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Experimentally Induced Systemic Hyperchloremic Acidosis in Calves

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Background: Among the various metabolic disturbances occurring in calves affected by neonatal diarrhea or ruminal acidosis, acidemia constitutes an important condition requiring specific therapy. Although various attempts have been made to estimate the degree of metabolic acidosis on the basis of clinical signs alone, some doubts have been raised regarding the accuracy and predictive value of the clinical variables suggested.

Hypothesis: The induction of metabolic acidosis in healthy calves via the infusion of hydrochloric acid (HCl) will lead to a clinical picture similar to that seen in neonatal calves with diarrhea or ruminal acidosis.

Animals: The study was carried out on 15 Holstein male calves between 5 and 19 days of age.

Methods: Hyperchloremic metabolic acidosis was induced over a period of 80 minutes by an IV infusion of 4,000 mL of 0.9% NaCl solution containing 400 mM HCl.

Results: Acidemia occurred rapidly and increased constantly up to a maximum value, which was reached in all calves by the end of the administration and amounted to a 22.4 mM/L mean base deficit (range from 17.0 to 33.1 mM/L).Despite the relatively severe acute acid-base imbalance during the entire observation period, no calves showed any clinical signs or depressed appetite.

Conclusions and Clinical Importance: Factors other than a disturbance of the acid-base balance should be considered to be primarily responsible for the clinical picture in calves affected by diarrhea or ruminal acidosis.

Key words: Acid-base; Clinical findings; D-lactate; Diarrhea; Hydrochloric acid; Ruminal acidosis.

The most important metabolic disturbances in calves with neonatal diarrhea[1] or ruminal acidosis caused by ruminal drinking[2,3] are dehydration, metabolic acidosis, hyper–L-/D-lactatemia, electrolyte imbalances, hypoglycemia, and hypothermia. Of these, dehydration and acidemia represent the principal conditions that require specific therapy.[4]

In order to decide upon the extent of fluid replacement and restoration of the physiologic acid-base balance, accurate and objective guidelines are necessary to assess the degree of the associated metabolic disturbances. Reliable clinical tests (skin elasticity and position of the eyeballs)[5] enable the clinician to satisfactorily estimate the degree of dehydration. Although various attempts have been made to estimate the severity of acidemia on the basis of clinical signs, some doubts have recently been raised regarding the validity of the clinical variables suggested.[6] Disturbance of posture, behavior, or both[7–13] and impairment of the sucking reflex[11,14] have been suggested as being correlated to systemic acidosis.

Recent observations on calves with naturally acquired diarrhea have shown that changes in demeanor and in posture seem to be better explained by an increase in the serum D-lactate concentrations than by a decrease in base excess.[14] Confirming these observations, experimentally induced hyper–D-lactatemia without acidemia reproduced some clinical findings once attributed to systemic acidosis, such as somnolence and staggering gait.[15] In particular, disturbances of the palpebral reflexes appeared to be strongly correlated to high levels of D-lactatemia. On the other hand, no impairment of the sucking reflex was observed as a consequence of hyper–D-lactatemia.

However, other authors have also demonstrated that D-lactate can produce acutely severe and wide-ranging neurological disturbances.[4,16]

The aim of this study was to investigate clinical findings in experimentally induced systemic acidosis and, therefore, to elucidate the role of the acid-base imbalance in the development of clinical manifestations in calves suffering from diarrhea or ruminal acidosis. The working hypothesis was that systemic acidosis induced by the infusion of HCl is associated with the clinical signs attributed to the spontaneous metabolic acidosis accompanying neonatal calf diarrhea.

Materials and Methods

Fifteen healthy Holstein-Friesian male calves were utilized for this study. Calves were managed in a free housing system with straw bedding and fed 1.5 L of milk replacer using nipple pails at 0800, 1300, and 1800 hours. The age of the calves on the day of the experiment ranged between 5 and 19 days (with a mean and SD of 11.2 and 3.6 days, respectively) and weights ranged between 25 and 52 kg (40.8 ± 8.5 kg). The calves had been acclimatized to their environment for a minimum of 2 days, and during this period no abnormalities in appetite or feces were noted.

After having fasted for a minimum period of 6 hours, the calves were fitted with 2 IV catheters,[a] each inserted into a jugular vein. The puncture sites were clipped and scrubbed for aseptic placement.

Metabolic acidosis was induced by a 4,000 mL IV infusion of a solution containing 400 mM of HCl and 559 mM of NaCl, and thus had a theoretical osmolarity of 479 mOsm/L. The infusion was completed after 80 minutes, corresponding to an infusion rate of 50 mL/min or 5 mM HCl/min. The total dose of the acid load was

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calculated in order to obtain a base deficit comparable to naturally occurring metabolic acidosis secondary to diarrhea of medium to severe grade. Assuming a distribution space of 0.6 L/kg, the mean expected decrease in bicarbonate concentration was 17.2 ± 4.2 mM/L.

After having been warmed, the solution was infused through 1 catheter. The contralateral jugular vein was used for the collection of blood samples before the beginning of the infusion (T0), at 20-minute intervals during the infusion period (T20, T40, T60, T80), and then at various intervals after the end of infusion (recovery period): T90, T100, T110, T120, T130, T140, T150, T180, T210, T270, and T390.

The IV catheter used for collecting the blood was rinsed by the aspiration and reinjection of blood for 30 seconds before each sampling.

Clinical variables were examined before and at short intervals during and after the injection, with the period of observation lasting until the last blood sample (T390). Special attention was paid to changes in posture and behavior, and to the palpebral and sucking reflexes. Immediately after the end of the infusion, the calves were set free from the halter restraint and allowed to walk or run on a lawn. Then, they were offered 1.5 L of milk replacer using nipple pails. Behavior, gait, and appetite, as well as milk-replacer consumption, were recorded.

Heparinized blood samples were immediately examined for blood gases[b] to assess any changes in the acid-base status that might have been responsible for any clinical signs. The following variables were determined: pH, pCO_2, plasma (HCO_3^-), and base excess (BE). In the same heparinized sample, plasma concentrations of L-lactate, sodium (Na^+), potassium (K^+), chloride (Cl^-), and ionized calcium (Ca^{2+}) were also determined[b]. The anion gap was calculated as follows: ($[Na^+] + [K^+]$) − ($[Cl^-] + [HCO_3^-]$). The decrease of plasma bicarbonate concentration as a consequence of the acid load was also calculated ($\Delta[HCO_3^-] = [HCO_3]_{-T80} - [HCO_3]_{-T0}$)

The concentrations of D- and L-lactate were determined in blood samples, which were centrifuged immediately, and the plasma was stored at −20 °C until analysis (spectrophotometric method).[17]

The data are presented as mean values ± SD. A Tukey's post hoc test was conducted whenever the F test was significant ($P < .05$). Simple linear regression analysis was used to test the relationship between the weight of the calves and the BE value at T80 and the decrease of the concentration of plasma bicarbonate at T80, respectively.

The study was carried out at the Faculty of Veterinary Medicine – University of Bologna, under the supervision of the local Centralised Veterinary Service for the Welfare of Experimental Animals and with the approval of the Ethical Review Committee of Animal Experimentation of the University of Bologna.

The clinical examinations of the experimental calves were carried out scrupulously by experienced clinicians with the intention of detecting any abnormalities in the animals. Therefore, the inclusion of an appropriate control group and the blinding of the observer, which are the recommended measures to avoid bias in clinical studies, were not considered necessary.

Results

The infusion of 400 mM HCl induced systemic acidosis in all calves (Table 1, Fig 1). The BE value started at 3.9 ± 2.9 mM/L (T0) and reached its maximum deviation (−22.4 ± 4.9 mM/L, with a range from −17.0 to −33.1 mM/L) at the end of the programmed 80 minutes of infusion (T80). The wide range was attributed to the different weights of the calves (negative correlation between weight and change in BE, $P < .05$, $r = 0.53$).

As a consequence of the acid load, the plasma bicarbonate concentration dropped from 29.6 ± 2.5 mM/L (T0) to 9.9 ± 2.8 mM/L (T80). The mean ± SD of the magnitude of this decrease ($\Delta[HCO_3^-]$) was 19.6 ± 3.3 mM/L. A negative correlation between body weight and (ΔHCO_3^-) was observed ($P < .001$, $r = 0.91$). A significant decrease of pCO_2 was observed, beginning from T60.

Despite the relatively severe acidemia, clinical and behavioral abnormalities could not be detected by observation or physical examination during the entire observation period (T0–T390), including the calf with the lowest determined BE (−33.1 mM/L). After having been set free from the restraint at the end of the infusion, they all showed physiological behavior and demonstrated normal appetite, running toward the bucket of the milk replacer. The milk offered was suckled readily and vigorously. A more in-depth neurological examination (including mental status, posture and balance, voluntary movement and reflexes, as well as cranial and peripheral nerve function) revealed no abnormalities (Fig 2). In particular, sucking and palpebral reflexes were physiological.

Frequent urination was observed after the infusions.

The acid-base disturbance was accompanied by a parallel increase in blood Cl^- concentration, whereas Na and K concentrations remained unchanged from baseline (T0) values. There was an increase in the blood concentration of Ca ions.

The condition of pure hyperchloremic metabolic acidosis without an increase in the concentration of organic acids was confirmed by the determination of the anion gap, whose deviation from the physiological range was slight and of short duration (T60–T80). Plasma L- and D-lactate concentrations were not significantly different from baseline (T0) values.

Discussion

Although empirical guidelines have been widely suggested to predict systemic acidosis in calves affected by diarrhea or by ruminal acidosis, our results make it even more doubtful that the degree of acidosis can be predicted accurately on the basis of clinical signs. This is supported by the fact that experimentally induced, non-complicated acidemia was not able to induce any clinically evident abnormalities, at least under the experimental condition of IV HCl infusion in healthy calves.

The total acid load of 400 mM over a period of 80 minutes (corresponding to 5 mM/min) caused acidemia, the severity of which can be considered medium to severe if compared with naturally occurring metabolic acidosis, secondary to diarrhea or ruminal acidosis. The amount of bicarbonate necessary to buffer the acid load ($\Delta[HCO_3^-]$) was slightly higher than what was expected from the experimental design, thus indicating a smaller bicarbonate distribution space (0.52 ± 0.07 L/kg instead of 0.60 L/kg). Some respiratory compensation was indicated by the lower pCO_2 values observed 60 minutes after the beginning of the infusion.

Table 1. Blood gas and electrolyte concentrations before (T0), during (infusion period) and after (recovery period) the infusion of 4000 mL of a solution containing 400 mM of HCl (50 mL/min).

	T0	Infusion period (minutes)				Recovery period (minutes)										
Blood variable	0	20	40	60	80	90	100	110	120	130	140	150	180	210	270	390
pH																
Mean	7.36	7.24*	7.15*	7.06*	6.96*	7.01*	7.05*	7.08*	7.10*	7.11*	7.12*	7.13*	7.14*	7.16*	7.20*	7.25*
SD	0.02	0.04	0.06	0.08	0.11	0.11	0.09	0.08	0.07	0.06	0.06	0.06	0.06	0.05	0.04	0.04
P_{CO_2}																
Mean	54.5	52.9	51.1	49.5†	46.9*	49.3†	47.9*	47.3*	47.2*	47.3*	47.6*	47.6*	48.7§	47.9*	46.3*	44.7*
SD	3.8	3.7	3.7	4.2	4.3	6.0	2.9	3.1	3.1	3.2	3.3	2.9	2.8	2.6	3.6	3.1
HCO_3^-																
Mean	29.6	21.1*	16.5*	12.8*	9.9*	11.6*	12.3*	13.0*	13.5*	13.9*	14.3*	14.6*	15.4*	16.1*	17.1*	18.1*
SD	2.5	2.7	2.8	2.8	2.8	2.1	2.3	2.3	2.4	2.4	2.4	2.4	2.4	2.3	2.1	1.7
BE																
Mean	3.9	−5.7*	−11.9*	−17.6*	−22.4*	−19.8*	−18.1*	−16.8*	−15.8*	−15.3*	−14.6*	−14.1*	−13.2*	−11.8*	−10.2*	−8.0*
SD	2.9	3.0	3.4	4.1	4.9	4.5	3.9	3.7	3.6	3.4	3.3	3.3	3.3	2.9	2.3	2.0
Cl^-																
Mean	98	108*	113*	119*	123*	120*	120*	119*	118*	117*	117*	117*	116*	114*	113*	111*
SD	3.74	3.93	3.90	4.15	4.42	4.05	4.43	3.79	3.69	3.65	3.34	3.41	3.11	2.91	3.03	2.56
K^+																
Mean	4.4	4.1	4.0	4.1	4.3	4.7	4.8	4.7	4.5	4.4	4.4	4.4	4.3	4.4	4.3	4.4
SD	0.34	0.32	0.33	0.35	0.31	0.46	0.47	0.42	0.42	0.34	0.33	0.34	0.24	0.24	0.31	0.33
Na^+																
Mean	136	137	137	138	139	139	138	138	138	138	138	138	139	138	138	137
SD	2.02	1.80	2.21	2.29	2.69	2.44	2.56	2.34	2.55	2.51	2.03	2.21	2.52	1.70	2.02	1.95
Ca^{++}																
Mean	1.24	1.20	1.34	1.47*	154*	1.53*	1.61*	1.60*	1.57*	1.53*	1.57*	1.57*	1.50*	1.46*	1.38†	1.36
SD	0.07	0.09	0.09	0.08	0.16	0.11	0.15	0.12	0.11	0.14	0.12	0.10	0.12	0.12	0.13	0.09
AGap																
Mean	12.7	11.9	11.1	10.4†	10.1§	11.5	10.9	10.9	10.9	11.6	11.3	11.2	11.6	12.4	12.6	12.4
SD	2.26	1.31	1.32	1.41	1.60	1.19	2.45	1.46	1.20	1.73	1.39	1.47	1.73	1.48	1.66	1.49

P_{CO_2} = mmHg.
HCO_3^-, BE, $[Cl^-]$, $[K^+]$, $[Na^+]$, $[Ca^{++}]$ = mM/L.
AGap (meq/1) = $[Na^+] + [K^+] - [HCO_3^-] - [Cl^-]$.
†Significantly different from baseline value (T0): $P < 0.05$.
§Significantly different from baseline value (T0): $P < 0.01$.
*Significantly different from baseline value (T0): $P < 0.01$.

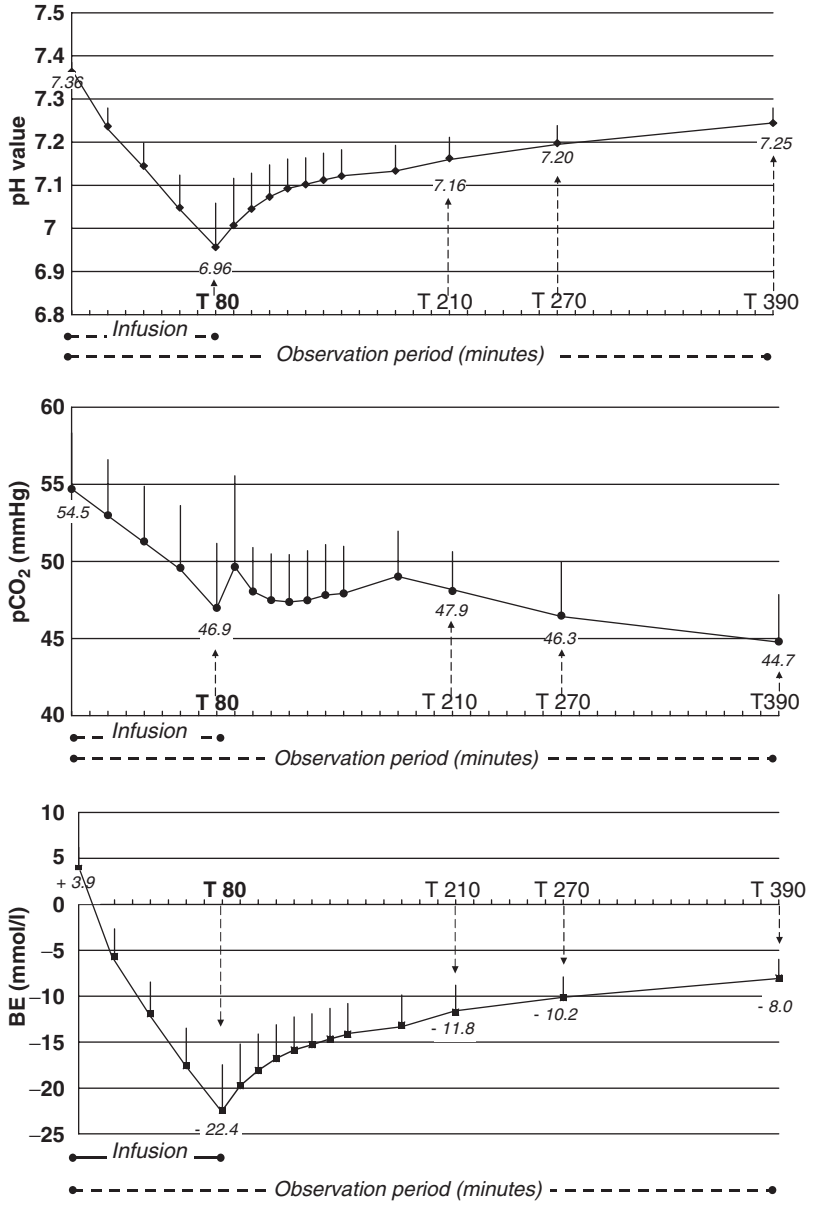

Fig 1. pH, pCO_2, and base excess values before (T0), during (80 minutes), and after the infusion of 4,000 mL of a solution containing 400 mM of HCl (50 mL/min). The overall observation period lasted for 390 minutes.

In naturally affected animals, factors other than a simple deviation of the acid-base balance should therefore be considered to be primarily responsible for general health impairment and the appearance of clinical findings; dehydration, prerenal azotemia, hyperlactatemia, hyponatremia, hyperkalemia, hypoglycemia, endotoxemia, and sepsis are mechanisms that are potentially responsible.

Of these mechanisms, the role of D-lactate in the development of clinical disease has been definitively verified.[4,14–16] The correlation between D-lactate concentrations and BE found in diarrheic calves[6] is the most probable reason for the fact that most studies regarding clinical signs in acidosis have revealed an influence of acidosis, especially on posture and behavior.[7–13]

L-Lactate, by contrast, seems to play a minor role, if any, in this respect. Thus, experimentally induced hyper–L-lactatemia did not provoke clinical disturbances in healthy calves (Lorenz and Gentile, unpublished data).

In the case of diarrhea, other factors that might influence the general state of calves are organic and functional alterations at the level of the intestinal tract itself, such as inflammation, spastic or clonic hypermotility, and wall distension. In calves with ruminal acidosis, organic alterations at the level of the forestomachs, such as necrotizing ruminitis/omasitis, and of the abomasum, eg, hemorrhagic abomasitis,[18] may play a role in inducing clinical distress.

The hydrochloric acidosis protocol used in this study produced severe hyperchloremic metabolic acidosis with

Fig 2. One of the calves has just been set free after the end of the 80 minute infusion period; despite severe acidosis (base excess: −33.1 mM/L), it shows normal mentation and activity.

no changes in other electrolytes such as K or Na. As evidenced by the unchanged anion gap, the acid-base disturbance was not influenced by an organic acid load as commonly occurs.

The absence of a hyperkalemic response in our experiment indicates the lack of a substantial "cation shift" in the face of an extracellular hydrogen load. This fact can be explained by the limited period of persistence of the acid-base imbalance. In an experiment with HCl-induced metabolic acidosis (an experiment performed on dogs), the preferential utilization of an extracellular buffer was observed in the initial phase,[19] with the contribution of intracellular buffers becoming more important in the latter part of the experiment (as the acidosis increased). On the basis of this interpretation, in our experiment, the time-limited acid load could have been completely buffered in the extracellular space, without requiring additional intracellular buffering. Thus, the exchange of unbuffered hydrogen ions with Na and especially K from the intracellular space may therefore not have been necessary.

Moreover, the renal excretion of K might have played a role in limiting the increase of the extracellular concentration of K and might, therefore, have masked the cation shift. Renal K clearance was not measured, however.

The notably higher ionized Ca concentration observed, beginning from T60, could be explained by the fact that the binding of Ca to plasma proteins (nonionized Ca), chiefly albumin, is pH dependent, the binding decreasing with decrease in pH. A negative correlation between ionized Ca concentration and pH has been confirmed in diarrheic calves, with the ionized Ca constituting approximately 35% of the total Ca in the healthy controls and 53% in the diarrheic animals.[20] Because neither total Ca nor albumin concentrations were included in the list of the variables to be evaluated, no additional detailed remarks can be made about the possible contribution of bone buffering. A net Ca efflux has been observed during in vitro metabolic acidosis as a result of a combination of inhibited osteoblastic synthesis and increased osteoblastic bone resorption.[21]

In the only study somewhat comparable to our experiment,[16] a loss of the suckle reflex was correlated to the degree of HCl-induced metabolic acidosis. On the basis of this observation, the possibility that acidemia may be directly toxic to some regions of the brain is an open question.[4] This may be particularly true in chronic acidemia where there is more time for the cerebrospinal fluid and serum pH to equilibrate; indeed a delay in the acid-base equilibration between the blood and the cerebrospinal fluid has been clearly demonstrated in calves with experimentally induced respiratory- and strong-ion acidosis.[22]

Our results suggest that severe, uncomplicated, hyperchloremic metabolic acidosis does not result in abnormalities detectable through routine physical examination. Hence, acidemia cannot always be predicted accurately on the basis of clinical signs, particularly if that acidemia is caused by hyperchloremia. The maintenance of good health in our animals, regardless of relatively severe acute systemic acidosis, confirms that factors other than a deviation of the acid-base balance should be considered to be primarily responsible for the disturbance of the general health usually observed in animals affected by spontaneous diarrhea or ruminal acidosis. Similar to D-lactate, whose role in the production of clinical distress has already been confirmed, the possible involvement of other factors should be the object of further clinical and pathogenetical studies.

Footnotes

[a] FEP radio-opaque, nonpyrogenic, G14 [2.0 × 70]; Delta Med Medical Devices, Viadana, Italy

[b] ABL 700, Radiometer, Copenhagen, Denmark

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Supplementary Material

The following supplementary material is available for this article online:

Video 1. One of the calves (Fig 2) has just been set free after the end of the infusion; despite severe acidosis (BE: −33.1 mmol/L) it seems "happy" to have been released from the constraints and it runs free in the meadow.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1939-1676.2007.0028.x (This link will take you to the article abstract).

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