Brief Communication

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Anion Gap Correlates with Serum D- and DL-Lactate Concentration in Diarrheic Neonatal Calves

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The objective of this study was to investigate the relationship between serum D- and L-lactate concentrations, and anion gap (AG) in neonatal calves. The association of AG with lactic acidosis in diarrheic calves has only been investigated by measurement of L-lactate in calves with experimentally induced diarrhea. D-lactate has recently been reported to be present in high concentrations in the serum of some diarrheic neonatal calves. The contribution of this acid to AG is not reported. The relationship between AG and L- and D-lactate concentrations was examined in 24 healthy calves and 52 calves with naturally occurring infectious diarrhea with metabolic acidosis. AG was calculated as $[Na^+ + K^+] - [Cl^- + HCO_{3-}]$. D- and L-lactate were quantified using high-performance liquid chromatography. There was no correlation between L-lactate and AG, contrary to previous reports in the literature. Moderate correlations between D-lactate concentration and AG ($r = .74$, $P < .0001$), and between DL-lactate and AG ($r = .77$), $P < .0001$) were detected. No differences existed due to the age or sex of the calf. This study indicates that AG provides information on the nature of acidosis in the diarrheic, neonatal calf and reinforces the importance of investigating clinical, in addition to experimental, populations.

Key words: Acid-base; Diarrhea; D-lactate; Metabolic acidosis.

Anion gap (AG) is widely used in diagnosing metabolic acidosis in humans, small animals, horses, and cattle. Calculation of AG is based on the principle of electroneutrality and is calculated as the difference between routinely measured cations (sodium and potassium) and routinely measured anions (chloride and bicarbonate).[1] The range of values in normal animals is 14–20 mmol/L,[1,2] representing the charge on serum proteins, phosphate, and strong anions, which are not routinely measured in clinical laboratories. Increases in AG generally are indicative of a gain of organic anions such as ketoacids or lactate.[1] Increased AG occurs in numerous syndromes and is used to differentiate metabolic acidosis caused by bicarbonate loss from that caused by accumulation of organic acid.[1] Metabolic acidosis observed in neonatal calves with enteric infections results from excessive losses of water and electrolytes, fecal bicarbonate loss, or titration of bicarbonate by organic acids.[3] Until recently, the major organic acid reported as a contributor to metabolic acidosis in diarrheic calves was L-lactate, which is produced by anaerobic metabolism resulting from hypovolemia and low oxygen supply to tissues.[4] However, some diarrheic calves have recently been shown to have high serum concentrations of D-lactate, with relatively low serum L-lactate concentrations.[5,6] D-lactate is produced almost exclusively by microbes. The pathogenesis of D-lactic acidosis in diarrheic calves is hypothesized to involve decreased absorption of substrate with subsequent fermentation of this material in the gastrointestinal tract.[6] The resultant drop in pH allows acid-resistant Gram-positive bacteria, particularly *Lactobacillus* spp., to proliferate and produce high concentrations of D- and L-lactate in the gut.[6] Because D-lactate is metabolized by mammals at approximately one fifth the rate of L-lactate, it accumulates in the blood.[7] Similar pathogeneses of D-lactic acidosis result from grain overfeeding of ruminants[8] and in short-bowel syndrome in humans.[9] The accumulation of milk in the reticulorumen of calves with abomasal volvulus[10] esophageal groove reflex disorders, reduced forestomach motility, or abomasoruminal reflux also result in high AG metabolic acidosis.[1]

The clinical utility of AG in calves has been questioned.[10] Thus far, however, only the association between AG and L-lactate has been investigated in calves with induced diarrhea resulting in moderate dehydration.[10] AG correlated moderately with serum L-lactate concentration ($r = .66$). To our knowledge, the association between AG and D-lactic acidosis has not been studied in a clinical setting.

Materials and Methods

A retrospective study of 26 healthy and 52 diarrheic mixed-breed calves (Red Angus, Charolais, Aberdeen-Angus, Simmental, Limousin, Holstein-Friesian, Hereford, and Gelbvieh) was carried out to assess the relationship between increased AG and serum D-, L- and DL-lactate concentrations. Diarrheic calves ranged from 5 to 35 days of age (mean = 16 days), and consisted of 25 females, 23 males, and 3 for which the sex was not recorded. Healthy calves ranged from 2 to 34 days of age (mean = 19 days). Diarrhea was defined as 3 or more profuse or watery stools per day. Samples were collected from diarrheic calves immediately after admission to the Large Animal Clinic at the Western College of Veterinary Medicine at the University of Saskatchewan, and from healthy calves at Goodale Farms and the Farm Department, University of Saskatchewan. None of the calves were treated intravenously with fluids prior to sampling. Blood was collected anaerobically from the jugular vein into a heparinized syringe and acid-base parameters were determined using an automated blood gas analyzer.[a] Simultaneous measurement of plasma sodium, potassium, and chloride concentrations was made using a spectrophotometric auto analyzer.[b] A second blood sample was collected and

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Table 1. Anion gap and lactate values from pooled data for healthy and diarrheic neonatal calves.

Factor		Mean ± SD	Range	Coefficient of Determination with Anion Gap[a]
Anion Gap (mmol/L)	Healthy	7.1 ± 2.6	3.0–11.1	—
	Diarrheic	23.4 ± 7.7	7.0–37.5	—
	Pooled	18.3 ± 10.0	3–37.5	—
D-Lactate (mmol/L)	Healthy	0.2 ± 0.3	nq–1.0	r = .41, $P = .04$
	Diarrheic	10.2 ± 7.7	nq–28.2	r = .57, $P < .0001$
	Pooled	7.0 ± 7.9	nq–28.2	r = .74, $P < .0001$
L-Lactate (mmol/L)	Healthy	2.2 ± 1.2	0.7–4.8	r = .24, $P = .23$
	Diarrheic	3.3 ± 2.9	0.4–12.9	r = 0, $P = .9$
	Pooled	2.9 ± 2.6	0.4–12.9	r = .14, $P = .16$
DL-Lactate (mmol/L)	Healthy	2.3 ± 1.3	0.7–5.0	r = .10, $P = .57$
	Diarrheic	13.4 ± 7.4	1.4–31.7	r = .58, $P < .0001$
	Pooled	9.9 ± 8.1	0.8–31.7	r = .77, $P < .0001$

nq, not quantifiable.
[a] significant at $P < .05$.

allowed to clot, was centrifuged, and the serum drawn off. The serum was frozen at −20°C until analysis. High-performance liquid chromatography (HPLC) was used for the stereospecific analysis of lactate enantiomers using a CHIRALPAK MA(+)[c] column and 2 mM copper sulphate in 1% acetonitrile as the mobile phase.[6] Two mM malonic acid was used as the internal standard. The HPLC system employed a Waters 715 Ultra WISP autosampler, a Waters 600 controller, and a Waters 486 Tunable Absorbance UV detector.[d] Prior to analysis, serum samples were deproteinized using ultrafiltration through an Ultrafree-MC centrifugal filter[e] with a 10,000 dalton cutoff. Quality-control samples were incorporated into each run. This assay demonstrates excellent linear relationships between peak area ratios and serum concentrations over a range of 0.5–20 mmoles/L. No interference was present between L- and D-lactic acid, as peaks were baseline resolved and without interfering peaks. The relative standard deviations for both enantiomers is <15%.

The study was approved by the animal care committee of the University of Saskatchewan.

Statistical Analyses

Statistical analyses were performed using SPSS version 11.[f] Simple linear regression analysis was used to test the relationship between AG and serum D-, L-, or DL-lactate concentrations at the 95% confidence level. AG values were subdivided into male and female groups and into age ranges to determine if any differences existed between these groups by using an independent *t*-test and 2-way ANOVA, respectively ($\alpha = 0.05$).

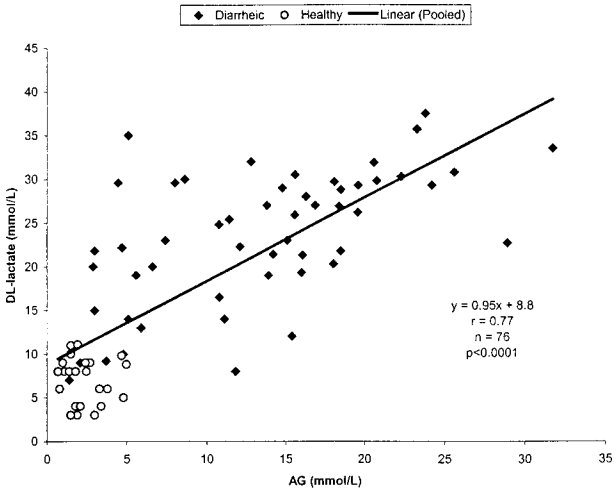

Fig 1. Relationship between anion gap (AG) and serum DL-lactate.

Results

Data from 2 healthy calves were omitted because AG values were negative. The mean AG (± SD) was 7.1 ± 2.6 mmol/L in healthy calves and 23.4 ± 7.7 mmol/L in diarrheic calves. The mean serum D- and L-lactate concentrations for healthy calves were 0.2 ± 0.3 mmol/L and 2.2 ± 1.2 mmol/L, respectively, and for diarrheic calves were 10.2 ± 7.7 mmol/L and 3.3 ± 2.9 mmol/L, respectively. The coefficients of determination were highly significant between serum D-lactate and AG (r = .74, $P < .0001$) and DL-lactate and AG (r = .77, $P < .0001$) in the pooled data (Table 1) (Fig 1). The coefficient of determination between L-lactate and AG was not significant (r = .14, $P > .1$) in the pooled data. Severity of AG did not differ significantly between male and female calves nor with the age of the calf.

Discussion

Results of this study indicate that AG significantly correlates with D- and total DL-lactate concentrations in serum but not with serum L-lactate concentration. This finding is contrary to a previous finding that L-lactate is significantly correlated with AG in diarrheic neonatal calves.[10] The finding of the present study reinforces the importance of obtaining data from a clinical population, as the diarrhea in the previous study was experimentally induced and resulted in dehydration and hypovolemia.

AG values obtained for healthy calves in this study are lower than some values in the literature (13.8–22.6 mmol/L,[1] 14–20 mmol/L[2]), but are consistent with AG values that are routinely obtained at the Western College of Veterinary Medicine,[6] and within the range of values (2–20 mmol/L) obtained by researchers in Berlin.[11]

The strong ion gap provides an estimate of the difference between unmeasured strong cations and strong anions and

is a more specific measure of change in unmeasured strong ions than AG,[12] but could not be calculated for the animals in this study because albumin, globulin, and phosphate measurements were not obtained. AG may also be influenced by variation in albumin, globulin, and phosphate and by other organic acids such as pyruvate, acetate, and 3-hydroxybutyrate.[1] Presumably, these account for much of the remaining 40% variation in AG that cannot be attributed to total DL-lactate concentration. We have shown that pyruvic acid and acetic acid levels are also slightly higher in diarrheic calves than in healthy calves (0.05 and 0.7 mmol/L increase, respectively).[6] Further evaluation of serum proteins, phosphate, and other organic acids would aid in understanding the increase in AG in diarrheic calves.

This study emphasizes the potential importance of D-lactic acidosis in infectious diarrhea, and demonstrates that AG is relevant to the practitioner, as much of the increase in AG observed in calf diarrhea is due to microbial fermentation in the gastrointestinal tract.

Footnotes

[a] Ciba-Corning Canada Inc., Markham, ON, Canada
[b] Abbot Spectrum System, North Chicago, IL, USA
[c] Chiral Technologies Inc., Exton, PA, USA
[d] Waters Limited, Mississauga, ON, Canada
[e] Millipore, Milford, MA, USA
[f] SPSS Inc., Chicago, IL, USA

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Erratum

The following abstract was mistakenly omitted from the Oral Research Communications of the 13th ECVIM-CA Congress which appeared in the *Journal* in Volume 17, Number 5 (September/October 2003). The *Journal* regrets the omission.

AN INTRANASAL KENNEL COUGH VACCINE PROTECTS DOGS AGAINST CLINICAL DISEASE CAUSED BY A COMBINED BORDETELLA BRONCHISEPTICA AND CANINE PARAINFLUENZA CHALLENGE FOR 1 YEAR. Linda Horspool, Ashley Gray, Ron Jaspers, Guntram Paul, Rob Theelen and Ton Jacobs Intervet International BV, PO Box 31, 5830AA Boxmeer,

The aim of this study was to determine whether an intranasal kennel cough vaccine offered protection against clinical disease in an experimental challenge model one year after vaccination.

12 dogs of 3 weeks of age were vaccinated with Nobivac KC (Intervet) by the intranasal route. A further 6 source- and age-matched dogs served as non-vaccinated controls. Fifty-six weeks after vaccination all dogs were challenged with wild-type *Bordetella bronchiseptica* (*Bb*) and Canine Parainfluenza (CPi) by the aerosol route. Oro-nasal swabs were taken regularly before and after challenge for isolation of *Bb* and CPi. All dogs were observed for clinical signs for 3 weeks after challenge.

Prior to challenge all dogs remained culture-negative for *Bb* and CPi. After challenge the control dogs became culture-positive for *Bb* and CPi. By contrast, isolation yields in the vaccinated group were significantly lower. Clinical scores of the vaccinated group post-challenge were also significantly lower than the control group (61% reduction, $p < 0.05$).

These results show that the kennel cough vaccine used in this experiment can protect dogs against clinical disease associated with *Bb* and CPi infection for 1 year.