma D-lactate, on the other hand, has low sensitivity and moderate specificity for the differentiation between nonstrangulating and strangulating obstructions. A previous study evaluating D-lactate in the serum of horses found no differences between controls and horses with colic.[26] Similarly, we did not find a difference between our healthy control horses and horses admitted with colic because of nonstrangulating, strangulating obstructions, or septic peritonitis. However, we found a difference when comparing with the horses in our gastrointestinal rupture group. Most likely horses with acute bowel rupture developed rapid absorption of either bacteria or bacterial byproducts by the peritoneal surface increasing values of D-lactate in the blood. The performance of a diagnostic variable can be quantified by calculating the area under the ROC curve. The ideal test should have and area under the curve of 1, whereas a random guess would have an area under the curve of 0.5.[33] Plasma D-lactate may be useful in horses with acute bowel rupture, but it seems that determination of plasma D-lactate concentrations may not be very useful (ROC low area under the curve, low sensitivity, and

Table 3 Significant Correlations of Peritoneal and Plasma D-Lactate Concentrations with Corresponding Clinical Examination and Clinicopathologic Variables (Continuous Variables)

	Clinical Exam			Blood			Peritoneal Fluid			
Variables	HR	CRT	Hrs	Ht	L-lact	Gluc	TP	L-lact	PI	pH
Peritoneal D-lactate	pos** r = 0.4	pos** r = 0.2	pos* r = 0.1	pos** r = 0.4	Not done	pos* r = 0.2	pos** r = 0.3	pos** r = 0.8	pos** r = 0.2	neg** r = 0.7
Plasma D-lactate	–	pos* r = 0.4	–	pos* r = 0.3	pos* r = 0.3	–	pos* r = 0.3	Not done	–	neg* r = 0.2

Clinical exam, blood and abdominal variables that showed significant correlations of peritoneal and plasma D-lactate concentrations are shown. Correlations are reported as positive (pos) or negative (neg) with r = correlation coefficient.

**$P < .001$,

*$P < .05$.

HR, heart rate; CRT, capillary refill time; Hrs, hours from first observation of colic signs; Ht, hematocrit; L-lact, L-lactate; Glu, glucose; TP, serum total protein; PI, calculated prediction of ischemia.

Table 4 Comparison of Selected Clinical Examination and Blood Variables According to Lesion Type in Horses with Colic

	NSO	SO	Peritonitis	Rupture
Heart rate (beats/min)	48.2 ± 12	69 ± 24	54 ± 7	85 ± 17
	(32–80)	(32–124)	(42–60)	(56–120)
	n = 43	n = 32	n = 5	n = 10
RR (breaths/min)	23 ± 9	26 ± 11	24 ± 12	36 ± 19
	(12–40)	(12–60)	(12–42)	(20–80)
	n = 43	n = 31	n = 5	n = 10
CRT (seconds)	2 ± 0.6	2.9 ± 0.9	2.6 ± 0.9	3.9 ± 1.3
	(1–3)	(1–5)	(2–4)	(3–6)
	n = 42	n = 31	n = 5	n = 10
Hematocrit (%)	37 ± 4	41 ± 10	38 ± 8	55 ± 10
	(24–64)	(23–60)	(30–49)	(36–76)
	n = 43	n = 32	n = 5	n = 10
Total protein (g/dL)	6.8 ± 0.8	7.1 ± 1.3	7.3 ± 1.1	6.7 ± 1.5
	(4.3–8.8)	(5.1–10.5)	(5.6–8.7)	(5–10.7)
	n = 43	n = 32	n = 5	n = 10
Nucleated cell count (10^3cells/μL)	8261 ± 3484	8503 ± 3580	8700 ± 3172	5236 ± 6984
	(1850–17,625)	(1141–17,570)	(4130–12,510)	(860–19,000)
	n = 43	n = 30	n = 5	n = 10
L-lactate (mmol/L)	2.3 ± 1.9	6 ± 6	2.3 ± 1	8 ± 3.5
	(0.6–9.8)	(0.9–24)	(1.1–3.5)	(1.6–13.7)
	n = 42	n = 32	n = 5	n = 10
Glucose (mg/dL)	132.7 ± 39	225.8 ± 115	120 ± 31	257.5 ± 74.8
	(69–254)	(84–584)	(88–169)	(142–390)
	n = 41	n = 31	n = 5	n = 10

Values are reported as mean ± SD (range), n = number of horses.
NSO, nonstrangulating obstruction; SO, strangulating obstruction; RR, respiratory rate; CRT, capillary refill time.

moderate specificity) in the more common causes of colic (nonstrangulating and strangulating obstructions).

In agreement with our final hypothesis, peritoneal D-lactate concentration was strongly correlated to peritoneal L-lactate. However, plasma D-lactate was weakly correlated with plasma L-lactate. The main purpose of the study was to determine if D-lactate concentration could be useful in clinical cases to differentiate various causes of equine colic. Recent studies have shown that plasma and peritoneal L-lactate concentrations are useful in assessment of various causes of equine colic.[1,2,4] Therefore, we also analyzed the data from this study to obtain a cut-off value

Table 5 Comparison of Selected Variables for Peritoneal Fluid According to Lesion Types in Horses with Colic

	NSO	SO	Peritonitis	Rupture
Total protein (g/dL)	2.4 ± 1.4	4.3 ± 2	5.3 ± 1.3	3.7 ± 1.9
	(0.8–5.8)	(1.2–9)	(4.4–7.6)	(0.6–6.5)
	n = 42	n = 31	n = 5	n = 10
Nucleated cell count (10^3cells/μL)	27,624 ± 78,652	20,855 ± 38,168	221,713 ± 208,095	8240 ± 16,255
	(210–374,000)	(74–151,000)	(223–456,040)	(0–50,000)
	n = 41	n = 31	n = 5	n = 10
L-lactate (mmol/L)	3 ± 2.8	8.8 ± 7.4	11.5 ± 3.2	12.5 ± 12.2
	(0.8–12.9)	(1.1–28)	(8.4–16)	(0.8–41)
	n = 43	n = 32	n = 5	n = 10
Glucose (mg/dL)	124 ± 49	183 ± 91	20 ± 36	66 ± 105
	(1–240)	(55–401)	(1–84)	(3–302)
	n = 39	n = 27	n = 5	n = 9
Blood to abdomen glucose difference	13.5 ± 19	73.2 ± 64	100 ± 17	190.2 ± 105
	(0–69)	(0–211)	(85–125)	(3–327)
	n = 37	n = 26	n = 5	n = 10
pH	7.6 ± 0.23	7.49 ± 0.2	7.05 ± 0.21	6.97 ± 0.4
	(7.1–8.2)	(7.1–7.8)	(6.8–7.3)	(6.1–7.3)
	n = 41	n = 32	n = 4	n = 8
Prediction of ischemia (%)	24 ± 33	72.8 ± 33	100 ± 0.01	87 ± 35
	(0–99)	(1.2–100)	(99.9–100)	(0–100)
	n = 40	n = 32	n = 4	n = 8

Values are reported as mean ± SD, (range), n = number of horses.
NSO, nonstrangulating obstruction; SO, strangulating obstruction.

for L-lactate to differentiate nonstrangulating from strangulating obstructions and compared those results with the results of the D-lactate. Both peritoneal D- and L-lactate had a better sensitivity than specificity to differentiate nonstrangulating from strangulating obstructions in our sample population. Peritoneal D- and L-lactate have a similar high sensitivity and moderate specificity. Plasma D- and L-lactate had the same low sensitivity; however, plasma L-lactate has the highest specificity of all test evaluated. The higher specificity of plasma L-lactate makes this analysis more useful to rule out the presence of a strangulating obstruction. The higher sensitivity of peritoneal D- and L-lactate makes these analyses useful to detect horses that do have a strangulating obstruction. Therefore determination of both plasma L-lactate and peritoneal D- and L-lactate concentrations in the same patient may provide high sensitivity and high specificity and may assist in the differentiation of horses with nonstrangulating from strangulating obstructions. Clinical variables used to evaluate horses with colic combine physical examination findings including degree of pain and abnormalities on abdominal palpation by rectum or abdominal ultrasound, with clinicopathologic changes such as those identified by abdominal fluid examination. Abdominal conditions producing colic signs are varied, each having specific characteristics, preventing the availability of a single test to provide a specific diagnosis. Determinations of L-lactate concentration can be performed by using bench top and portable analyzers and results are obtained within minutes allowing it use in the field. However, increases of L-lactate concentrations are not specific, because it is a byproduct of glycolysis and is commonly elevated because of poor systemic tissue perfusion and anaerobic glycolysis associated with circulatory shock. Determination of D-lactate concentrations as we performed requires several steps including filtration by high speed centrifugation and the assay may take up to 2 hours for its determination, preventing it use in field conditions. However, D-lactate is a specific bacterial byproduct and therefore high values may indicate cases where bacterial translocation has occurred.

Several methods have been reported to measure D-lactate concentrations in body fluids.[18,29,34] The D-lactic acid spectrophotometer assay is the most widely used and should be easily adaptable for use in most clinical laboratories as it is simple to perform and only requires a D-lactate assay kit and a spectrophotometer. The analysis was initiated by a centrifugation step to remove oxido-reductase proteins, including L-LDH, since it has been shown the nonspecific transformation of NAD^+ to NADH would result in an overestimation of D-lactate measurement. This is particularly important in samples with high L-lactate, and L-LDH that will result in inaccurate calculations of D-lactate concentrations. This step, however, prolongs the assay time from 20 to 110 minutes. A study confirmed the importance of deproteination by ultrafiltration to remove L-LDH in samples with high L-lactate concentrations; and reported a cut-off value for L-lactate of 4 mmol/L above which deproteination is indicated.[35] Plasma and peritoneal fluid samples from horses admitted for colic are likely to have elevated L-lactate concentrations and therefore in this study deproteination was indicated. Another method for deproteination requires administration of perchloric acid instead of ultrafiltration, which would have shortened the procedure time of the assay. However, this may have produced sample dilution making D-lactate assay at physiologic concentrations inaccurate.[35] Instead we used the ultrafiltration method to be able to detect horses with low D-lactate concentrations.

A study limitation is the relatively small sample size particularly for horses with rupture or septic peritonitis that prevented calculation of cut-off points for these conditions. Peritoneal D-lactate has been used in the diagnosis of septic peritonitis in people that have received antimicrobials, and therefore are more likely to have no bacterial growth in cultures or a negative gram stain in their suspected body fluids.[27,28,36] To determine the diagnostic and prognostic value of D-lactate concentration in horses with septic peritonitis, a study using a larger population of horses with this condition is warranted.

Different than reported in people, plasma D-lactate concentration could not accurately distinguish the presence of ischemic bowel in horses. Peritoneal fluid D-lactate, however, showed high sensitivity in detecting horses with strangulating obstructions. The value of D-lactate concentration in other septic cavities or synovial structures would be of interest.

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