AN ABSTRACT OF THE THESIS OF

Montague N. Saulez for the degree of Master of Science in Veterinary Science presented on October 23 2003.
Title: The Determination of Alkaline Phosphatase Activity and Analysis with a Portable Clinical Analyzer of Serum and Peritoneal Fluid from Horses Suffering Colic.

Abstract approved:

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Dr. Christopher K. Cebra

Alkaline phosphatase (ALP) is an enzyme present in intestinal mucosa, bile, bone and renal tubule cells. Bile acids have been shown to decrease ALP activity from bone and kidney but not those from intestinal origin. This action can be mimicked in serum and peritoneal fluid samples by the use of an L-phenylalanine buffer which specifically measures intestinal ALP activity only; while the standard buffer measures total ALP activity. We sought to assess the diagnostic and prognostic relationship of intestinal and total ALP activity between serum and peritoneal fluid in 126 horses with acute colic. Blood and peritoneal fluid samples were analyzed for ALP activity using both the standard and L-phenylalanine based buffers. Neither total nor intestinal serum ALP activity was useful in classifying type or severity of intestinal damage. Total and intestinal peritoneal fluid ALP activity were lowest in horses suffering simple medical colic and non-strangulated surgical lesions, and highest in surgical cases with suspected ulceration, strangulation, peritonitis and intestinal rupture. High total and intestinal

peritoneal fluid ALP activity was associated with greater intestinal damage, increased probability of surgical intervention and a worse prognosis while low total and intestinal peritoneal fluid ALP activity was unable to accurately differentiate between simple medical colics and surgical colics. The use of L-phenylalanine buffer in both serum and peritoneal fluid did not improve the sensitivity of the test. Based on these results, determination of total ALP activity in peritoneal fluid may be helpful in identifying ischemic or inflammatory bowel lesions in horses with acute colic.

A portable clinical analyzer (PCA) was used for the determination of venous blood and peritoneal fluid pH value, glucose, lactate and electrolyte concentrations in a hospital setting. Blood and peritoneal fluid glucose, lactate, sodium, chloride and potassium concentrations, and pH value were determined using both a portable clinical analyzer with test cartridges and an in-house analyzer in 56 horses with acute abdominal disease. Results were compared by the Bland-Altman method of comparison and linear regression. The PCA yielded higher blood and peritoneal pH values, with greater variability in the alkaline range and lower pH values in the acidic range. The PCA glucose concentrations (<150 mg/dL) were significantly lower, and were higher in the high range (>150 mg/dL). Venous lactate concentration (<5 mmol/dL) and peritoneal fluid lactate concentration (<2 mmol/dL) had the smallest variability. On average, the PCA underestimated peritoneal lactate and glucose concentration. Peritoneal fluid sodium and chloride concentration had higher bias and variability than venous

sodium and chloride concentration. Venous and peritoneal fluid potassium concentration was closely clustered around the mean with a low bias and variability. Correlation coefficients were >0.80 for all values except venous and peritoneal sodium concentration; venous chloride concentration and venous pH value. The PCA may be suitable for point-of-care biochemical analysis of blood and peritoneal fluid for horses suffering colic and may provide further diagnostic and prognostic information. The PCA may be of help in diagnosing metabolic acidosis, uroperitoneum, septic and non-septic peritonitis and intestinal ischemia. This may be of benefit to ambulatory equine clinicians.

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The Determination of Alkaline Phosphatase Activity and Analysis with a Portable Clinical Analyzer of Serum and Peritoneal Fluid from Horses suffering Colic.

by
Montague N. Saulez

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Montague N. Saulez, Author

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CONTRIBUTION OF AUTHORS

Dr. Christopher K. Cebra assisted with the data interpretation. Dr. Susan Tornquist helped with sample analysis. Statistical support was provided by Ms. Megan Dailey.

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DEDICATION

To my parents,
Newton and Jo Saulez,
Whose guidance,
And support,
Always ever present.

THE DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY AND ANALYSIS WITH A PORTABLE CLINICAL ANLYZER OF SERUM AND PERITONEAL FLUID FROM HORSES SUFFERING COLIC.

MONTAGUE N. SAULEZ, BVSc, MRCVS

GENERAL INTRODUCTION

Colic due to an abdominal disorder is the leading cause of mortality within the equine industry worldwide. Methods to examine horses suffering acute abdominal disease include: visual inspection, oral examination, auscultation and percussion of the abdomen, nasogastric intubation, and rectal examination. Ancillary diagnostic aids include: endoscopy, abdominal radiography and ultrasonography, and clinicopathological tests. These tests may include a complete blood count, serum biochemistry and determination of acid-base balance.

Abdominocentesis is a quick, minimally-invasive procedure which can yield valuable information about the condition of the peritoneal surfaces in the abdomen. Abdominal fluid is typically assessed for appearance, and determination of total nucleated cell count and protein concentration. Abdominal fluid should always be interpreted with clinical and laboratory findings, and can be used prognostically as well as an aiding the selection of medical or surgical intervention.

Few reports exist of peritoneal fluid enzymology. Alkaline phosphatase (ALP) activity is present in bone, intestinal mucosa, renal tubular cells, and biliary epithelium. Specific intestinal ALP activity may be determined in blood and peritoneal fluid through the inhibition of non-intestinal phosphatases by the addition of L-phenylalanine and has been shown to increase following intestinal ischemia. Determination of ALP activity may thus yield diagnostic and prognostic information about the severity of intestinal ischemia.

Biochemical analysis of abdominal fluid for glucose and lactate concentration and pH value has been performed. These analyses are performed on non-portable, automated chemical analyzers that are costly to purchase and maintain, and can not be used for point-of-care analysis of samples. Recently, portable clinical analyzers (PCA) have become available to veterinarians and have been used in hospital as well as ambulatory settings for the analysis of equine blood. These analyzers have been used routinely for the analyses of whole blood, but have not previously been used to analyze abdominal fluid.

Therefore, this study had two purposes. Firstly, it aimed to aid in the prognosis of horses with intestinal insults by the measurement of ALP activity in both serum and abdominal fluid. Secondly, should the PCA yield results comparable with an in-house analyzer in the measurement of abdominal fluid constituents, the PCA could be used by veterinarians for enhanced diagnostic evaluation and triage of horses with intestinal disease in an ambulatory setting.

ACTIVITY OF ALKALINE PHOSPHATASE IN SERUM AND PERITONEAL FLUID FROM HORSES WITH COLIC

BY

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Introduction

The equine acute abdomen provides a diagnostic challenge to clinicians worldwide. Among other tests, abdominocentesis is a rapid, inexpensive, minimally-invasive procedure which provides the investigator with a wealth of information. Routine peritoneal fluid analysis involves visual appraisal for appearance and determination of total nucleated cell count and protein concentration.[1] Previous studies also have reported the usefulness of measuring certain biochemical parameters in peritoneal fluid such as lactate, glucose, and pH as indicators of intestinal ischemia or peritonitis.[2-6]

Studies on peritoneal enzymology are less common.[7-9] Alkaline phosphatase (ALP) activity is present in bone, intestinal mucosa, renal tubular cells and biliary epithelium. Some component of bile inhibits ALP activity of bone and kidney origin while not interfering with that of intestinal origin.[10] This action can be mimicked *in vitro* by dilution of serum and peritoneal fluid in buffering solutions containing L-phenylalanine before analysis, thereby allowing more accurate measurement of intestinal-specific ALP activity.[11]

Increases in both serum and peritoneal ALP activity occur after experimental ischemia of the ileum,[7] suggesting that intestinal ALP activity could be used as a prognostic indicator for bowel health and potentially be employed as a marker for strangulating versus non-strangulating lesions. This has not been tested in a population with naturally occurring disease. The purpose of this study was to assess whether determination of total ALP activity or intestinal ALP activity in

serum or peritoneal fluid yielded diagnostic and prognostic information about the severity of intestinal insult in horses with colic.

Materials and Methods

Sample Population: 126 horses admitted to the Oregon State University Veterinary Teaching Hospital for acute colic.

Sample Collection and Processing: Blood and peritoneal fluid samples were collected into clot tubes after routine venipuncture and abdominocentesis during the standard initial evaluation of these clinical horses. Samples were centrifuged and the pellet discarded. Serum and peritoneal fluid were analyzed for ALP activity using an automated chemical analyzer,[a] using both the standard magnesium buffer solution[b] and a second buffer containing 0.27% L-phenylalanine.[c,11] All serum and peritoneal fluid samples were refrigerated at 7°C for a maximum of 12 hours before analysis.

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[a] Hitachi 717 Serum Biochemical Analyzer, Boehringer Mannheim Diagnostics. Division of Roche Diagnostics Corp, Indianapolis, IN.

[b] Boehringer Mannheim Diagnostics Division of Roche Diagnostics Corp, Indianapolis, IN.

[c] L-Phenylalanine, Sigma Diagnostics, St Louis, MO.

Data Analysis: Horses were grouped based on clinical findings and management. Horses managed without surgery were categorized into horses without suspected ulcerative or inflammatory disease (Med) – this included fecal impaction, intestinal tympany and ileus; horses with suspected ulcerative disease (MedU) - this included sand impaction, colitis and proximal duodenitis jejunitis (DPJ); and peritonitis (MedP). Peritonitis was diagnosed based on peritoneal fluid culture and cytology (nucleated cell count > 5000 cells/μl and total protein > 2.5 g/dl)[12] in the absence of a lesion for another category.

Patients that were explored surgically were classified based on the lesion found. Surgical patients were divided into horses without either intestinal strangulation or mucosal trauma (Sx) – this included intestinal displacements or entrapments; non-strangulating lesions with mucosal damage (SxU) – which included DPJ, sand impaction, enterolithiasis, or colitis; intestinal strangulation requiring resection (SxS), which included intestinal volvulus (>360°), intussusception, strangulating lipoma or herniation; peritonitis (SxP) due to inflammatory bowel disease or diffuse intra-abdominal abscesses; and intestinal rupture following a non-iatrogenic event (SxR) due to impaction by feed or sand, enterolithiasis, diverticulosis or volvulus. Horses were thus classified into 8 mutually exclusive groups.

Additionally, in order to address differences between horses that survived compared to horses that died, all horses were divided into medical and surgical groups and then four groups were created by splitting the horses into a medical

alive (MedA) and dead group (MedD); and a surgical alive (SxA) and dead group (SxD).

Statistical Analysis: Statistical analyses were performed using a commercial software program.[d] Descriptive statistics were calculated for each group. Differences in both total ALP activity (without l-phenylalanine) and intestinal ALP activity (with l-phenylalanine) between groups were evaluated for both blood and peritoneal fluid via Analysis of Variance (ANOVA) on natural log transformed data. If ANOVA revealed evidence of at least one group difference (statistical significance was defined as $P < 0.05$), all pair wise group differences were assessed using the Tukey-Kramer procedure for multiple comparisons. This method of analysis was performed separately for the 8 group classification and for the 4 group classification. Due to the log transformation, estimates of group differences were reported as ratios of the medians of the two groups, with standard errors.

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[d] SPlus 6.1, Department of Statistics, Corvallis, OR.

Results

The medians and standard errors for serum and peritoneal ALP activity with and without l-phenylalanine are summarized (Table 1). There was no significant difference among the 8 groups in serum total ($P = 0.14$) or intestinal ALP activity ($P = 0.28$; Figure 1), nor in the serum intestinal ALP activity in the 4 group classification ($P = 0.26$; Figure 2). In the 4 group classification, total serum ALP activity was significantly lower in MedA than MedD ($P < 0.003$).

Significant differences in peritoneal intestinal ALP activity were found in the 4 and 8 group classifications ($P < 0.0001$ for both groups), (Figures 2, 3, and 4); SxU, SxS, SxP and SxR were significantly higher than Med ($P < 0.001$ for all), MedU ($P = 0.001$ for all), and Sx ($P < 0.003$). Additionally, Med was significantly lower than MedP ($P = 0.007$) and Sx ($P = 0.009$).

In the 4 group comparison, horses that died during or after surgery (SxD) had significantly higher peritoneal intestinal ALP activity than the other 3 groups (MedA, $P < 0.001$; MedD, $P = 0.003$; SxA, $P = 0.0024$). Also, horses that survived surgery (SxA) had significant higher peritoneal intestinal ALP activity than MedA ($P = 0.003$).

Significant differences in total peritoneal ALP activity were found in the 8 and 4 group classifications ($P < 0.0001$ for both groups); SxU, SxS, SxP and SxR were significantly higher than Med ($P < 0.001$ for all), MedU ($P < 0.006$ for all), and Sx ($P < 0.002$ for all). Med was significantly lower than MedP ($P = 0.004$) and Sx ($P < 0.001$). Horses that died during and after surgery (SxD) in the 4 group

comparison had higher total peritoneal ALP activity than the other 3 groups (MedA, $P < 0.001$; MedD, $P = 0.002$; SxA, $P = 0.0024$). Those horses that survived surgery (SxA) had higher total peritoneal ALP activity than MedA ($P = 0.001$).

Table 1: Total and intestinal serum and peritoneal ALP activity.

Group Classification	Total serum ALP activity (IU/L)		Intestinal serum ALP activity (IU/L)		Total peritoneal ALP activity (IU/L)		Intestinal peritoneal ALP activity (IU/L)		Number of cases
	Median	Quantiles 0.25 to 0.75	Median	Quantiles 0.25 to 0.75	Median	Quantiles 0.25 to 0.75	Median	Quantiles 0.25 to 0.75	
Medical: Without ulcerative process	186	141 to 186	155	117 to 193	39 [a]	19 to 60	31 [a]	16 to 44	56
Medical: With ulcerative process	286	162 to 874	236	132 to 683	41 [ab]	32 to 55	33 [ab]	25 to 45	6
Medical: Peritonitis	259	216 to 808	208	176 to 699	293 [bc]	188 to 580	230 [bc]	131 to 277	3
Surgical: Non-strangulated, non-mucosal	189	159 to 294	159	132 to 236	45 [b]	30 to 62	36 [b]	23 to 54	24
Surgical: Non-strangulated, mucosal	212	189 to 232	166	153 to 192	109 [c]	60 to 1113	83 [c]	52 to 970	6
Surgical: Strangulated	181	141to 276	145	105 to 211	114 [c]	69 to 240	94 [c]	56 to 191	20
Surgical: Peritonitis	308	184 to 437	265	158 to 383	313 [c]	110 to 2227	283 [c]	91 to 1800	4
Surgical: Rupture	261	158 to 312	219	128 to 265	687 [c]	205 to 852	564 [c]	166 to 732	7
Medical: All	191	141to 259	160	118 to 207	39	24 to 63	31	17 to 48	65
Medical: Alive	186 [a]	139 to 238	155	113 to 199	39 [a]	20 to 62	31 [a]	17 to 47	58
Medical: Dead	259 [b]	191 to 820	207	157 to 646	50 [ab]	39 to 74	41 [ab]	27 to 60	7
Surgical: All	201	151 to 293	157	124 to 232	96	43 to 226	77	36 to 185	61
Surgical: Alive	189	159 to 275	156	124 to 206	49 [b]	33 to 178	43 [b]	26 to 131	32
Surgical: Dead	217	149 to 293	179	127 to 245	123 [c]	69 to 320	102 [c]	56 to 240	29
All Groups	195	146 to 274	158	121 to 228	52	28 to 141	42	22 to 100	126
All Alive	186	142 to 250	155	116 to 203	42	25 to 78	33	18 to 57	90
All Dead	235	148 to 363	188	126 to 311	114	58 to 226	91	50 to 181	36

1. Values within a comparison with different superscripts are significantly different.

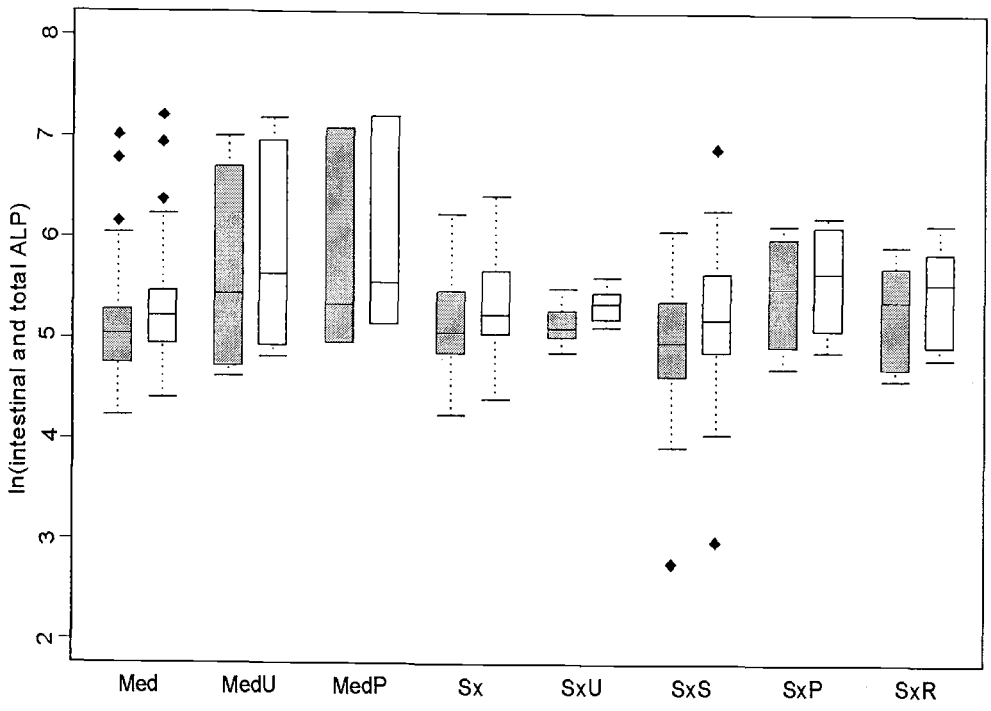

Figure 1: Box plots of log transformed serum ALP activity.

(Med = medical group without ulcerative process, MedU = medical group with ulcerative process, MedP = medical group with peritonitis, Sx = surgical group without both strangulation and mucosal trauma, SxU = surgical group without strangulation but with mucosal damage, SxS = surgical group with strangulation, SxP = surgical group with peritonitis, SxR = surgical group with intestinal rupture)

(Box plots were used to display the data and allow visual comparisons between groups. The box encloses the middle half of the data and is bisected by a line at the value for the median. The box length is referred to as the inter-quartile range [IQR] and is defined as the space between the upper and lower quartiles. The vertical lines at the top and bottom of the box indicate the "range" of typical data values. Extreme values are displayed as "♦" for possible outliers)

Shaded box plots depict serum intestinal ALP activity (with L-phenylalanine)
Clear box plots depict serum total ALP activity (without L-phenylalanine)

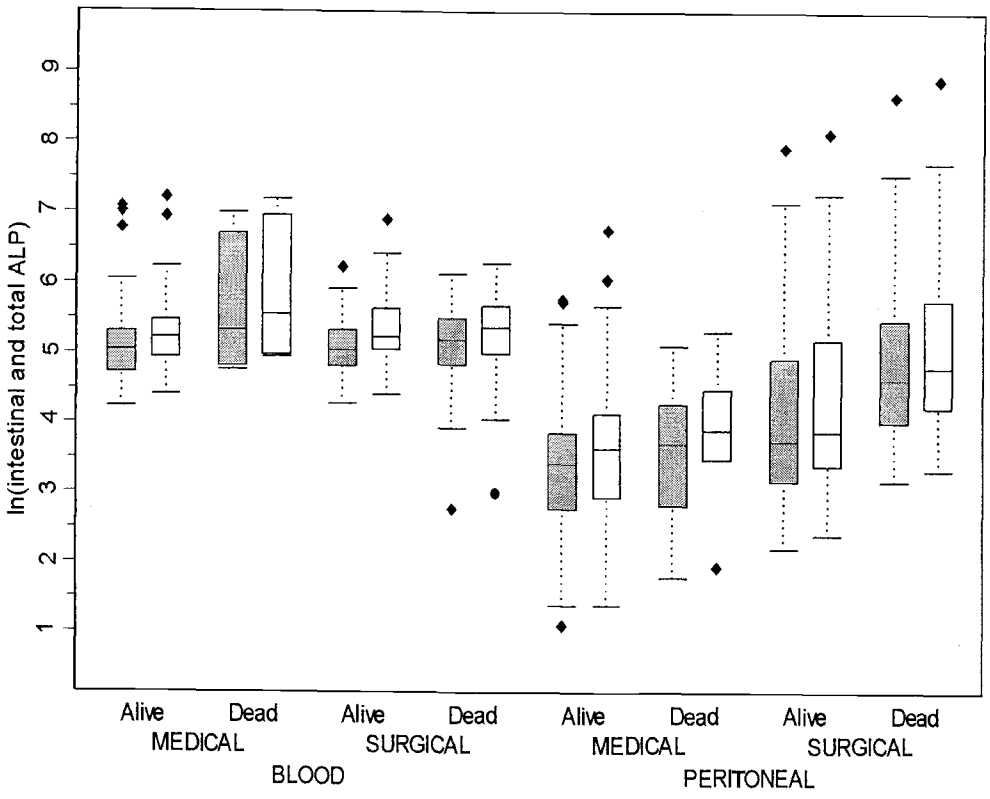

Figure 2: Box plots of log transformed serum and peritoneal ALP activity with classification into medical alive and dead groups, and surgical alive and dead groups.

(Box plots were used to display the data and allow visual comparisons between groups. The box encloses the middle half of the data and is bisected by a line at the value for the median. The box length is referred to as the inter-quartile range [IQR] and is defined as the space between the upper and lower quartiles. The vertical lines at the top and bottom of the box indicate the "range" of typical data values. Extreme values are displayed as "♦" for possible outliers)

Shaded box plots depict serum or peritoneal intestinal ALP activity (with L-phenylalanine)
Clear box plots depict serum or peritoneal total ALP activity (without L-phenylalanine)

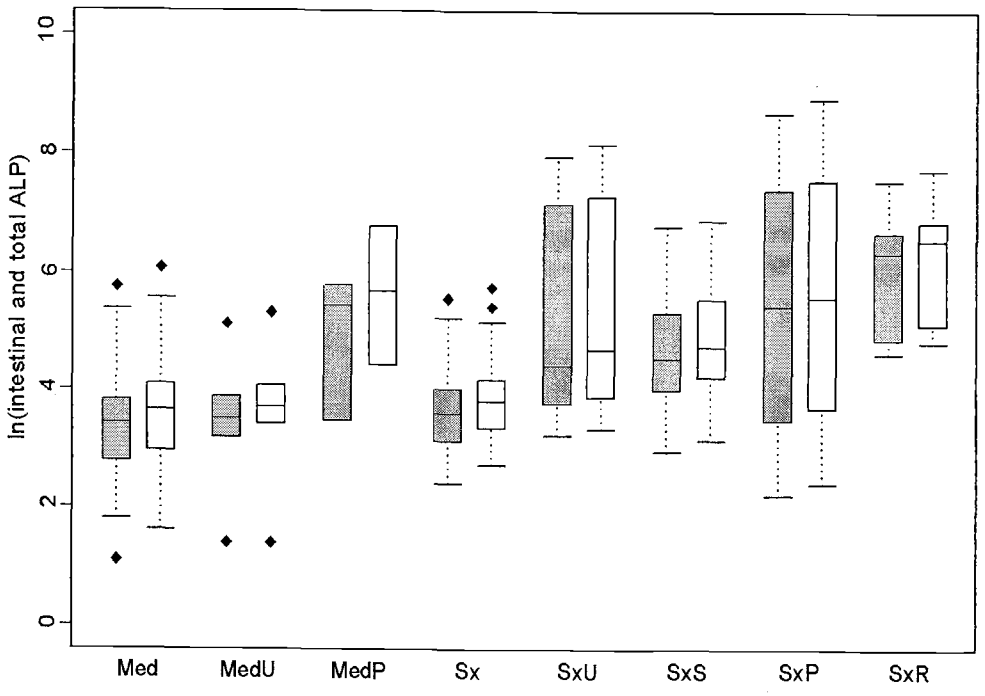

Figure 3: Box plots of log transformed peritoneal ALP activity.

(Med = medical group without ulcerative process, MedU = medical group with ulcerative process, MedP = medical group with peritonitis, Sx = surgical group without both strangulation and mucosal trauma, SxU =surgical group without strangulation but with mucosal damage, SxS = surgical group with strangulation, SxP = surgical group with peritonitis, SxR = surgical group with intestinal rupture)

(Box plots were used to display the data and allow visual comparisons between groups. The box encloses the middle half of the data and is bisected by a line at the value for the median. The box length is referred to as the inter-quartile range [IQR] and is defined as the space between the upper and lower quartiles. The vertical lines at the top and bottom of the box indicate the "range" of typical data values. Extreme values are displayed as "♦" for possible outliers)

Shaded box plots depict peritoneal intestinal ALP activity (with L-phenylalanine)
Clear box plots depict peritoneal total ALP activity (without L-phenylalanine)

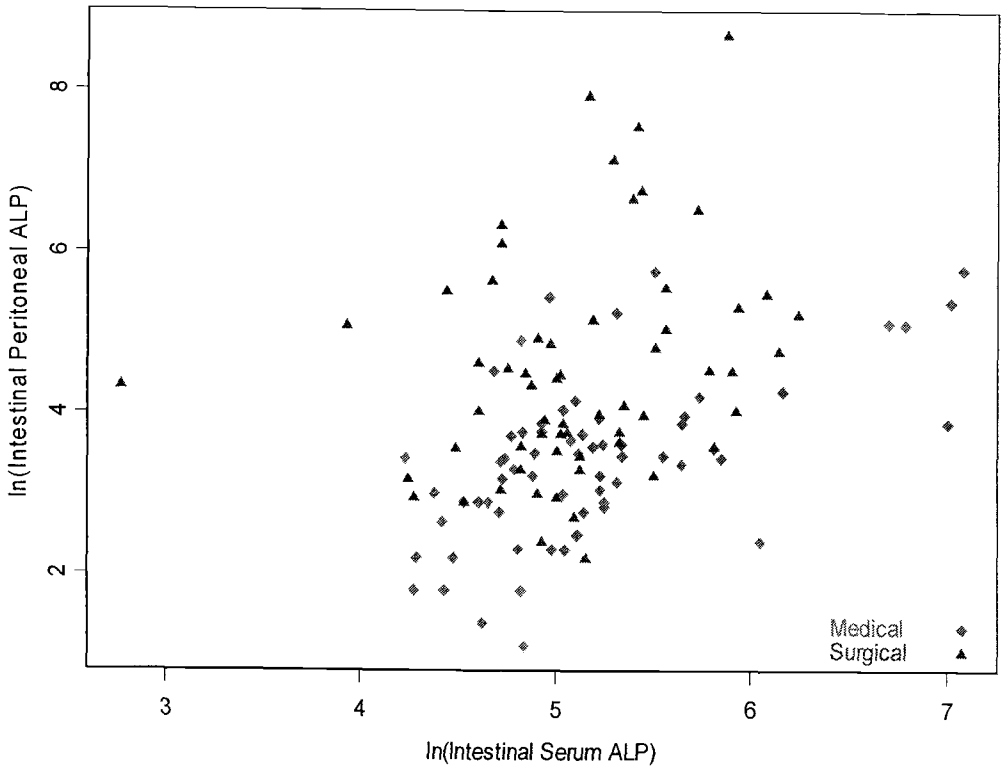

Figure 4: Scatter plot of medical and surgical classification of cases by comparison of serum and peritoneal ALP activity.

Conclusions

Serum ALP activity was of limited usefulness in identifying type or severity of intestinal lesions. The highest medians were found in horses with ulcerative disease, peritonitis, or poor outcome, but there was substantial overlap between these groups and others in the study. The use of the l-phenylalanine buffer in the serum did not improve the diagnostic value. In the regression model, serum intestinal ALP also appeared to play a minor role in predicting the need for surgery. Previous studies have demonstrated conflicting results: one described no changes in serum intestinal ALP activity following experimentally-induced ischemia of the small intestine.[7] Another study, however, found increases in serum intestinal ALP activity which was associated with clinical intestinal damage.[11] Based on our findings, due to overlap among groups, serum ALP activity with or without l-phenylalanine appears to be a poor prediction of type of lesion, type of intervention or outcome.

In contrast, peritoneal ALP activity aided in identification of more severe lesions and horses needing surgery. Both horses with medical peritonitis and different types of surgical lesions often had high total peritoneal ALP activity whereas horses with more simple medical or surgical conditions typically had low values. Previous studies have yielded similar results: experimental intestinal ischemia leads to increases in peritoneal fluid ALP activity;[7] whereas equine

dysautonomia, which can cause severe colic signs without inflammation or ischemia, does not.[8,9]

The use of the l-phenylalanine buffer did not appear to improve the diagnostic usefulness of either serum or peritoneal ALP determination. The value of this buffer was to remove the confounding effects of non-intestinal tissue such as bone or biliary epithelium, but previous studies have not performed comparative analysis of intestinal and total ALP concentration. The current study suggests that confounding effects are rare in the diverse population of horses with colic.

For a single horse, differentiation between categories based solely on peritoneal ALP activity was not possible. However, higher values correlated with more severe lesions, greater chance of surgery, and worse outcome. Horses with low peritoneal ALP activity could still require surgical intervention but as a group tended to have a more favorable outcome. This could possibly reflect the well perfused, non-inflammatory nature of these lesions or that inflammatory or ischemic lesions were less extensive or shorter in duration.

Higher concentrations of intestinal ALP activity reflecting increased intestinal damage were not specific for intestinal strangulation or the need for surgery. Thus, other parameters should be used in conjunction with ALP to determine appropriate clinical management.

Bibliography

1. Cowell RL, Tyler RD, Clinkenbeard KD, MacAllister CG. Collection and evaluation of equine peritoneal fluid and pleural effusion. *Vet Clinics of North Am* 1987;3:543-561.

2. Ruggles AJ, Freeman DE, Acland HM, FitzSimmons M. Changes in fluid composition on the serosal surface of jejunum and small colon subjected to venous strangulation obstruction in ponies. *Am J Vet Res* 1993;54:333-340.

3. Parry BW, Anderson GA, Gay CC. Prognosis in equine colic: A study of individual variables used in case assessment. *Equine Vet J* 1983;15:337-344.

4. Parry BW. Use of Clinical Pathology in the evaluation of horses with colic. *Vet Clinics of North Am* 1987;3:529-542.

5. Hoogmoed L, Rodger LD, Spier SJ, et al. Evaluation of peritoneal fluid pH, glucose concentration and lactate dehydrogenase activity for the detection of septic peritonitis in horses. *J Am Vet Med Assoc* 1999;214:1032-1036.

6. Brownlow M, Hutchins DR, Johnston KG. Reference values for equine peritoneal fluid. *Equine Vet J* 1981;13:127-130.

7. Davies J, Gerring EL, Goodburn R, Manderville P. Experimental ischemia of the ileum and concentrations of the intestinal isoenzyme of alkaline phosphatase in plasma and peritoneal fluid. *Equine Vet J* 1984;16:215-217.

8. Barrelet A. Peritoneal fluid: Part 1--Laboratory analyses. *Equine Vet Ed* 1993;5:81-83.

9. Milne EM, Doxey DL, Gilmour JS. Analysis of peritoneal fluid as a diagnostic aid in grass sickness (equine dysautonomia). *Vet Record* 1990;127: 162-165.

10. Bodansky O. Are phosphatases of bone, kidney, and serum identical? Use of bile acids in their differentiation. *J of Biological Chemistry* 1937;118: 341-362.

11. Blackmore DJ, Palmer A. Phenylalanine inhibited p-nitrophenol phosphatase activity in the serum as an indication of intestinal cellular disruption in the horse. *Res in Vet Science* 1977;23:146-152.

12. Hawkins J. Peritonitis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. Philadelphia, PA: Saunders; 2003:153-158.

ANALYSIS OF EQUINE BLOOD AND PERITONEAL FLUID WITH A PORTABLE CLINICAL ANLYZER

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Introduction

Analysis of peritoneal fluid is an important tool in the evaluation of horses with acute abdominal disease. Analysis can reveal evidence for specific problems, such as septic peritonitis [1-3] or uroperitoneum,[4] or provide general information concerning the overall health of the abdominal contents, such as the presence of strangulating versus non-strangulating lesions of the bowel.[5-14] This information becomes important in the clinical management of affected horses, especially influencing decisions concerning medical treatment, surgical exploration, or humane euthanasia.

The most common analyses of equine peritoneal fluid are determination of protein content and cytological evaluation of cells. Although biochemical analyses have been shown to be of diagnostic and prognostic value,[3,5-10] they are rarely requested because historically these analyses have been cumbersome, time-consuming, expensive, or impractical to perform. Specific biochemical constituents that have been shown to be useful include pH value and glucose content for septic peritonitis;[3] and lactate concentration for overall prognosis.[9,10] These analyses typically are performed on non-portable, automated chemistry analyzers, which require removal of cells and fibrin from peritoneal fluid in addition to transport of the sample to a clinical laboratory. Lactate measurement requires special reagents that have a short shelf life and are thus expensive to maintain in the machine. Newer blood gas analyzers are able to determine lactate

and glucose on cellular fluid samples, but are still hampered by the expense and lack of convenience for lactate analysis (probes last approximately 10-14 days). None of these aforementioned analyzers are portable, thus removing them from the hands of the equine primary care veterinarian, who must then make difficult decisions at the stable or barn without complete information.

Portable clinical analyzers (PCA) present a possible solution to the problems with the point-of-care biochemical analysis of equine peritoneal fluid. Most are designed for whole blood, and their analytical range suggests they may be useful in analysis of anticoagulant-prepared cellular, proteinaceous body fluids. Analyte probes are individually stored, and thus have longer shelf life. Probes are also disposable, preventing damage to the analyzer itself. PCA require minimal warm-up time, and thus can be available at all times of the day. Results are obtained quickly, usually within minutes of sample collection. Most importantly, the analyzer is completely portable, and thus can be taken to the site of the horse. There are, however, no reports on PCA analysis of equine peritoneal fluid. If this analyzer could be demonstrated to yield accurate results of biochemical analyses of equine peritoneal fluid comparable to the in-house analyzer, it could become an invaluable tool in the field diagnostic evaluation and triage of horses with acute abdominal disease.

Materials and Methods

Sample Population: The study population consisted of fifty-six horses admitted for evaluation of acute abdominal disease.

Sample Collection and Processing: On admission, blood was collected by venipuncture from the jugular vein and peritoneal fluid by free-flow through a teat canula. Samples were collected into tubes containing lithium heparin. These samples were analyzed immediately using the PCA[a] with 6+® and CG 4+® cartridges[b] and an in-house blood gas analyzer.[c] Both analyzers yielded results for pH, glucose, lactate, sodium, chloride, and potassium.

Statistical Analysis: Using a computer software program,[d] observed differences for the two methods were calculated and evaluated for normality.[15] Given an approximately normal distribution, differences were then plotted against the average of the two measurements.[15] Bias (mean difference between values obtained on the same sample using different methods) and variability (SD of the differences) were determined for all values.

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[a] i-STAT® Portable Clinical Analyzer, Heska Corporation, Fort Collins, CO.

[b] i-STAT® cartridges, Heska Corporation, Fort Collins, CO.

[c] Chiron Diagnostics 865 Blood Gas Analyzer®, Bayer Corporation, Norwood, MA.

[d] SPlus 6.1, Department of Statistics, Corvallis, OR.

The variability in observed differences was found to be relatively constant for all variables except lactate and glucose, where the variance differed across the range of the measurement values. Thus, the ranges were split into two (glucose) or three (lactate) bins, each displaying constant variance individually. The 2 analyzers had different reportable ranges (Table 2).

Paired t-tests were also performed to test if the differences between the two analyzers were different from zero. Additionally, the relationship between the two analyzers for the different variables was described with least squares regression analysis.

Results

The PCA produced values that were consistently within ± 2 SD and evenly distributed about the line of the mean of differences for all variables tested (Figures 5-12) with bias and variability for the variables displayed (Table 3 and 4).

All venous pH differences were < 0.1 unit (Figure 5a), as compared to the peritoneal fluid pH values (Figure 5b) which showed greater variability in the alkaline range and consistently reported lower pH values in the acidic range. Both venous blood and peritoneal fluid pH values were slightly but significantly higher on the PCA.

Venous glucose concentration (>150 mg/dL) had greater variability as concentrations increased (Figure 6b) as compared to venous glucose concentrations (<150 mg/dL) which showed smaller variability (Figure 6b). All results in this bin differed by <20%. The PCA venous glucose concentrations in the low range (<150 mg/dL) were significantly lower, and were higher ($P = 0.05$) in the high range (>150 mg/dL). The PCA yielded significantly lower peritoneal fluid glucose concentrations (Figure 7). All but 2 of the glucose values measured by the PCA were within 20% of glucose values measured by the in-house analyzer.

88% of values for venous lactate concentration (<5 mmol/dL) were within 0.5 unit of the mean (Figure 8); however, lactate values (>5 mmol/dL) had greater variability. Peritoneal fluid lactate concentration (<2 mmol/dL) had the smallest

variability as compared to higher lactate ranges which showed increased variability (Figure 9). On average, the PCA underestimated peritoneal fluid lactate concentration especially in the higher ranges, as compared to blood lactate concentration.

Both venous sodium and chloride concentration showed closer clustering around the mean as compared to peritoneal fluid sodium and chloride concentration, which had a higher bias, and greater variability (Figures 10 and 11).

Venous and peritoneal fluid potassium concentration had a low bias and small variability (Figure 12).

Regression parameters are displayed in Table 5. Correlation coefficient (r^2) values were > 0.80 for all variables, with the exception of venous pH and chloride concentrations; and venous and peritoneal sodium concentrations.

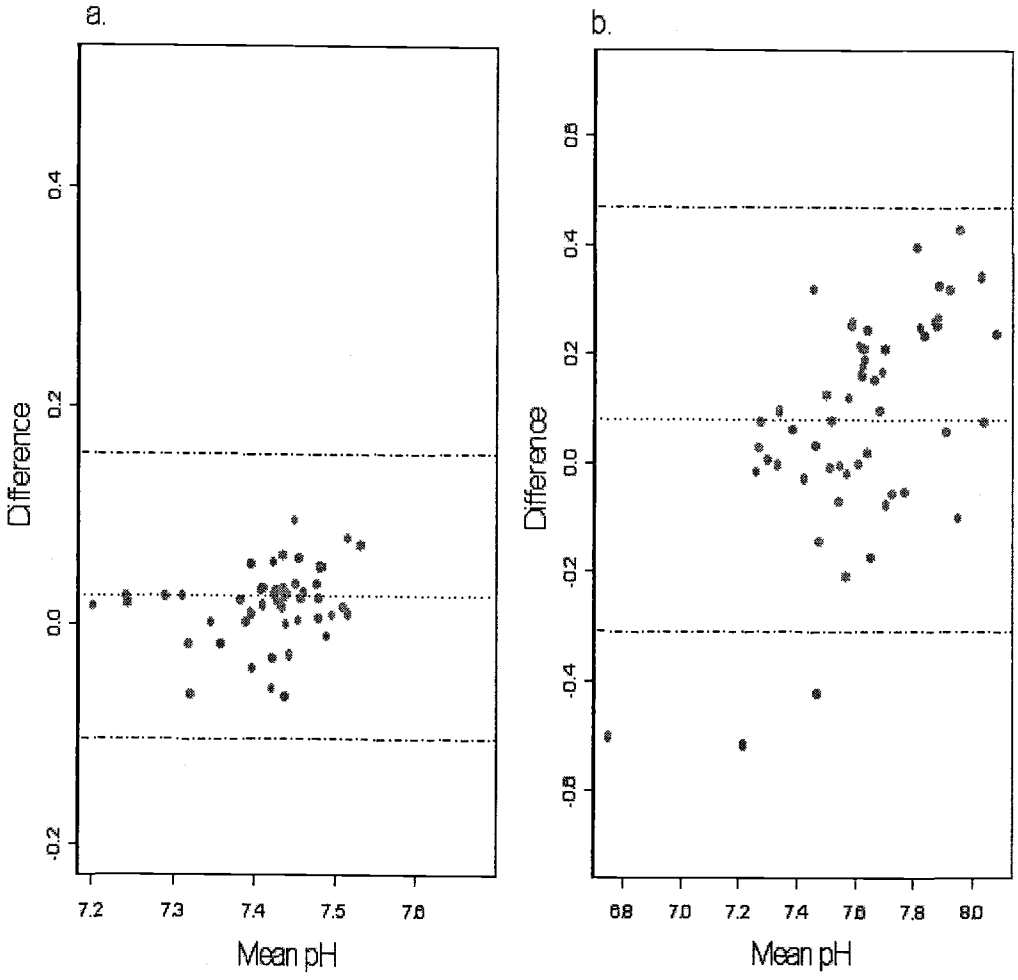

Figure 5: Plots depicting the difference between the pH values obtained by portable and in-house analyzers from equine venous blood (a) and peritoneal fluid (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

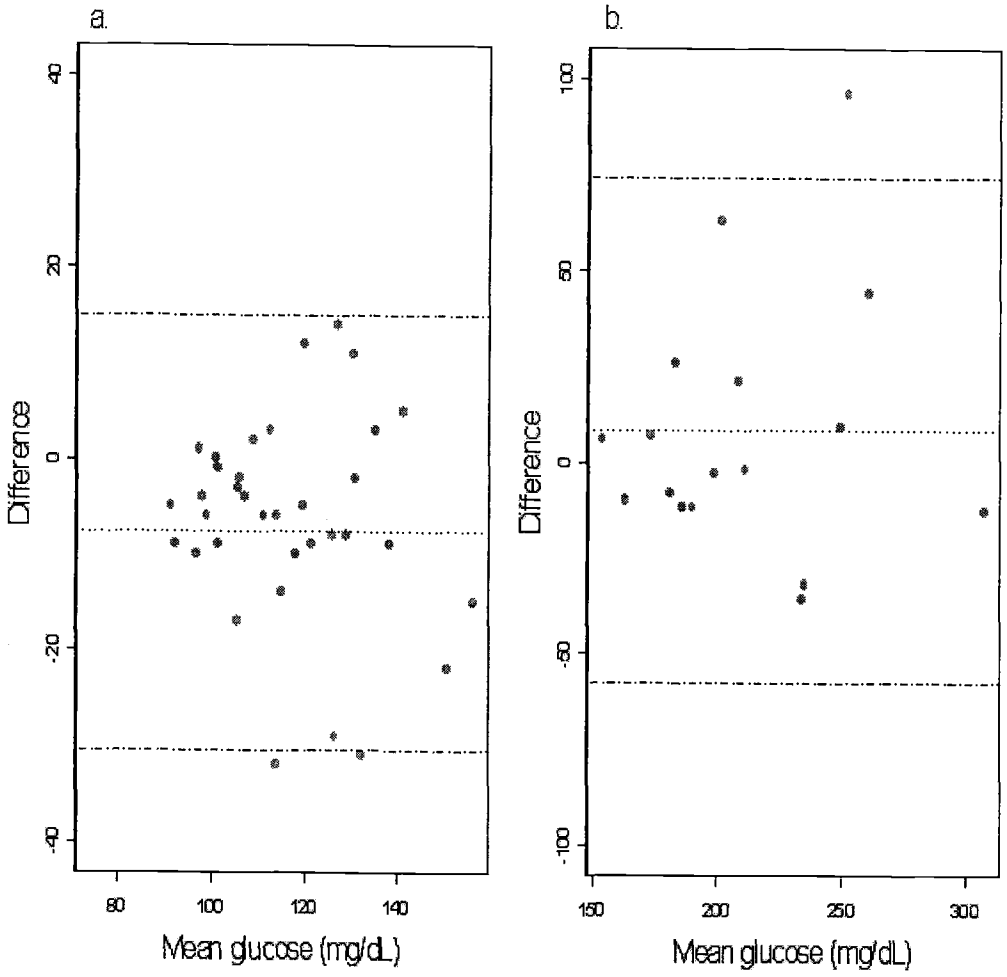

Figure 6: Plots depicting the difference between glucose values obtained by portable and in-house analyzers from equine venous blood with glucose values < 150 mg/dL (a) and glucose values > 150 mg/dL (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

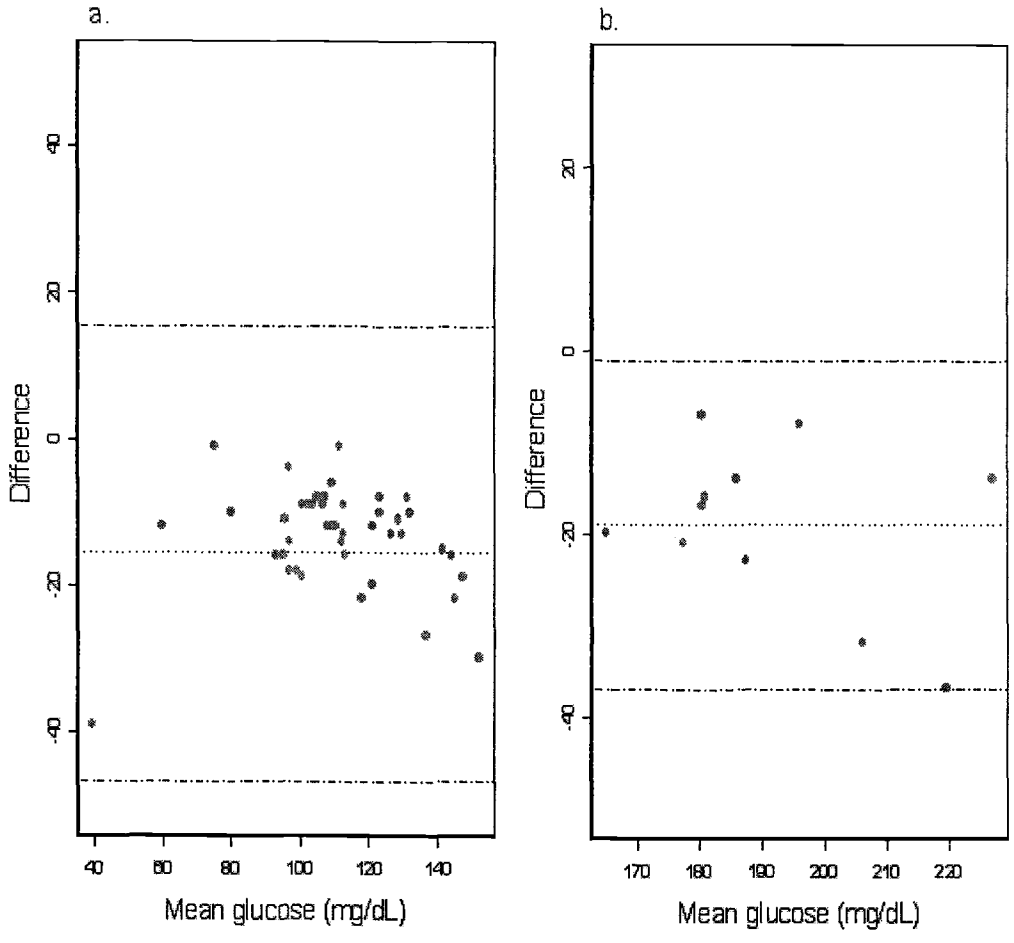

Figure 7: Plots depicting the difference between the glucose values obtained by portable and in-house analyzers from equine peritoneal fluid with glucose values < 150 mg/dL (a) and glucose values > 150 mg/dL (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

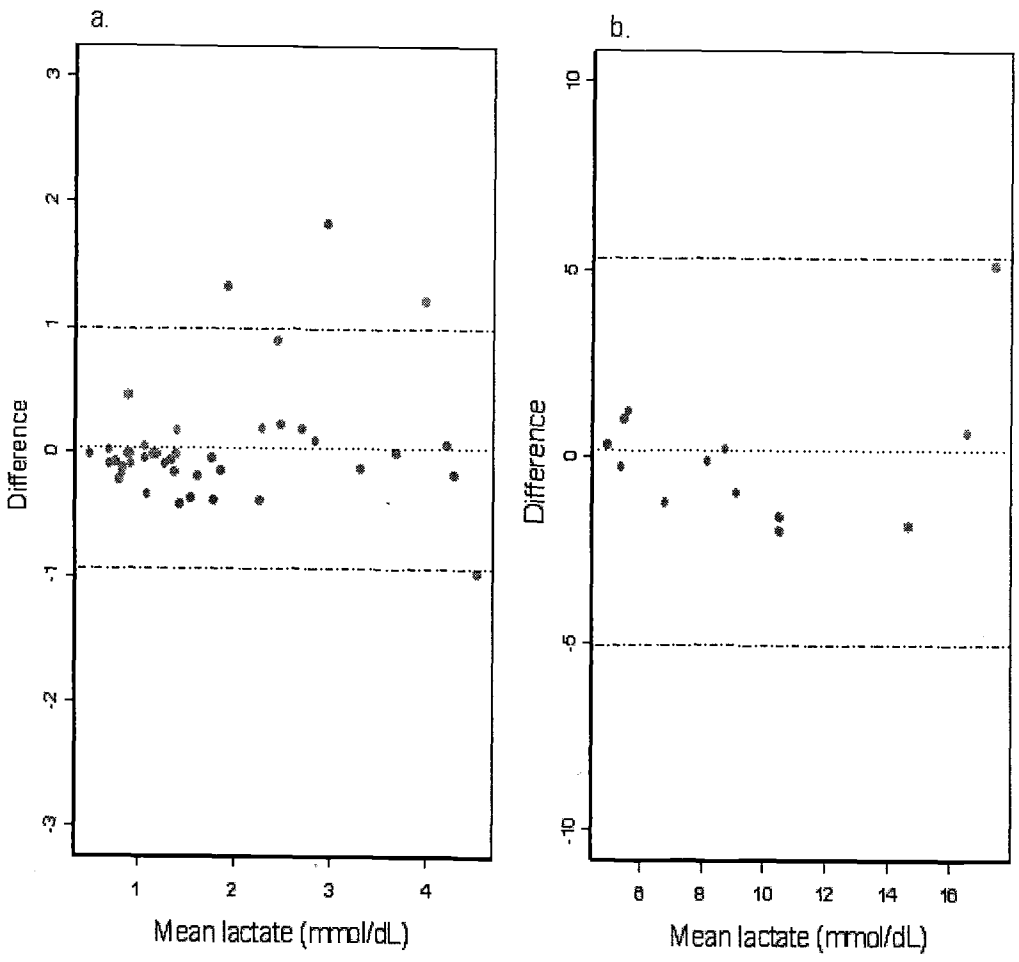

Figure 8: Plots depicting the difference between the lactate values obtained by portable and in-house analyzers from equine venous blood with lactate values < 5 mmol/dL (a) and lactate values > 5mmol/dL (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

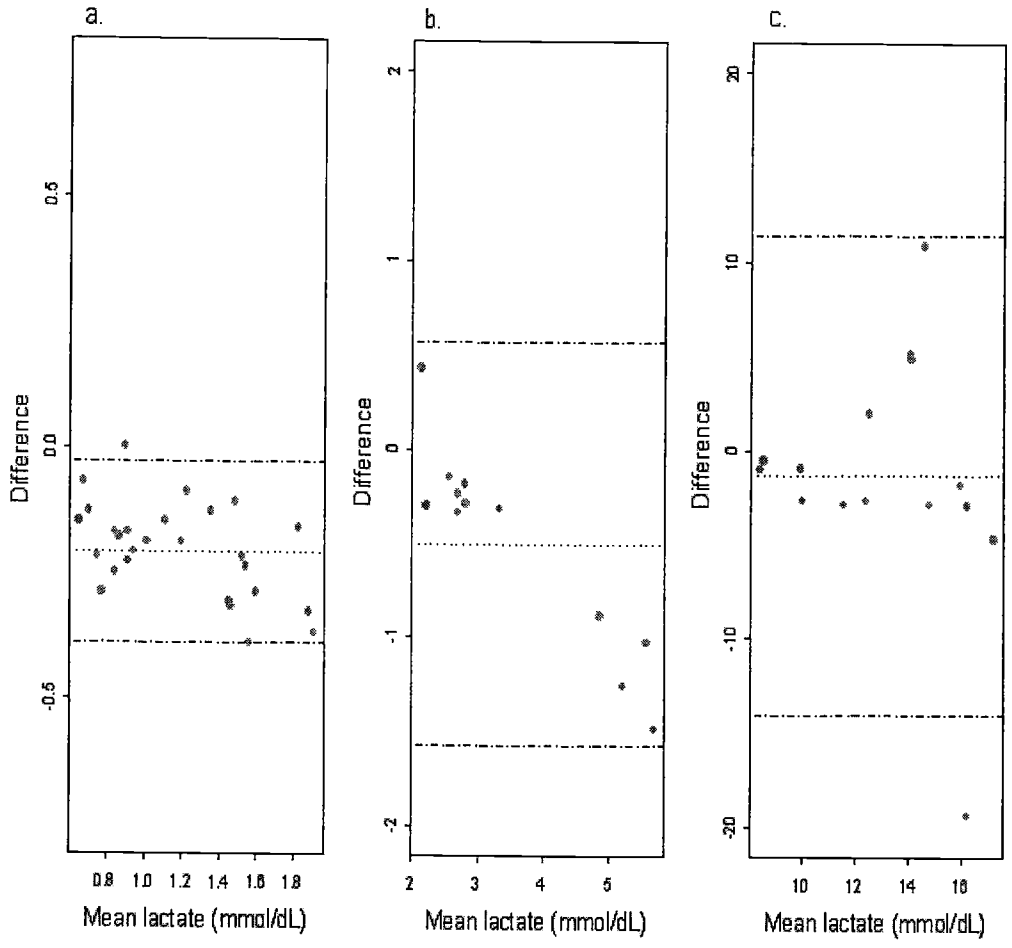

Figure 9: Plots depicting the difference between the lactate values obtained by portable and in-house analyzers from equine peritoneal fluid with lactate values < 2 mmol/dL (a), 2 mmol/dL – 7 mmol/dL (b) and > 5mmol/dL (c). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

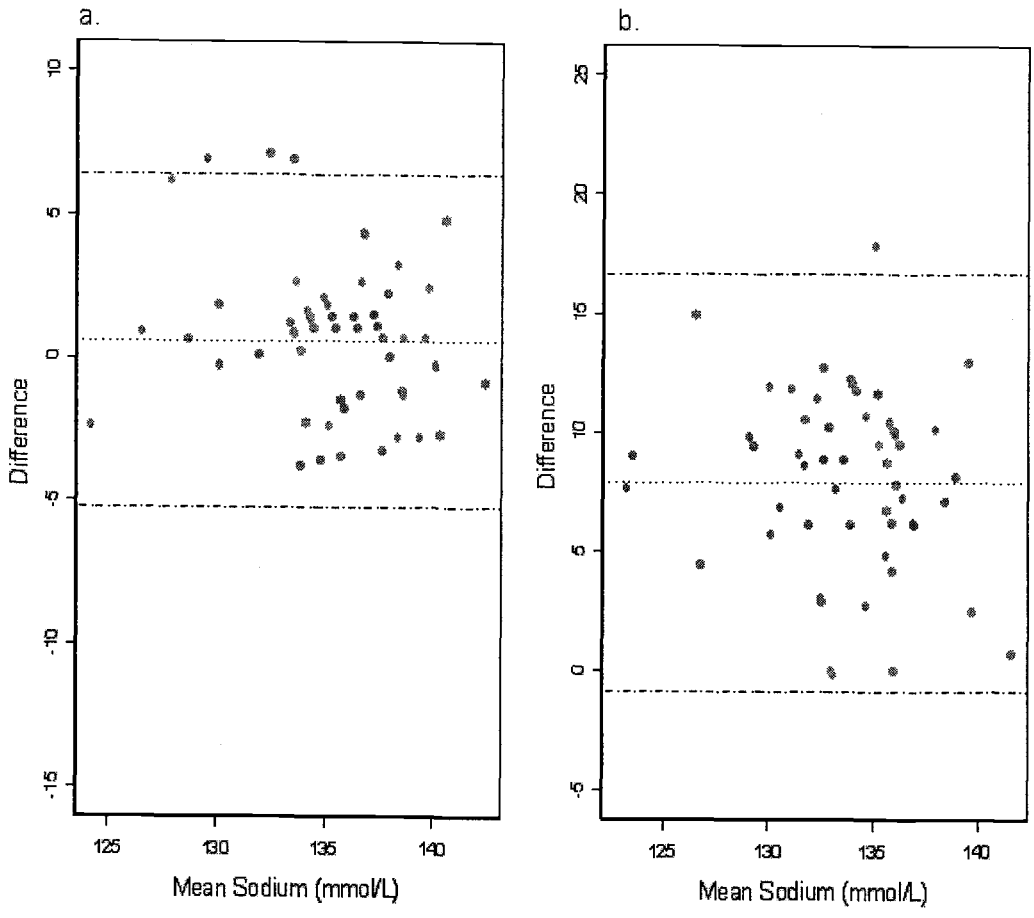

Figure 10: Plots depicting the difference between the sodium values obtained by portable and in-house analyzers from equine venous blood (a) and peritoneal fluid (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

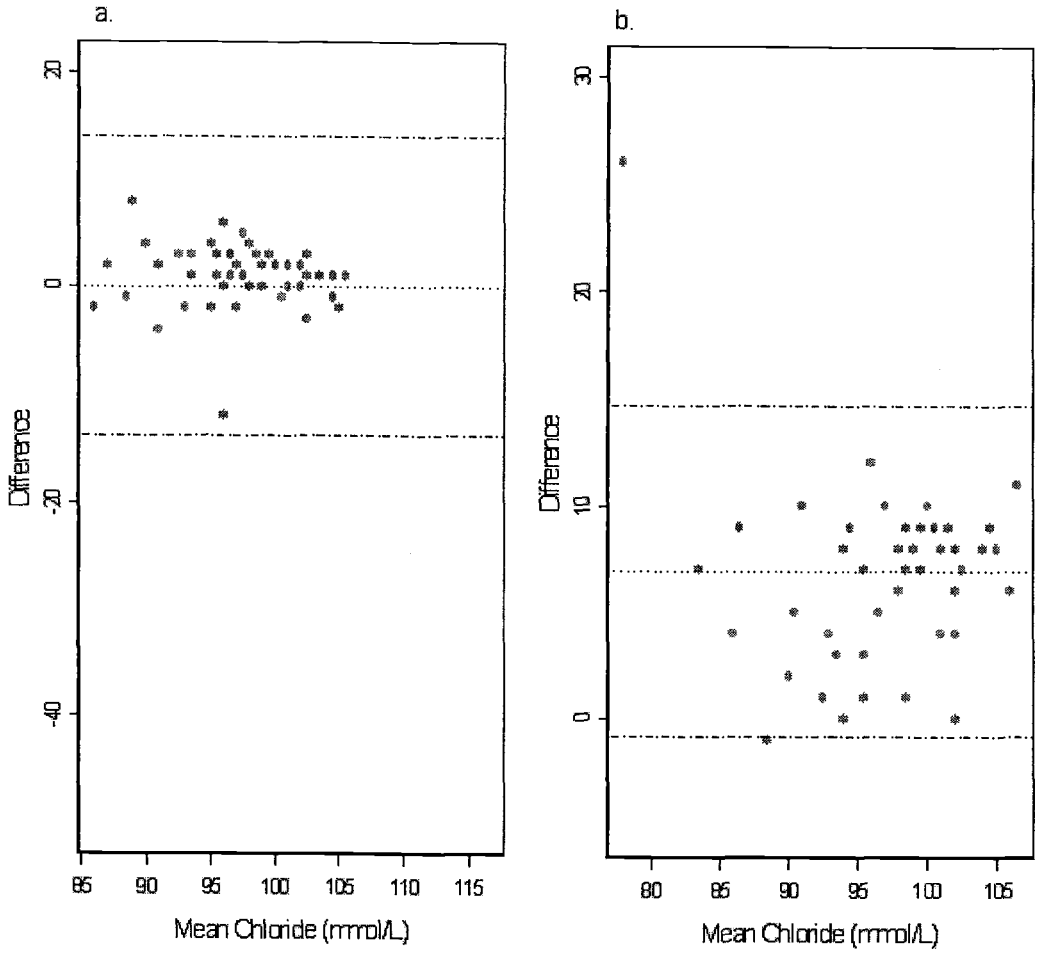

Figure 11: Plots depicting the difference between the chloride values obtained by portable and in-house analyzers from equine venous blood (a) and peritoneal fluid (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

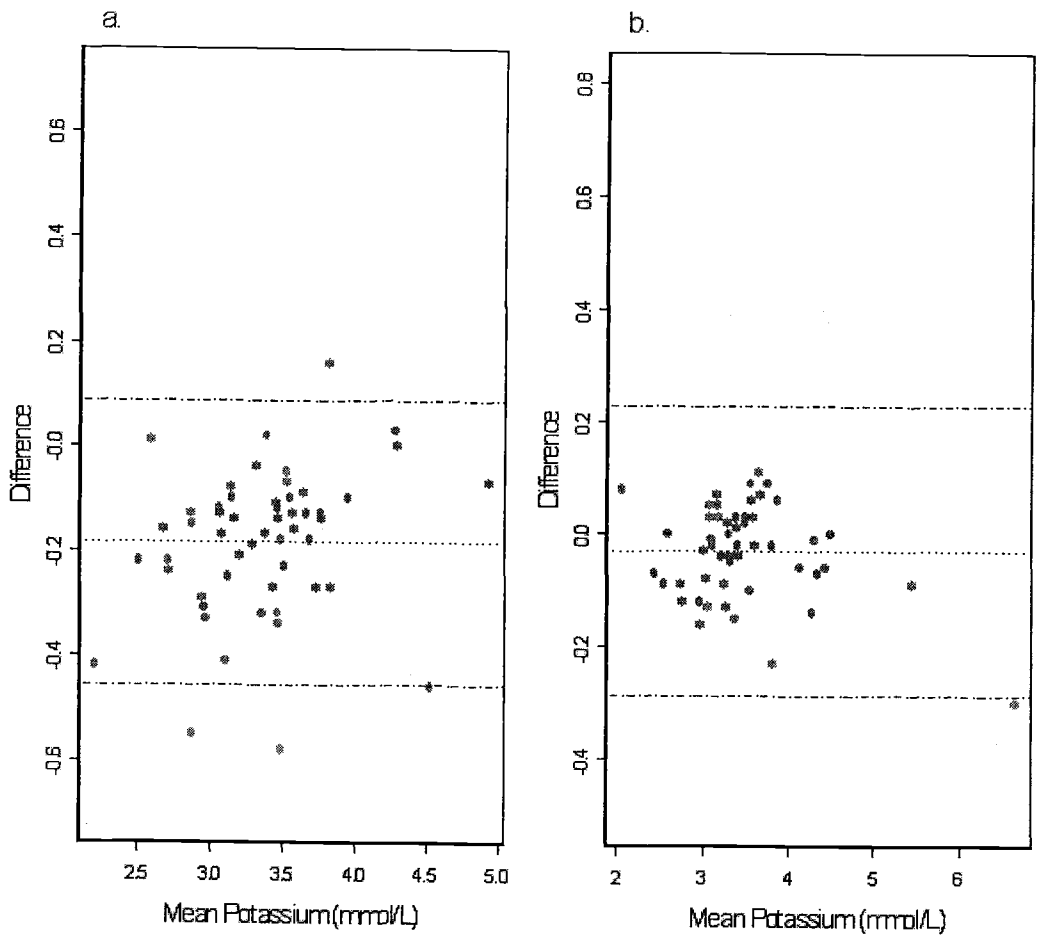

Figure 12: Plots depicting the difference between the potassium values obtained by portable and in-house analyzers from equine venous blood (a) and peritoneal fluid (b). Dotted horizontal line indicates mean difference (bias) and dashed horizontal line indicates the estimated upper and lower limits of agreement (± 2 SD of the mean difference).

Table 2: Comparison of reportable ranges for the portable clinical analyzer and the in-house analyzer.

Analyte	Reportable range for portable clinical analyzer	Reportable range for in-house analyzer
pH value	6.5-8.0	6.0-8.0
Glucose (mg/dL)	20-700	10-999
Lactate (mmol/L)	0.3-20	0-30
Sodium (mmol/L)	100-180	70-200
Chloride (mmol/L)	40-160	65-140
Potassium (mmol/L)	2.0-9.0	0.50-9.99

Table 3: Comparisons between the portable clinical analyzer and in-house analyzer for equine venous blood.

Analyte	Ranges compared using the in-house analyzer	No. of samples	Mean[1] and agreement interval
pH value	6.80-8.00	56	0.02* (-0.11 to 0.15)
Glucose (mg/dL)	0-149.99	37	-7.60* (-30.36 to 15.14)
	150-350	19	8.5 (-57.52 to 72.46)
Lactate (mmol/dL)	0-4.99	41	0.03 (-0.93 to 1.00)
	5-20.00	15	0.14 (-5.03 to 5.30)
Sodium (mmol/L)	120-150	56	0.57 (-5.26 to 6.40)
Chloride (mmol/L)	85-120	56	-0.07 (-13.98 to 13.84)
Potassium (mmol/L)	2-5	56	-0.18 (-0.46 to 0.09)

[1]Mean of the observed differences between the portable clinical analyzer and the in-house analyzer

*$P < 0.05$, from a paired t-test.

Table 4: Comparisons between the portable clinical analyzer and in-house analyzer for equine peritoneal fluid.

Analyte	Ranges compared using the in-house analyzer	No. of samples	Mean[1] and agreement intervals
pH value	6.80-8.00	56	0.08* (-0.31 to 0.47)
Glucose (mg/dL)	0-149.99	46	-15.60* (-46.71 to 15.51)
	150-300	10	-19.00* (-36.99 to -1.01)
Lactate (mmol/dL)	0-1.99	29	-0.21 (-0.39 to -0.03)
	2-6.99	13	-0.51 (-1.58 to 0.57)
	7-20	14	-1.30 (-14.02 to 11.42)
Sodium (mmol/L)	120-150	56	7.88 (-0.90 to 16.67)
Chloride (mmol/L)	85-120	56	6.91 (-0.83 to 14.65)
Potassium (mmol/L)	2-5	56	-0.03 (-0.29 to 0.22)

[1]Mean of the observed differences between the portable clinical analyzer and the in-house analyzer.
*$P < 0.05$, from a paired t-test.

Table 5: Least-squares regression comparison of mean differences between the portable clinical analyzer and the in-house analyzer.

Analyte	n	r^2	y =
pH			
Venous	56	0.736	1.087x - 0.621
SE			(0.135) (1.004)
Peritoneal	56	0.806	1.156x - 1.096
SE			(0.115) (0.873)
Glucose			
Venous	56	0.924	1.065x - 12.152
SE			(0.061) (9.338)
Peritoneal	56	0.93	0.903x - 3.251
SE			(0.049) (6.825)
Lactate			
Venous	56	0.945	0.999x + 0.068
SE			(0.047) (0.262)
Peritoneal	56	0.829	0.744x + 0.760
SE			(0.068) (0.532)
Sodium			
Venous	56	0.722	-0.131x + 135.929
SE			(1.179) (4.13)
Peritoneal	56	0.522	0.403x + 85.327
SE			(0.09) (11.605)
Chloride			
Venous	56	0.43	0.685x + 30.770
SE			(0.2) (19,63)
Peritoneal	56	0.796	0.845x + 8.758
SE			(0.087) (8.844)
Potassium			
Venous	56	0.964	1.032x - 0.295
SE			(0.039) (0.138)
Peritoneal	56	0.984	0.954x + 0.133
SE			(0.024) (0.084)

(x = in-house analyzer, y = portable clinical analyzer)

Conclusions

This study was designed to compare the biochemical analysis of both blood and peritoneal fluid through the use of a PCA and an in-house analyzer. Point-of-care testing comparing a PCA and in-house-analyzer has been previously reported; however, these reports only used whole blood.[16-24] Reports on the use of a PCA in an ambulatory setting in human[16,17] and veterinary medicine[18] exist. Of particular interest was the PCA, as it would be most useful to the equine ambulatory veterinarian. Both analyzers were easy to use, displayed results with venous reference ranges, and can store data. Results may be printed providing hard copies for the patient record, or easily recalled from the analyzer's memory. It should be noted that the analysis of peritoneal fluid by either analyzer was beyond the manufacturer's intended use.

Although there have been numerous comparisons of methods to analyze equine blood,[18-20] there is a paucity of articles concerning biochemical analyses of equine peritoneal fluid. Thus, a "gold standard" is lacking. Correlation coefficients and techniques such as regression analysis have been previously used to compare measurements between different methods.[15,25] However, the use of correlation coefficients is unsuitable because it measures the strength of a relationship between two measures and not the clinical agreement between them, making it possible to observe high correlations in data with poor agreement.[15] Therefore, using the Bland-Altman method of comparison,[15] data was compared by plotting

the difference against the means, allowing the new measurement modality (PCA) to be compared with the in-house analyzer.

The PCA yielded slightly higher venous pH values, which concurred with one previous report,[20] but not with another study that underestimated the pH values.[19] This discrepancy is likely to have minimal clinical significance. Following analysis of peritoneal fluid, the PCA yielded higher pH values in the alkaline range and lower pH values in the acidic range. Horses with septic peritonitis tend to have lower peritoneal fluid pH value than horses with non-septic peritonitis and or healthy horses[3] due to bacterial production of acid metabolite and lactate production by the peritoneal fluid neutrophils.[26-28] Peritoneal fluid pH may thus be used to differentiate septic from non-septic peritonitis; however, venous pH has not been shown to be of value in such differentiation.[3] Due to the agreement in the acidic pH range in peritoneal fluid, both analyzers appear to yield similar information and this would lead to similar clinical decisions.

Lactic acidosis is a common metabolic disorder in horses suffering colic.[29] Lactate accumulation in blood and peritoneal fluid may occur in cases of hypoperfusion or intestinal strangulation and is associated with increased mortality.[29-31] The PCA had greater agreement with the in-house analyzer at low lactate concentrations; however, the higher lactate concentrations would be of greater clinical relevance. Even though there appeared to be less agreement between the two analyzers at high lactate values, especially in the peritoneal fluid, both analyzers yielded high values. Most variation was due to 3 peritoneal fluid

lactate values which were higher than the reportable range for the PCA. Intestinal ischemia due to torsion, strangulation or thrombo-embolic infarction may lead to lactate crossing the affected intestinal wall and accumulating in the peritoneal cavity.[30] If peritoneal lactate concentration is high, particularly if there is a disparity between venous and peritoneal fluid lactate concentrations, the clinician should be alerted to the possibility of intestinal ischemia,[30,32] and the necessity for possible surgical exploration.

The PCA underestimated venous glucose concentrations in the lower range as reported previously;[19] however, not in the higher range. Generally, these differences in blood glucose concentration were not clinically relevant. Typically, peritoneal fluid glucose concentrations should be higher than in peripheral blood.[33,34] However, should intestinal ischemia and bacterial invasion into the abdomen occur, peritoneal fluid glucose concentration will decrease.[3,6] This may be due to consumption of glucose by either bacterial or phagocytic cells, glycolytic enzymatic activity in peritoneal fluid, or low transport of glucose from the blood to peritoneal fluid.[35] Although the study population did not contain many horses with very low peritoneal fluid glucose concentration, the lower values obtained by the PCA suggest the analysis of equine peritoneal fluid by the PCA may overestimate septic peritonitis. Therefore, the lower glucose values reported by the PCA must be considered when making treatment decisions.

Electrolyte disturbances are not well described factors in equine colic but are seen in bladder rupture[4] and colitis.[36] Peritoneal fluid potassium concentration

increases following small colon ischemia.[37] Hyponatremia, hypochloremia and hyperkalemia have been reported in horses with ruptured bladders.[4] The PCA was able to identify electrolyte disturbances and thus aided in the diagnosis of uroperitoneum. Overall, there seemed to be greater agreement between the two machines for blood than peritoneal fluid electrolyte concentration and the clinician may need to adjust the reference value for the PCA when assessing peritoneal fluid.

Certain limitations should be recognized despite the clinical accuracy of the portable analyzer. The portable analyzer works under optimum temperatures (16°C – 30°C), thus if exposed to prolonged cold, the analyzer would require time to warm up to become operational. The analyzer uses cartridges contained in pouches which required storage at 2°C – 8°C until used. Upon selection of the desired cartridge, the cartridge was brought to room temperature before opening the pouch and filling. Moreover, the analyzer does not have a display illuminator and could not be read in the dark No alphanumeric keypad was provided so patients had to be recorded by numerical identification.

Although the PCA was used in a hospital environment, results of our study indicate that the PCA may be suitable for point-of-care blood and peritoneal fluid analysis for horses suffering colic. The PCA as compared to the in-house analyzer, provided comparable results on average, and was able to further help diagnose metabolic acidosis, uroperitoneum, septic and non-septic peritonitis and intestinal

ischemia. The PCA may aid in determining the prognosis of horses with acute abdominal crisis in an ambulatory setting.

Bibliography

1. Hawkins JF, Bowman KF, Roberts MC, et al. Peritonitis in horses: 67 cases (1985-1990). *J Am Vet Med Assoc* 1993; 203:284-288.

2. Dyson S. Review of 30 cases of peritonitis in the horse. *Equine Vet J* 1993; 15:25-30.

3. Van Hoogmoed L, Rodger LD, Spier SJ, et al. Evaluation of peritoneal fluid pH, glucose concentration, and lactate dehydrogenase activity for the detection of septic peritonitis in horses. *J Am Vet Med Assoc* 1999; 214:1032-1036.

4. Kablack KA, Embertson RM, Bernard WV, et al. Uroperitoneum in the hospitalised equine neonate: retrospective study of 31 cases, 1988-1997. *Equine Vet J* 2000; 32:505-508.

5. Arden WA, Stick JA. Serum and peritoneal fluid phosphate concentrations as predictors of major intestinal injury associated with equine colic. *J Am Vet Med Assoc* 1998; 193:927-931.

6. Parry BW. Use of clinical pathology in evaluation of horses with colic. *Vet Clin North Am Equine Pract* 1987; 3:529-542.

7. Hjortkjaer RK, Svendson CK. Simulated small intestinal volvulus in the anesthetized horse. *Nord Vet Med* 1979; 31:466-483.

8. Parry BW, Anderson GA, Gay CC. Prognosis in equine colic: a study of individual variables used in case assessment. *Equine Vet J* 1983; 15:337-344.

9. Genn HJ, Hertsch B. Diagnostical and prognostical value of blood lactate concentration and its level in peritoneal fluid in colic of the horse. *Dtsch Tierarztl Wochenschr* 1982; 89:295-229.

10. Svendson CK, Hjortkjaer RK, Hesselholt M. Colic in the horse. A clinical and clinical chemical study of 42 cases. *Nord Vet Med* 1979; 31:Suppl 1-32.

11. Fischer AT. Diagnostic and prognostic procedures for equine colic surgery. *Vet Clin North Am Equine Pract* 1989; 2:335-350.

12. Allen D, White NA, Tyler DE. Factors for the prognostic use in equine obstructive small intestinal disease. *J Am Vet Med Assoc* 1986; 189:777-780.

13. Furr MO, Lessard P, White NA. Development of a colic severity score for predicting the outcome of equine colic. *Vet Surg* 1995; 24:97-101.

14. Reeves MJ, Curtis CR, Salman MD, et al. Prognosis in equine colic patients using multivariable analysis. *Can J Vet Res* 1989; 53:87-94.

15. Bland J.M., Altman D.G. Statistical methods for assessing the agreement between two methods of clinical measurement. *Lancet* 1986;1:307-310.

16. Herr DM, Newton NC, Santrach PJ, Hankins DG, Burrit MF. Airborne and rescue point-of-care testing. *Am J Clin Pathol* 1995; 104:S54-S58.

17. Burrit MF, Santrach PJ, Hankins DG, et al. Evaluation of the i-STAT portable clinical analyzer for use in a helicopter. *Scand J Clin Lab Invest* 1996; 56: 121-128.

18. Silverman SC, Birks EK. Evaluation of the i-STAT hand-held chemical analyser during treadmill and endurance exercise. *Equine Vet J* Suppl. 2002; 34:551-554.

19. Grosenbaugh DA, Gadawski JE, Muir WW. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc* 1998; 213:691-694.

20. Looney AL, Ludders JL, Erb HN, et al. Use of a handheld device for the analysis of blood electrolyte concentration and blood gas partial pressures in dogs and horses. *J Am Vet Med Assoc* 1998; 213:526-530.

21. Erickson KA, Wilding P. Evaluation of a Novel Point-of-Care System, the i-STAT Portable Clinical Analyzer. *Clin Chem* 1993; 39, 2:283-287.

22. Tortella BJ, Lavery RF, Doran JV, et al. Precision, Accuracy, and Managed Care Implications of a Hand-Held Whole Blood Analyzer in the Prehospital setting. *Clin Chem* 1996; 106:124-127.

23. Sediame S, Zerah-Lancner F, d'Ortho MP, et al. Accuracy of the i-STAT bedside blood gas analyzer. *Eur Respir J* 1999; 14:214-217.

24. Jacobs E, Vadasdi E, Sarkozi L, et al. Analytical Evaluation of i-STAT Portable Clinical Analyzer and Use by Nonlaboratory Health-Care Professionals. *Clin Chem* 1993; 39:1069-1074.

25. Altman DG, Bland J.M. Measurement in medicine: The analysis of method comparison studies. *Statistician* 1983; 32:307-317.

26. Gitlin N, Stauffer JL, Silvestri RC. The pH of ascitic fluid in the diagnosis of spontaneous bacterial peritonitis in alcoholic cirrhosis. *Hepatology* 1982;2:408-411.

27. Potts DE, Wilcox MA, Good JT et al. The acidosis of low glucose pleural effusions. *Am Rev Resp Dis* 1978; 117:665-671.

28. Lloyd KCK, Stover SM, Pascoe JR, et al. Synovial fluid pH, cytologic characteristics, and gentamicin concentration after intra-articular administration of the drug in an experimental model of infectious arthritis in horses. *Am J Vet Res* 1990; 51:1363-1369.

29. Donawick WJ, Ramberg CF, Paul SR et al. The diagnostic and prognostic value of lactate determinations in horses with a cute abdominal crisis. *J S Afr Vet Assoc* 1975; 46:127.

30. Moore JN, Owen R, Lumsden JK. Clinical evaluation of blood lactate levels in equine colic. *Equine Vet J* 1976; 8:49-54.

31. Gosset KA, Cleghorn B, Martin GS, et al. Correlation between anion gap, blood L-lactate concentration and survival in horses. *Equine Vet J* 1987; 19:29-30.

32. Moore JN, Traver DS, Turner MF, et al. Lactic acid concentration in peritoneal fluid of normal and diseased horses. *Res vet Sci* 1977; 23:117-118.

33. Brownlow MA, Hutchins DR, Johnston KG. Reference values for equine peritoneal fluid. *Equine Vet J* 1981; 13:127-130.

34. Nelson AW. Analysis of equine peritoneal fluid. *Vet Clin North Am Large Anim Prac* 1979; 1:267-274.

35. Chaffin MK, Carter GK, Relford RL. Equine bacterial pluropneumonia. Part II. Clinical signs and diagnostic evaluation. *Compend Contin Educ Pract Vet* 1994; 16:362-379.

36. Corley KTT, Marr CM. Pathophysiology, assessment and treatment of acid-base disturbances in the horse. *Equine Vet Ed* 1998; 10:255.

37. Ruggles AJ, Freeman DE, Acland HM, et al. Changes in fluid composition. on the serosal surface of the jejunum and small colon subjected to venous strangulation obstruction in ponies. *Am J Vet Res* 1993; 54:333-340.

GENERAL CONCLUSIONS

Abdominal fluid analysis can provide the investigator with information which can be used diagnostically and prognostically in horses suffering acute abdominal pain. When deciding on a therapeutic plan, these results need to be integrated with clinical and laboratory findings in order to offer the best possible therapeutic plan. The clinician should be aware that on rare occasions, abdominal fluid may not reflect the severity of intestinal insult creating a false impression of a less severe or absent intestinal lesion.

This study demonstrated that ALP activity may be used as an indicator of the degree of intestinal ischemia in peritoneal fluid, helping the clinician in deciding whether surgical intervention was necessary and allowing further determination of the prognosis for a horse with colic.

Although no "gold standard" exists with regard to the use of an automated clinical analyzer for the biochemical analysis of peritoneal fluid, this study shows that the PCA yields consistent results which were comparable to the in-house analyzer. The PCA helps diagnose metabolic acidosis, dehydration, intestinal ischemia and uroperitoneum and is able to differentiate between septic and non-septic peritonitis. The PCA may thus be beneficial to the ambulatory veterinarian when performing point-of-care analysis biochemical analysis of blood and peritoneal fluid in horses suffering colic.

BIBLIOGRAPHY

Allen D, White NA, Tyler DE. Factors for the prognostic use in equine obstructive small intestinal disease. *J Am Vet Med Assoc* 1986; 189:777-780.

Altman DG, Bland J.M. Measurement in medicine: The analysis of method comparison studies. *Statistician* 1983; 32:307-317.

Arden WA, Stick JA. Serum and peritoneal fluid phosphate concentrations as predictors of major intestinal injury associated with equine colic. *J Am Vet Med Assoc* 1998; 193:927-931.

Barrelet A. Peritoneal fluid: Part 1–Laboratory analyses. *Equine Vet Ed* 1993; 5:81-83.

Blackmore DJ, Palmer A. Phenylalanine inhibited p-nitrophenol phosphatase activity in the serum as an indication of intestinal cellular disruption in the horse. *Research in Vet Science* 1977;23:146-152.

Bland J.M., Altman D.G. Statistical methods for assessing the agreement between two methods of clinical measurement. *Lancet* 1986;1:307-310.

Bodansky O. Are phosphatases of bone, kidney, and serum identical? Use of bile acids in their differentiation. *J of Biological Chemistry* 1937;118: 341-362.

Brownlow MA, Hutchins DR, Johnston KG. Reference values for equine peritoneal fluid. *Equine Vet J* 1981; 13:127-130.

Burrit MF, Santrach PJ, Hankins DG, et al. Evaluation of the i-STAT portable clinical analyzer for use in a helicopter. *Scand J Clin Lab Invest* 1996; 56: 121-128.

Chaffin MK, Carter GK, Relford RL. Equine bacterial pluropneumonia. Part II. Clinical signs and diagnostic evaluation. *Compend Contin Educ Pract Vet* 1994; 16:362-379.

Corley KTT, Marr CM. Pathophysiology, assessment and treatment of acid-base disturbances in the horse. *Equine Vet Ed* 1998; 10:255.

Cowell RL, Tyler RD, Clinkenbeard KD, MacAllister CG. Collection and evaluation of equine peritoneal fluid and pleural effusion. *Vet Clinics of North Am* 1987;3:543-561.

Davies J, Gerring EL, Goodburn R, Manderville P. Experimental ischemia of the ileum and concentrations of the intestinal isoenzyme of alkaline phosphatase in plasma and peritoneal fluid. *Equine Vet J* 1984;16,3:215-217.

Donawick WJ, Ramberg CF, Paul SR et al. The diagnostic and prognostic value of lactate determinations in horses with a cute abdominal crisis. *J S Afr Vet Assoc* 1975; 46:127.

Dyson S. Review of 30 cases of peritonitis in the horse. *Equine Vet J* 1993;15:25-30.

Erickson KA, Wilding P. Evaluation of a Novel Point-of-Care System, the i-STAT Portable Clinical Analyzer. *Clin Chem* 1993; 39:283-287.

Fischer AT. Diagnostic and prognostic procedures for equine colic surgery. *Vet Clin North Am Equine Pract* 1989; 2:335-350.

Furr MO, Lessard P, White NA. Development of a colic severity score for predicting the outcome of equine colic. *Vet Surg* 1995; 24:97-101.

Genn HJ, Hertsch B. Diagnostical and prognostical value of blood lactate concentration and its level in peritoneal fluid in colic of the horse. *Dtsch Tierarztl Wochenschr* 1982; 89:295-229.

Gitlin N, Stauffer JL, Silvestri RC. The pH of ascitic fluid in the diagnosis of spontaneous bacterial peritonitis in alcoholic cirrhosis. *Hepatology* 1982;2:408-411.

Gosset KA, Cleghorn B, Martin GS, et al. Correlation between anion gap, blood L-lactate concentration and survival in horses. *Equine Vet J* 1987; 19:29-30.

Grosenbaugh DA, Gadawski JE, Muir WW. Evaluation of a portable clinical analyzer in a veterinary hospital setting. *J Am Vet Med Assoc* 1998; 213:691-694.

Hawkins J. Peritonitis. In: Robinson NE, ed. *Current Therapy in Equine Medicine 5*. Philadelphia, PA: Saunders; 2003:153-158.

Hawkins JF, Bowman KF, Roberts MC, et al. Peritonitis in horses: 67 cases (1985-1990). *J Am Vet Med Assoc* 1993; 203:284-288.

Herr DM, Newton NC, Santrach PJ, Hankins DG, Burrit MF. Airborne and rescue point-of-care testing. *Am J Clin Pathol* 1995; 104:S54-S58.

Hjortkjaer RK, Svendson CK. Simulated small intestinal volvulus in the anesthetized horse. *Nord Vet Med* 1979; 31:466-483.

Jacobs E, Vadasdi E, Sarkozi L, et al. Analytical Evaluation of i-STAT Portable Clinical Analyzer and Use by Nonlaboratory Health-Care Professionals. *Clin Chem* 1993; 39:1069-1074.

Kablack KA, Embertson RM, Bernard WV, et al. Uroperitoneum in the hospitalised equine neonate: retrospective study of 31 cases, 1988-1997. *Equine Vet J* 2000; 32:505-508.

Lloyd KCK, Stover SM, Pascoe JR, et al. Synovial fluid pH, cytologic characteristics, and gentamicin concentration after intra-articular administration of the drug in an experimental model of infectious arthritis in horses. *Am J Vet Res* 1990; 51:1363-1369.

Looney AL, Ludders JL, Erb HN, et al. Use of a handheld device for the analysis of blood electrolyte concentration and blood gas partial pressures in dogs and horses. *J Am Vet Med Assoc* 1998; 213:526-530.

Milne EM, Doxey DL, Gilmour JS. Analysis of peritoneal fluid as a diagnostic aid in grass sickness (equine dysautonomia). *Vet Record* 1990; 127:162-165.

Moore JN, Owen R, Lumsden JK. Clinical evaluation of blood lactate levels in equine colic. *Equine Vet J* 1976; 8:49-54.

Moore JN, Traver DS, Turner MF, et al. Lactic acid concentration in peritoneal fluid of normal and diseased horses. *Res vet Sci* 1977; 23:117-118.

Nelson AW. Analysis of equine peritoneal fluid. *Vet Clin North Am Large Anim Prac* 1979; 1:267-274.

Parry BW. Use of clinical pathology in evaluation of horses with colic. *Vet Clin North Am Equine Prac* 1987; 3:529-542.

Parry BW, Anderson GA, Gay CC. Prognosis in equine colic: A study of individual variables used in case assessment. Eq Vet J 1983;15,4:337-344.
Parry BW. Use of Clinical Pathology in the evaluation of horses with colic. *Vet Clin North Am* 1987; 3:529-542.

Potts DE, Wilcox MA, Good JT et al. The acidosis of low glucose pleural effusions. *Am Rev Resp Dis* 1978; 117:665-671.

Reeves MJ, Curtis CR, Salman MD, et al. Prognosis in equine colic patients using multivariable analysis. *Can J Vet Res* 1989; 53:87-94.

Ruggles AJ, Freeman DE, Acland HM, FitzSimmons M. Changes in fluid composition on the serosal surface of jejunum and small colon subjected to venous strangulation obstruction in ponies. *Am J Vet Res* 1993; 54:333-340.

Sediame S, Zerah-Lancner F, d'Ortho MP, et al. Accuracy of the i-STAT bedside blood gas analyzer. *Eur Respir J* 1999; 14:214-217.

Silverman SC, Birks EK. Evaluation of the i-STAT hand-held chemical analyser during treadmill and endurance exercise. *Equine Vet J* Suppl. 2002; 34:551-554.

Svendson CK, Hjortkjaer RK, Hesselholt M. Colic in the horse. A clinical and clinical chemical study of 42 cases. *Nord Vet Med* 1979; 31:Suppl 1-32.

Tortella BJ, Lavery RF, Doran JV, et al. Precision, Accuracy, and Managed Care Implications of a Hand-Held Whole Blood Analyzer in the Prehospital setting. *Clin Chem* 1996; 106:124-127.

Van Hoogmoed L, Rodger LD, Spier SJ, et al. Evaluation of peritoneal fluid pH, glucose concentration, and lactate dehydrogenase activity for the detection of septic peritonitis in horses. *J Am Vet Med Assoc* 1999; 214:1032-1036.