Development of a diagnostic diagram for rapid field assessment of acidosis severity in diarrheic calves

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Conclusions and Clinical Relevance—Use of the diagnostic diagram may aid differentiation between severe and nonsevere acidosis patterns as determined on the basis of clinical signs. (J Am Vet Med Assoc 2012;240:312–316)

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mal calves and 20 diarrheic calves; age, < 30 days) from a local farm were used in another preliminary experiment to evaluate interobserver agreement for the assessment of clinical signs. Eighty-three client-owned Piedmontese calves (49 males and 34 females; age, 1 to 28 days) referred to the Teaching Hospital of the Faculty of Veterinary Medicine of the University of Turin by veterinary practitioners because of diarrhea were used in the primary experiment. Calves that had clinical signs of other diseases were excluded from the study. A control group of 27 healthy Piedmontese calves, age matched against the calves with diarrhea, was included in the primary experiment to obtain data for comparison. The study protocol was approved by the Bioethics and Animal Welfare Board of the Faculty of Veterinary Medicine of the University of Turin. Calves were client-owned animals, and informed consent was obtained from all owners.

Preliminary experiments—Blood samples were collected from 8 clinically normal Piedmontese calves to assess the stability of blood gas values at various times after collection. Blood samples were collected into 2.5-mL heparinized plastic syringes, and the tip of the syringe was immediately capped (time 0). Care was taken to exclude air from each sample. Blood samples were stored in an ice bath (0° to 4°C), and blood gas analysis was performed to obtain pH, Pco2, HCO3− concentration, and BeECF values for samples at 0, 15, 30, 45, and 60 minutes after collection. Syringes were gently rolled to mix the blood before each analysis.

Interobserver agreement for the assessment of clinical signs was evaluated in another preliminary experiment in which 10 clinically normal calves, 10 diarrheic calves that did not have metabolic acidosis, and 10 diarrheic calves that had metabolic acidosis were examined separately by 3 examiners (C Bellino, FA, and AD). Interobserver agreement for the assessment of clinical signs was calculated.

Primary experiment—History and physical examination findings were recorded for all calves on a standard data collection form. Special attention was given to the clinical signs (mental status, posture, gait, suckling reflex, menace reaction, palpebral reflex, character of oral mucosal membranes, and rectal temperature) described in the literature12–15,17,22,23 as the best predictors for metabolic acidosis. Clinical signs were assessed and scored separately by 3 examiners (C Bellino, FA, and AD).

Jugular venipuncture was performed in all calves in the primary experiment. Blood samples were collected into 2.5-mL heparinized plastic syringes, and the tip of the syringe was immediately capped (time 0). Care was taken to exclude air from each sample. The syringes were stored in an ice bath (0° to 4°C) and analyzed within 60 minutes after collection for measurement of blood gas (pH, Pco2, HCO3− concentration, and BeECF) values and electrolyte (Na+, Cl−, ionized Ca2+, and K+) glucose, and lactate concentrations. Blood samples were collected into 10-mL test tubes with coagulation activator and allowed to clot at 20°C; they were then centrifuged (2,500 g for 5 minutes at 20°C), and total protein concentration was determined by use of a temperature-compensated handheld refractometer. Blood samples were collected into 5-mL EDTA-coated tubes, and WBC concentration, Hb concentration, and Hct were determined by use of an automated cell counter.

Data and statistical analysis—Diarrheic calves were allocated into 2 groups: calves with metabolic acidosis (HCO3− concentration < 22 mmol/L) and calves without metabolic acidosis (HCO3− concentration ≥ 22 mmol/L). Extracellular base excess was automatically calculated with the blood analysis instrument according to the following equation: BeECF = ([HCO3− concentration − 23 mmol/L] + [16.2 mmol/L × (pH − 7.40)]).

Acidosis was considered severe when BeECF was more negative than −10 mmol/L.

Statistical analysis was performed by use of a freeware statistical software package. Interobserver agreement in the preliminary experiment was determined by use of the Cohen κ test. The median was calculated for metric data, and nominal data were expressed as a percentage. Data from healthy control calves were reported as 5th to 95th percentiles. Metric data were tested for significance by use of the Wilcoxon rank sum test. Values of P ≤ 0.05 were considered significant. An initial logistic regression was performed to determine the association between clinical signs and acidosis status. Enophthalmos was considered a dichotomous variable (present or visible = 1; absent or not visible = 0) in this model. Variables that had significant odds ratios and 95% CIs that excluded 1 were considered in the final logistic regression model. In this model, a BeECF more negative than −10 mmol/L was the dependent variable and clinical signs were the independent variables. The final model was selected via backward elimination, and there was good agreement in the final logistic regression model. In this model, a BeECF more negative than −10 mmol/L were ordered into a diagnostic diagram. Diagnostic sensitivity and specificity of the diagnostic diagram were calculated for use in identification of calves with severe metabolic acidosis.

Results

Differences in blood gas and acid-base variables for samples maintained in an ice bath until analysis 0, 15, 30, 45, and 60 minutes after blood collection were not clinically important (Table 1), and there was good

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Change in pH</th>
<th>Change in Pco2 (mm Hg)</th>
<th>Change in HCO3− (mmol/L)</th>
<th>Change in BeECF (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>−0.001 ± 0.003</td>
<td>0.4 ± 0.7</td>
<td>−0.1 ± 0.1</td>
<td>−0.2 ± 0.1</td>
</tr>
<tr>
<td>0–30</td>
<td>−0.001 ± 0.002</td>
<td>0.4 ± 1.0</td>
<td>−0.1 ± 0.1</td>
<td>−0.1 ± 0.2</td>
</tr>
<tr>
<td>0–45</td>
<td>−0.003 ± 0.003</td>
<td>0.6 ± 0.6</td>
<td>−0.2 ± 0.3</td>
<td>−0.3 ± 0.3</td>
</tr>
<tr>
<td>0–60</td>
<td>−0.005 ± 0.003</td>
<td>0.7 ± 0.9</td>
<td>−0.4 ± 0.2</td>
<td>−0.5 ± 0.3</td>
</tr>
</tbody>
</table>

Table 1—Mean ± SD changes in blood gas values for blood samples collected from each of 8 clinically normal Piedmontese calves and maintained in an ice bath (0° to 4°C) until 15, 30, 45, and 60 minutes after sample collection.
interobserver agreement for assessment of clinical signs ($\kappa = 0.90$; 95% CI, 0.77 to 1.00) in the preliminary experiments.

Blood samples obtained from diarrheic calves were analyzed within 30 (77/83 samples) or 60 (6/83 samples) minutes after collection. Of the 83 diarrheic calves, 47 (57%) had metabolic acidosis ($HCO_3^-$ concentration $< 22$ mmol/L). Acidosis was severe ($HCO_3^-$ concentration $< 22$ mmol/L; $Be_{\text{ECF}}$ more negative than $-10$ mmol/L) in 16 of 83 (19%) calves. Laboratory findings for diarrheic calves were more negative than $-10$ mmol/L. Total protein, Cl$^-$, and Hb concentrations and Hct were significantly higher in diarrheic calves with metabolic acidosis than in diarrheic calves without acidosis.

The relationships between metabolic acidosis and physical examination findings were determined by use of multivariate analysis (Table 3). The clinical signs associated with metabolic acidosis were dry oral mucosa, cold ears, cold oral mucosa, abnormal mental status (signs of depression or comatose), enophthalmos, abnormal posture or gait (wobbly or recumbent), pale mucosal color, and abnormal suckle reflex (delayed or

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinical sign</th>
<th>Diarrheic calves without metabolic acidosis (n = 36)</th>
<th>Diarrheic calves with metabolic acidosis (n = 47)</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dampness of oral mucosa</td>
<td>Dry</td>
<td>2</td>
<td>12</td>
<td>5.8</td>
<td>1.1–56.4</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>34</td>
<td>35</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Warmth of ears</td>
<td>Cold</td>
<td>4</td>
<td>18</td>
<td>1.4–22.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cool</td>
<td>4</td>
<td>18</td>
<td>1.4–22.1</td>
<td></td>
</tr>
<tr>
<td>Warmth of oral mucosa</td>
<td>Cold mucosa</td>
<td>4</td>
<td>11</td>
<td>1.4–473.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cool mucosa</td>
<td>35</td>
<td>36</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Enophthalmos</td>
<td>Present</td>
<td>3</td>
<td>35</td>
<td>3.0</td>
<td>1.1–8.3</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>33</td>
<td>12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mental status</td>
<td>Comatose</td>
<td>0</td>
<td>5</td>
<td>4.7</td>
<td>1.1–400.0</td>
</tr>
<tr>
<td></td>
<td>Signs of depression</td>
<td>12</td>
<td>25</td>
<td>3.0</td>
<td>1.1–8.3</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>24</td>
<td>17</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Posture or gait</td>
<td>Recumbent</td>
<td>2</td>
<td>9</td>
<td>5.6</td>
<td>1.1–56.0</td>
</tr>
<tr>
<td></td>
<td>Wobbly</td>
<td>6</td>
<td>16</td>
<td>3.3</td>
<td>1.1–11.9</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>28</td>
<td>22</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Color of oral mucosa</td>
<td>Pale</td>
<td>12</td>
<td>28</td>
<td>3.0</td>
<td>1.1–8.5</td>
</tr>
<tr>
<td></td>
<td>Hyperemic</td>
<td>2</td>
<td>2</td>
<td>1.3</td>
<td>0.8–15.5</td>
</tr>
<tr>
<td></td>
<td>Pale pink</td>
<td>22</td>
<td>17</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Suckle reflex</td>
<td>Absent</td>
<td>1</td>
<td>9</td>
<td>14.7</td>
<td>1.7–700.0</td>
</tr>
<tr>
<td></td>
<td>Delayed</td>
<td>11</td>
<td>24</td>
<td>3.7</td>
<td>1.3–11.1</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>24</td>
<td>14</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Represents results for comparison of values between diarrheic calves with and without metabolic acidosis; values were considered significant at $P \leq 0.05$.

Data from diarrheic calves are expressed as median values, whereas data from control calves are expressed as 5th to 95th percentiles.

Table 2—Results of laboratory analysis of blood samples from diarrheic calves with or without metabolic acidosis and reference data from healthy control calves.
absent). In the final multivariate analysis, mental status (signs of depression or comatose), suckle reflex (delayed or absent), posture or gait (wobbly or recumbent), enophthalmos, and cold oral mucosa were independent predictors of severe metabolic acidosis (B_{ecf} more negative than –10 mmol/L).

Independent predictors of severe metabolic acidosis obtained from the regression model were arranged into a diagnostic diagram (Figure 1). The sensitivity and specificity of the diagnostic diagram for the determination of B_{ecf} more negative than –10 mmol/L was 88% and 79%, respectively.

**Discussion**

In the study reported here, the choice to use Piedmontese cattle was based on their economic importance in Italy. This breed is characterized by extremely high food conversion, feed efficiency that is superior to all other regional beef breeds, and muscular hypertrophy (double-muscle factor). For these reasons, suckling calves are valuable.

Diarrhea is a leading cause of morbidity and death of neonatal calves in North America and Europe, and there has been no reported change in the mortality rate of female dairy calves in the United States between 1995 and 2001.8 Proper treatment with fluids reduces diarrhea-associated death in calves by correcting the pathological state that led to the metabolic acidosis.8 Food animal practitioners generally decide whether treatment is necessary in sick calves on the basis of clinical examination. Most food animal practices do not use laboratory blood gas analyzers because they are expensive and because sample collection methods and handling are critically important to obtaining accurate results.8

It is preferred that samples for blood gas analysis be collected in heparinized plastic syringes, maintained at ambient temperature (21° to 25°C), and analyzed within 30 minutes after collection.24 Blood sample analysis within 30 minutes after collection could not be guaranteed in the present study. For this reason, samples were maintained in an ice bath (0° to 4°C), thereby standardizing handling conditions and limiting changes related to prolonged transport or variations in weather conditions. Moreover, results of the preliminary experiment in the study reported here indicated that maintaining blood samples in an ice bath and analysis at 15, 30, 45, or 60 minutes after collection did not have a clinically important effect on blood gas and acid-base variables.

Subjective methods for determining acidosis severity in neonatal calves appear to be clinically effective.7,14,22,23 The depression score is useful for estimat-
ing the severity of metabolic acidosis in the absence of obvious clinical dehydration. However, in diarrheic calves with clinical dehydration, the same scoring system does not correlate well with acidosis severity. Low correlations between clinical signs and acid-base status have been reported when calves are dehydrated. In the present study, the group of diarrheic calves with metabolic acidosis included both dehydrated calves and calves that were not dehydrated; 14 of 16 calves with severe acidosis (Be\textsubscript{ecf} < –10 mmol/L) and 14 of 31 calves with nonsevere acidosis (Be\textsubscript{ecf} 0 to –10 mmol/L) were dehydrated as determined by clinical signs, Hct, and total protein concentration. The diagnostic diagram developed in the present study provided a method to predict the severity of the base deficit even in the dehydrated calves.

Severe acidosis (Be\textsubscript{ecf} more negative than –10 mmol/L) was related to alterations in mental status (signs of depression or comatose), posture or gait (wobbly or recumbent), suckle reflex (delayed or absent), enophthalmos, and cold oral mucosa. In contrast to observations in another study,\textsuperscript{7} we found that tactile menace response, menace response, and warmth of the extremities were not independent predictors of metabolic acidosis. In the present study, examiners interpreted the menace reaction with caution in calves < 2 weeks old because it is generally absent until the first or second week after birth.\textsuperscript{21}

The significant increase in total protein and Hb concentrations and Hct in diarrheic calves with metabolic acidosis (compared with results for diarrheic calves without metabolic acidosis) can be explained by dehydration attributable to severe diarrhea. The difference in Cl\textsuperscript{−} concentration between the 2 groups was minimal, and although the concentrations did differ significantly (P < 0.001), they were still within the range of concentrations for comparison data of control calves.

The diagnostic diagram developed in the present study may provide a useful tool in field situations for prediction of base deficit and may aid in selection of appropriate treatment for diarrheic calves with metabolic acidosis. Use of the diagnostic diagram may allow immediate differentiation between a diarrheic calf with severe acidosis (HCO\textsubscript{3} concentration < 22 mmol/L; Be\textsubscript{ecf} more negative than –10 mmol/L) and a diarrheic calf with nonsevere acidosis (HCO\textsubscript{3} concentration < 22 mmol/L; Be\textsubscript{ecf} 0 to –10 mmol/L) or without acidosis, thus aiding practitioners in selection of appropriate treatment. However, accurate clinical examination is necessary to exclude other common diseases (eg, sepsis, pneumonia, peritonitis, and arthritus) that could confound the clinical assessment.

References