

Standard Article

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The Effect of Inadequate Presample Blood Volume Withdrawal from Intravenous Catheter and Extension Sets on Measured Circulating L-Blood Lactate Concentration in Horses Receiving Lactated Ringer's Solution

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Background: Circulating L-lactate concentration is commonly measured in hospitalized horses by sampling from indwelling intravenous (IV) catheters. However, there are no published evidence-based recommendations to prevent contamination by lactated Ringer's solution (LRS).

Hypothesis: Withdrawing 10 mL of blood from the LRS-containing extension set connected to the IV catheter before obtaining the sample for analysis should be adequate to obtain accurate measurement of blood lactate concentration (BLC).

Animals: Thirty-three adult hospitalized horses receiving constant rate infusion of LRS.

Methods: Immediately after disconnecting the LRS, 5 sequential 5 mL blood samples were obtained by aspiration from an extension set connected to an indwelling IV catheter, followed by 3 samples collected by direct venipuncture of the contralateral jugular vein. Samples were analyzed with 1 portable blood lactate analyzer. A linear mixed model was used to examine differences in lactate concentrations among samples collected from the catheter and by direct venipuncture.

Results: After considering differences in age, breed, sex, and reason for hospitalization, BLCs were higher ($P < .001$) in the first and second 5 mL samples collected through the extension set/catheter than in all other extension set/catheter samples or the direct venipuncture samples. The largest difference observed between the third and subsequent catheter or venipuncture samples was 0.34 mmol/L with an upper 95% CI of 1.12 mmol/L.

Conclusions and Clinical Importance: Withdrawing 15 mL of blood from a LRS-containing extension set connected to an IV catheter (5.9 mL total volume capacity) before obtaining the sample for blood lactate analysis is suggested to optimize accuracy of BLC measurements.

Key words: Blood collection; Clinical pathology; Critical care; Lactate metabolism; Monitoring; Reliability.

L-lactate is produced by mammalian cells and is the end point of anaerobic glycolysis.¹ Circulating lactate concentration can reflect an abnormal oxygen delivery/consumption ratio at the tissue level (hyperlactatemia type A). Hyperlactatemia in critically ill horses is generally a result of suboptimal tissue perfusion and hypoxia as seen in shock, sepsis, hypovolemia, and low cardiac output states. When oxygen delivery to tissues is inadequate, anaerobic metabolism allows for the continued production of energy, and energy production becomes predominantly dependent on glycolysis with the subsequent formation of lactate.^{1,2} The measurement of circulating lactate concentration is widely used in human and veterinary medicine to assess the systemic

Abbreviations:

BLC	blood lactate concentration
CRI	continuous rate infusion
ICC	intraclass correlation coefficient
LRS	lactated Ringer's solution

repercussions of various disease states, to monitor treatments, and to determine prognosis.^{2–4}

Intravenous fluids are administered to increase perfusion and effective blood flow resulting in improved oxygen delivery to peripheral tissues. Lactated Ringer's solution (LRS), a lactate-containing solution, is routinely used to treat patients with hypovolemia, abnormal oxygen delivery/consumption ratio, and metabolic acidosis. Blood samples for clinicopathological evaluation are commonly obtained from the intravenous (IV) catheter or extension set used to administer IV fluids,⁵ and thus, the question has been raised as to whether the lactate contained in the LRS will influence measured blood lactate concentrations (BLC). A previous study has shown that even small concentrations of LRS in IV catheters in humans, if inadequately cleared from the catheter, can cause a false increase in the measured circulating lactate concentration.³

The potential for bias in measuring BLC has not been investigated in horses. The objective of this study was to identify the minimum volume of fluid that must be withdrawn and discarded from an IV catheter before collecting a sample to accurately measure BLC in horses receiving LRS. We hypothesized that withdrawing 10 mL of blood from the LRS-containing extension

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All work for this study was performed at the Veterinary Medical Centre, Western College of Veterinary Medicine, University of Saskatchewan, SK, Canada.

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set connected to the IV catheter before obtaining the sample for blood lactate analysis would be adequate to obtain an accurate measured BLC. Results of this study are important to provide recommendations to equine clinicians regarding collecting and interpreting samples for measuring BLC in hospitalized horses receiving LRS.

Materials and Methods

Study Design

Client-owned adult horses admitted to the Veterinary Medical Centre (VMC), Western College of Veterinary Medicine, University of Saskatchewan, and treated with continuous rate infusion (CRI) of intravenous LRS were recruited for the study. All procedures were approved by the Animal Research Ethics Board, University Committee on Animal Care and Supply (UCACS), University of Saskatchewan, and followed the guidelines of the Canadian Council on Animal Care (CCAC).

Procedures

The study population comprised a total of 33 horses treated as in-patients for diverse medical reasons and admitted to the VMC from June 2015 to April 2016. The minimum number of horses required for the study ($n = 25$) was calculated to estimate the difference between samples with precision of 0.2 mmol/L with 95% confidence assuming the standard deviation of the differences was <0.5 mmol/L (<http://epitools.ausvet.com.au/content.php?page=1Mean>)^a.

Only horses receiving CRI of LRS^b through a 14 Ga \times 5.25 inch IV catheter^c connected to a rotating luer extension set^d as part of their treatment were included. All horses received a CRI of plain LRS or LRS with potassium chloride added for a minimum of 1 hour. During hospitalization and while receiving CRI of LRS, the extension set was clamped at its most distal clamp, and immediately after, 5 blood samples (5 mL each collected in 6 mL syringes^e) were obtained sequentially from the distal port of the extension set. BLC was measured stall-side immediately after obtaining each sample. The total volume capacity (dead space) of the 14 Ga \times 5.25 inch IV catheter connected to the 43 IN (109 cm) extension set was 5.9 mL (0.2 and 5.7 mL for the catheter and extension set volume capacity, respectively) calculated by a displacement technique *ex vivo*. After obtaining the 5 consecutive samples from the extension set connected to the IV catheter, they were analyzed and then 3 additional sequential samples (5 mL each, collected in 6-mL syringes^e) were obtained by venipuncture of the contralateral jugular vein with a 22 G \times 1½" needle^c and analyzed to serve as controls.

All samples were analyzed with a portable blood lactate analyzer (Lactate PlusTM)^f validated for use in horses⁶ and with a high intraclass correlation coefficient (ICC) compared to a bench top analyzer.⁶ According to manufacturer specifications, the blood lactate analyzer provides the test result within 13 seconds, with a test range of 0.3–25 mmol/L. The total time required to analyze 5 consecutive samples obtained from the extension set connected to the IV catheter was within 1 minute and 15 seconds. The 3 additional sequential samples were obtained immediately from the contralateral jugular vein and analyzed within 45 seconds.

Statistical Analysis

The data were managed in a commercial spreadsheet program^g and imported into a commercial statistical software program^h for

analysis. Lactate concentrations were described for each sequential sample collected from the extension set connected to the catheter and then for samples collected by direct venipuncture. Other variables of interest, including age (years), breed type (light horse versus warm blood), sex (mares versus geldings), and reason for hospitalization (colic versus other), also were summarized.

The average of the differences in lactate concentrations across the sequential samples from the catheter extension set and direct venipuncture within each horse was examined by a linear mixed model with a random intercept for each horse.⁷ The repeated measures within each horse were modeled using an autoregressive (AR1) correlation structure. The sequential sample identifiers, representing the source and order of the samples (catheter 1–5 and direct venipuncture 1–3), were considered in the model as fixed effects. The first venipuncture sample was designated as the reference category for the analysis. Horse age, breed type, sex, and reason for hospitalization were examined to see whether they confounded the differences among the ordered samples from the catheter extension set and then direct venipuncture. Confounding was assessed by adding each variable to the model containing the sample identifiers and evaluating whether any of the effect estimates changed by more than 20%. Variables such as age, breed, sex, and reason for hospitalization were not included in the model unless they were statistically significant ($P < .05$) or acted as an important confounder. BLCs below the detection limit were imputed at half the reported detection limit for the analyzer (<0.3 mmol/L). Model residuals were examined for normality and homogeneity of variance as well as for the presence of extreme outliers.

Agreement among measured lactate concentrations was estimated using 1-way random effects ICCs for samples collected by venipuncture as well as for samples collected from the catheter extension set after the values appeared to have stabilized based on the results of the linear mixed model.

Results

Study Population

The study sample consisted of 33 client-owned horses ranging in age from 2 to 30 years (mean, 12.3 years; standard deviation [SD] 6.7; median, 12.0; interquartile range [IQR], 8–15). There were 14 mares and 19 geldings in this group which included the following: 4 Arabians, 5 warm blood breeds, 13 Quarter Horses and crosses, 5 Thoroughbreds and crosses, and 5 Paints and 1 Appaloosa. Twenty-five of the horses were admitted for colic-related disorders and the remainder for other complaints including rhabdomyolysis (2), retained placenta, tarsal joint infection, choke (2), cellulitis, and nonsteroidal anti-inflammatory drug toxicity.

Analysis

Blood lactate concentrations varied from below the detection limit (<0.3 mmol/L) to 14.8 mmol/L (Table 1). In the unconditional analysis, BLC varied among sample numbers ($P < .001$), reflecting the order of collection and whether the sample was from the catheter extension set or direct venipuncture. There was no significant difference in lactate concentrations based on age ($P = .52$), breed type ($P = .88$), sex ($P = .63$), or reason for hospitalization ($P = .82$), and none of these

Table 1. Description of blood lactate concentration (mmol/L) among sequential 5 mL samples collected from an extension set connected to an intravenous jugular catheter and direct venipuncture.

	Catheter Sample	Catheter Sample	Catheter Sample	Catheter Sample	Catheter Sample	Direct jugular Venipuncture		
	1 (0–5 mL)	2 (5–10 mL)	3 (10–15 mL)	4 (15–20 mL)	5 (20–25 mL)	Sample 1	Sample 2	Sample 3
Mean	9.5	5.7	1.2	0.9	0.9	0.9	0.9	0.9
Standard deviation	3.0	3.2	0.7	0.4	0.4	0.4	0.4	0.4
Median	10.1	5.7	1.0	0.9	0.8	0.8	0.7	0.8
25th percentile	7.7	3.6	0.8	0.6	0.7	0.6	0.6	0.6
75th percentile	11.9	7.9	1.4	1.2	1.2	1.1	1.0	1.1
Minimum	2.1	1.0	0.5	0.4	0.3	<0.3	0.3	<0.3
Maximum	14.8	12.5	3.7	2.2	2.2	2.3	2.3	2.2
Count	33	33	33	33	31	33	31	31

Table 2. Final model of the differences in blood lactate concentration (mmol/L) among sequential 5 mL samples collected from an extension set connected to an intravenous jugular catheter and direct venipuncture.

	Coefficient	Upper 95% CI	Lower 95% CI	<i>P</i> > z
Intercept	0.87	0.31	1.43	.002
Sequentially collected blood samples				<.001 ^a
Catheter sample				
1 (0–5 mL)	8.62	7.83	9.40	<.001
2 (5–10 mL)	4.79	4.02	5.56	<.001
3 (10–15 mL)	0.33	–0.42	1.09	.38
4 (15–20 mL)	0.07	–0.63	0.76	.84
5 (20–25 mL)	0.07	–0.53	0.66	.83
Direct jugular venipuncture				
1 (0–5 mL)	Reference category			
2 (5–10 mL)	–0.002	–0.60	0.60	.99
3 (10–15 mL)	0.02	–0.70	0.74	.96

^aOverall type 3 Wald test for difference among sequential blood samples.

variables was an important confounder of the association between sample order and source and BLC.

In the final model, the average BLC was significantly higher (*P* < .001) in the first and second 5 mL samples collected from the catheter than from the first direct venipuncture sample (Table 2). The BLC for the first and second 5 mL samples from the catheter also was significantly higher than for each of the other catheter samples and the other 2 direct venipuncture samples (*P* < .001; Fig 1) based on additional post hoc pairwise comparisons (data not shown).

There were no other significant differences among the measured lactate concentrations of third to the fifth 5 mL samples and the 3 samples collected by direct venipuncture (*P* > .38). The smallest average difference was between the third and fourth catheter samples (0.26 mmol/L; 95% CI, –0.32 to 0.85, *P* = .38), and the largest average difference was between the third catheter sample and the second venipuncture sample (0.34 mmol/L; 95% CI, –0.45 to 1.12; *P* = .40).

Agreement among the 3 samples collected by venipuncture was good (ICC, 0.934; 95% CI, 0.884–

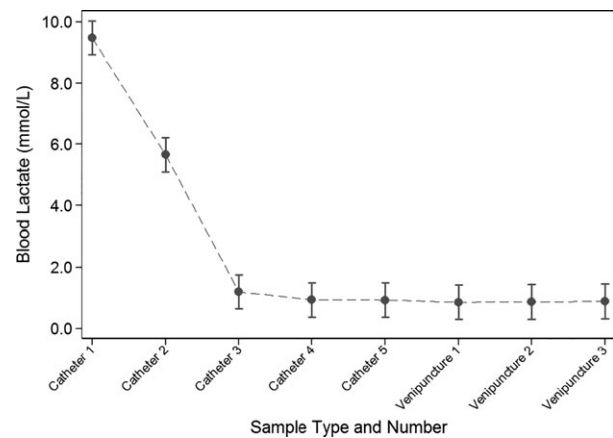


Fig 1. Predicted blood lactate concentrations (95% confidence intervals) for a series of 5 mL sequential blood samples collected from an extension set connected to an intravenous jugular catheter followed by three 5 mL blood samples collected directly from the contralateral jugular vein.

0.963). Agreement among the final 3 samples from the catheter was lower (ICC, 0.696; 95% CI, 0.537–0.819). Agreement between the final 2 samples from the catheter (4 [15–20 mL] and 5 [20–25 mL]) was substantially better (ICC, 0.945; 95% CI, 0.893–0.972).

Discussion

The aim of this study was to investigate the extent to which inadequate IV catheter clearance influences measurement of BLC in horses receiving LRS. Although in some horses potassium chloride was added to the LRS, it was not considered to have the potential to substantially alter BLC. Samples were purposely obtained from an extension set connected to the IV catheter, because it is common practice for horses having an indwelling IV catheter to have an extension set connected, and blood samples for laboratory analyses to be withdrawn from its distal port. Because the total volume capacity of the IV catheter and extension set was 5.9 mL, the hypothesis was that withdrawing 10 mL of blood before obtaining the sample would be adequate to obtain an accurate measured BLC. Age, breed type, and reason of hospitalization were recorded, but were not important

confounders of the association between sample sequence/source and BLC.

Our study showed that there was a significant difference in BLC in the first 5 mL sample obtained from the catheter extension set (“catheter 1”) compared to all subsequent samples obtained from the catheter extension set and from the contralateral vein. Similarly, when withdrawing an additional 5 mL of blood from the catheter extension set (“catheter 2”), BLC was also significantly higher than in all subsequent samples obtained from the catheter extension set and from the contralateral vein by venipuncture. This effect was not seen for the 3rd sample obtained from the catheter extension set (“catheter 3” [10–15 mL]). However, the agreement among the final 3 samples from the catheter extension set was not as good as that between the final 2 of the 5 catheter extension set samples. This suggests that the third sample (10–15 mL) potentially was different than the fourth or fifth although the difference was not statistically significant.

One of the study limitations that needs to be taken into account when interpreting the lack of statistical difference in the BLC from sample 3 obtained from the catheter extension set compared to other subsequent samples is whether the number of horses included in the study limited statistical power. The largest of the differences observed between the third catheter sample and the subsequent catheter or venipuncture samples was 0.34 mmol/L with an upper 95% CI of 1.12 mmol/L. Although a 0.34 mmol/L difference in BLC would be unlikely to result in a change in treatment plan or the prognosis for the patient, the 95% CI suggests that a 1.12 mmol/L difference from subsequent samples also was possible. Because normal BLC in healthy adult horses usually is <1.5 mmol/L,² a potential measurement difference of 1.2 mmol/L typically would be considered clinically important. Based on this information, withdrawing and discarding 15 mL of blood before obtaining a sample for BLC measurement are advisable.

Erythrocytes normally produce lactate and continue to produce lactate *ex vivo* if samples are not stored properly. Blood samples should be analyzed for lactate concentrations within 5–10 minutes of collection.² The potential for processing delays to bias these results was limited by analyzing all samples immediately after obtaining each sample. The BLC measurements were performed by the stall where the horse was kept to minimize the time between sampling and analysis.

In conclusion, withdrawing 15 mL of blood from an LRS-containing extension set connected to an IV catheter (5.9 mL total volume capacity) before obtaining the sample for blood lactate analysis is suggested to optimize the accuracy of measured BLC.

Footnotes

- ^a Sergeant, ESG, 2016. Epitools epidemiological calculators. Aus-Vet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease.
- ^b Lactate Ringer’s Injection USP. Baxter Corporation. 7125 Mississauga Rd. Mississauga, ON
- ^c BD Angiocath, Becton Dickinson Infusion Therapy Systems Inc, Sandy, UT
- ^d 43 IN (109 cm), 5.7 mL capacity, 2 clamps, rotating luer Catheter Extension Set, ICU Medical Inc, San Clemente, CA
- ^e Monoject, Covidien Ltd, Mansfield, MA
- ^f Lactate Plus, Nova Biomedical, Waltham, MA
- ^g Microsoft Excel, Microsoft Corporation, Redmond, WA
- ^h Stata/SE ver 14.0 for Windows, StataCorp LP, College Station, TX
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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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