Clinical examination, diagnostic testing, and treatment options for neonatal calves with diarrhea: A review

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Abstract

Primary diarrhea and septicemia are the 2 most common disease syndromes in neonatal calves. Differentiating between the 2 is difficult but vital, in order to determine prognosis and appropriate treatment. Both syndromes generally present with watery diarrhea, acid-base derangements, and/or negative energy balance. Depressed mentation and recumbency can occur with either disease if acidosis, hypoglycemia, hypokalemia, or dehydration is severe. In this review, we discuss criteria that distinguish primary diarrhea from diarrhea secondary to septicemia and present guidelines for the assessment, care, and management of diarrheic calves. Physical examination is the single-most powerful tool in differentiating the syndromes in individual calves. Therefore, particular emphasis is placed on the assessment of the calf’s demeanor, including mentation and suckling ability, its ability to stand, and the presence or absence of systemic infection. The value of laboratory diagnostics that assess acid-base and hydration status, and the adequacy of passive transfer are described. Strategies for fluid and electrolyte therapy and the use of antimicrobial, anti-inflammatory, and anticonvulsant medications are also discussed. Whether aggressive diagnostic techniques and therapeutic interventions are employed depends on the value of the individual animal and the case prognosis. This review provides the basis for optimal decision-making, thus ensuring that optimal care is provided for the calf while meeting the goals of the owner.

Key words: diarrhea, septicemia, passive transfer, fluid therapy, acid-base status

Résumé

La diarrhée primaire et la septicémie sont les deux syndromes de maladies les plus communs chez les veaux nouveau-nés. Il est difficile mais vital de différencier les deux syndromes afin d’établir le pronostic et le traitement approprié. Les deux syndromes sont associés avec de la diarrhée liquide, des déséquilibres acidobasiques et/ou un bilan énergétique négatif. Une diminution de l’éveil et le décubitus s’observent avec les deux syndromes si l’acidose, l’hypoglycémie, l’hypokaliémie ou la déshydratation sont sévères. Dans ce survol, nous discutons des critères pour distinguer la diarrhée primaire de la diarrhée secondaire associée à la septicémie et fournissons des recommandations pour l’évaluation, les soins et la gestion des veaux diarrhéiques. L’examen physique est l’outil le plus solide pour distinguer les syndromes chez un veau. Par conséquent, il est important de mettre l’accent sur l’évaluation de l’allure du veau, incluant l’éveil, la capacité de téter et de se tenir debout, et la présence ou l’absence d’infection systémique. On décrit la valeur des diagnostics de laboratoire qui servent à évaluer l’équilibre acidobasique et le niveau d’hydratation et la suffisance du transfert passif. On discute aussi les stratégies de thérapie hydro-électrolytique et l’utilisation de médicaments antimicrobiens, antiinflammatoires et anticonvulsants. L’utilisation de techniques de diagnostic et d’interventions thérapeutiques agressives dépend de la valeur de chaque animal et du pronostic du cas. Ce survol fournit les éléments pour une prise de décision éclairée qui assurera de prodiguer un soin optimal au veau tout en rencontrant les buts du propriétaire.
Introduction

Neonatal diarrhea has a huge economic impact on both dairy and beef cattle operations worldwide.\textsuperscript{11,13,55} In the United States Department of Agriculture 2007 National Animal Health Monitoring System survey, 56.5\% of deaths observed in pre-weaned dairy heifers (8.2 weeks of age on average) was attributed to diarrhea or other digestive problems, despite improved management practices such as timely colostrum administration.\textsuperscript{72} During that same period, losses in the beef cattle industry totaled 17.7\%.\textsuperscript{73}

In calves, diarrhea has been associated with risk factors related to management of the dam and/or calf, virulence or infectious load of the enteric pathogen, the environment (namely wildlife), type of housing, degree of animal contact, and weather.\textsuperscript{60} While poor management of the pre-calving dam will impact her ability to produce the appropriate quantity of quality colostrum, failure of the neonate to acquire sufficient colostral antibodies remains the single most important calf-related risk factor.\textsuperscript{60} Gestational immaturity, dystocia-related loss of vigor, age of the neonate, and ingestion of poorly reconstituted or inferior milk replacers also play a significant role.\textsuperscript{60} These factors frequently interact with each other.\textsuperscript{12}

Unlike the quick and successful recovery often observed in calves with primary diarrhea in response to appropriate therapy, treatment of septic calves is often difficult and expensive and survival rates are low.\textsuperscript{42} Based on the authors' experience, and that of others, if an appropriate diagnosis is made and intervention is timely, cure rates in calves with primary diarrhea will approach 90\%, while survival rates in calves with diarrhea secondary to sepsis range from 12 to 29.5\%.\textsuperscript{1,42,54} These marked differences in prognosis emphasize the importance of differentiating between the 2 syndromes early in the course of disease. Table 1 summarizes presenting clinical signs which may be observed in patients with either syndrome.

This review will focus on differentiating the clinical signs presented by the non-septicemic calf with primary diarrhea, and the calf with diarrhea secondary to septicemia. The goal is to present scientifically based, cost-effective, and medically optimal approaches that will optimize care and improve case outcome.

In addition to physical examination of the patient, inspection of the calf's environment and subsequent use of in-field diagnostics are useful in differentiating the 2 syndromes and determining appropriate therapeutic strategies. The diagnostic approach described in this review will guide practitioners through a decision-making process which will minimize the negative impact of diarrhea on individual patients, and ultimately its economic impact on the cattle industry as a whole.

Primary Diarrhea

Although some calves may be discovered several days after the onset of clinical signs, the clinical presentation of a non-septicemic diarrheic patient is typically that of a 1 to 5 day-old poorly conditioned calf, with profuse watery diarrhea.\textsuperscript{60} Affected calves often have accompanying signs attributable to dehydration and derangements in acid-base and electrolyte status. Necropsy findings rarely include gross mucosal intestinal lesions.\textsuperscript{60} Since multiple factors contribute to its development, this disease complex is often referred to as undifferentiated or primary diarrhea.\textsuperscript{60}

<table>
<thead>
<tr>
<th>Clinical sign or test result</th>
<th>Primary diarrhea</th>
<th>Septicemia with secondary diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scleral injection</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Omphalophebitis (navel enlargement, purulent discharge, enlarged umbilical structures on abdominal palpation)</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Polyarthritis (lameness, swollen joints)</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Hypopyon</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Fever</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Bacterial meningitis (teeth grinding, opisthotonus, convulsions, head pressing)</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Serum protein &lt; 5.5 g/dl</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Negative sodium sulfite test</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Loss of joint fluid viscosity and/or clarity</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acidosis</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Table 1. Clinical signs and expected test results which contribute to the challenge of distinguishing between calves with primary diarrhea and those with diarrhea secondary to septicemia.
Despite the fact that clinical cases of primary diarrhea are often the result of mixed or multiple infections,19,41,55,58,78 it has become customary to attribute a case of calf diarrhea to a single, specific etiologic agent.22 While this approach facilitates the development of preventative programs and safeguards against zoonotic exposure (Table 2), it is important to note that most diarrhea pathogens are endemic on many farms and can be isolated from both healthy and sick calves. In other words, proof does not necessarily lie in the discovery of an inciting cause.41,55,60 The distribution and occurrence of enteric pathogens depends on the age of the animal, the production system under which calves are being raised, and the geographical location. The ability of the diagnostic laboratory to isolate or demonstrate specific pathogens is also implicit.60

Major etiologic agents of primary diarrhea in calves less than 1 month of age include enterotoxigenic Escherichia coli (E. coli) [ETEC], Cryptosporidium parvum (C. parvum), rotavirus, coronavirus, and Salmonella serotypes.34 The mechanisms by which each of these pathogens cause diarrhea have been reviewed extensively.20 In brief, the K99 antigen associated with ETEC produces a heat-stable enterotoxin (STa) which induces intestinal hypersecretion resulting in secretory diarrhea. Clinical disease is typically seen in calves less than a week old because attachment of the organism to the intestinal mucosa is age-dependent.58,66 The malabsorptive diarrhea which develops from infection with C. parvum results from severe villous atrophy and impaired sodium chloride absorption in the face of prostaglandin-induced chloride (Cl−) and bicarbonate (HCO₃⁻) secretion.25 Based on a recent report, C. parvum–associated illness may develop in calves as early as 3 days old.46 Rotavirus and coronavirus destroy mature small-intestinal villi in calves up to 2 weeks and 1 month of age, respectively. This causes a malabsorptive/maldigestive diarrhea with an osmotic component. The osmotic force is generated by the poorly absorbed and/or undigested sugars (glucose and lactose) which are retained within the intestinal tract, subsequently pulling fluid into the lumen.19,34 Coronavirus also targets crypt cells of the small and large intestines, eliciting widespread destruction of cells of the colonic ridges, resulting in the release of mucous and blood.34 In general, loss of sodium, chloride, bicarbonate, and potassium ions in the fluid stool, coupled with decreased renal excretion of hydrogen ions, and accumulation of circulating unidentified organic acids, contribute to the commonly-observed clinical signs of weakness and/or inability to stand, neurologic depression, and tachycardia.7

**Diarrhea with Septicemia**

Septicemia is defined as an acute invasion of the circulatory system by pathogenic bacteria and their products.12,22 Left untreated, it may result in sepsis, a deleterious, non-resolving inflammatory host response to infection that leads to organ dysfunction,75 and ultimately septic shock. In 1 study, failed detection of bacteremia ultimately culminated in septicemia in 31% of the calf population.42

Risk factors which predispose animals to primary diarrhea (inadequate transfer of passive immunity, exposure to pathogens, and age at exposure) have also been incriminated in cases of septicemia. Failure of passive transfer (FPT) of colostral immunity—the failure of a calf to attain a serum IgG concentration of ≥ 1000 mg/dL—may occur when an inadequate immunoglobulin mass or volume of colostrum is fed to the calf, or when calves ingest colostrum which is heavily contaminated with bacteria.20,57 Inadequate mass can occur when either colostrum or its replacement product contains <50 g/L of IgG (total immunoglobulin mass <150 g IgG) or contaminating bacteria which are hypothesized to bind and therefore inhibit immunoglobulin absorption by enterocytes.21,58,59 Inadequate volume may be the result of colostrum shortage or the calf’s unwillingness to nurse an adequate amount.58 Proper handling of colostrum is therefore essential, with key feasible critical control points being appropriate hygiene during colostrum collection and administration, and the establishment of suitable protocols for colostrum storage.57 Low concentrations of circulating fetal cortisol subsequent to dystocia-related fetal stress, cesarean section, premature birth, and cold stress have also been linked to inefficient IgG absorption.8,10 In addition to protecting neonatal calves from environmental stressors such as overcrowding, excessive fecal contamination, and inclement weather, separating them from biological incubators and amplifiers, namely animals which are older by 2 or more weeks of age, as well as subclinically ill or sick herd mates, is key to limiting the exposure of individual calves to potentially pathogenic viral, bacterial, and protozoal enteric species.2,41 Like infection exposure, age at exposure significantly influences the outcome of

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**Table 2. Age of onset and zoonotic potential of agents that commonly cause diarrhea in neonatal calves.**

<table>
<thead>
<tr>
<th>Etiologic agent</th>
<th>Age of onset</th>
<th>Zoonosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>1 - 4 days</td>
<td>No</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>4 - 30 days</td>
<td>No</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>4 - 14 days</td>
<td>No</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>3’ - 28 days</td>
<td>Yes</td>
</tr>
<tr>
<td>Salmonella serotype</td>
<td>All ages</td>
<td>Yes</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>&lt;10 days</td>
<td>No</td>
</tr>
</tbody>
</table>

an infection. Despite being immunocompetent, calves possess a relatively naive and limited humoral immune system at birth, while other components of the immune system fail to become fully functional until close to 4 weeks of age. For instance, decreased opsonic activity of serum derived from FPT postpartum calves (i.e. prior to colostrum ingestion) has been identified as the reason for the limited ability of neonatal phagocytes to recognize and ingest bacteria. Risk of development of septicemia in primary diarrhea patients is also compounded by impaired gastrointestinal perfusion and motility. Recumbency and poor suckle reflex in clinically-ill calves, as well as the presence of focal sites of infection (umbilical abscesses, septic arthritis, pneumonia, and overwhelming enteritis) are associated with increased risk of septicemia and will negatively impact prognosis. In 1 study, only 12% of septicemic calves recovered, with the survivors rarely attaining full productivity.

In calves, septicemia-related diarrhea is most commonly caused by gram-negative bacteria. While E. coli remains the primary infective agent, Pasteurella and Salmonella spp. have frequently been isolated. Most infections occur during the postpartum period, with either the respiratory or enteric systems, respectively, serving as the point of entry, or alternatively, the umbilicus. Within the United States, clinical salmonellosis commonly results from infection with 1 or a combination of Salmonella serotypes (serovars) (Typhimurium, Dublin, Enteritidis, Kentucky, Montevideo, Newport, Anatum, or Muenster), and can occur within hours of the calf’s birth. Besides direct environmental exposure, clinical illness may result from either in utero infection or the ingestion of contaminated water, feed, or either of the mammary secretions. During an investigation of calf diarrhea in less than 1-week-old calves, Salmonellae-contaminated colostrum was incriminated for increased morbidity and mortality rates, despite enhanced colostrum-feeding. Calves died within 8 to 10 days following development of clinical signs 3 to 5 days post-infection. Peracute death or septicemia with or without pyrexia and diarrhea is characteristic.

Despite a preponderance of gram-negative pathogens in cases of calf septicemia, gram-positive pathogens are increasing in significance. In a retrospective study, Clostridium perfringens, Clostridium septicum, and Listeria monocytogenes were incriminated in 10% of septicemic calves.

Baseline Physical Examination

The importance of a thorough history and complete systematic physical examination cannot be over-emphasized. Detailed recordkeeping is therefore encouraged. Not only does this practice afford the practitioner a focused and objective view of the animal’s initial status, but it also facilitates continued monitoring by the producer throughout the productive life of the animal. A Calf Health Scoring Chart has been developed for on-farm use. Knowledge of the age of the animal and the onset of disease (Table 2), as well as disease incidence on the farm, is useful in ruling out potential causes of diarrhea.

Similar to animals with primary diarrhea, septicemic animals exhibit dehydration, depressed mentation, diarrhea, poor suckle reflex, and weakness. Recumbency and evidence of disordered coagulation may also be observed. In most cases, infections of the gastrointestinal tract, respiratory system or umbilicus which occur during the postpartum period lead to the development of septicemia. Multiple scoring systems have been developed for use in sick calves. Table 3 illustrates a system developed by Fecteau et al which is adaptable to field use.

Practitioners are cautioned against making assumptions based on generalities. For instance, while hypothermia is frequently observed in the calf with primary diarrhea, concurrent with dehydration, neither a subnormal temperature nor pyrexia is a consistent finding in septicemic patients. Sustained tachycardia and tachypnea in septic calves typically develop later in the course of disease progression.

Management of the Diarrheic Calf

Physical Examination Procedures

Evaluation of the calf’s hydration status, the animal’s eyes, joints, umbilical structures, and demeanor, will help the practitioner to correctly categorize the calf and identify required treatment interventions.

Evaluation of Hydration Status

Enophthalmos, skin elasticity (the skin turgor test) either over the lateral neck, thorax, upper or lower eyelid, and plasma protein concentration, have all been proposed as methods of assessing hydration status in calves. Guidelines generated through an experimental model demonstrate degree of enophthalmos and skin tent duration in the neck region to be superior, and both have proven usefulness in field investigations. The degree of enophthalmos is defined as an estimate of the distance between the globe and palpebral conjunctiva. This measurement can be evaluated by gently everting the lower eyelid. Skin elasticity is best evaluated by pinching a fold of skin over the lateral mid-cervical region, rotating it 90 degrees and determining the length of time (in seconds) it takes for the fold to disappear (Table 4). Since enophthalmos may be confounded by cachexia, skin tent duration is recommended in cases of chronic diarrhea.

Assessing the animal’s weight may be useful since weight loss in the face of acute fluid loss is an accurate predictor of hydration. Dehydration less than 5% of body weight cannot be reliably detected on physical examination.
Closer examination of the cornea, sclera, and anterior chamber of the eyes is particularly helpful in differentiating diarrhea associated with septicemia from primary diarrhea. Increased congestion or hyperemia in scleral vessels (scleral injection), or vascular rupture within the sclera (scleral ecchymosis) and petechial hemorrhaging, are rarely seen in calves with primary diarrhea, and are indicative of septicemia. Hypopyon, the presence of purulent material and debris within the anterior chamber of the eye, is more likely to develop in calves with septic meningitis, and is considered a grave prognostic indicator.

**Joint Evaluation**

Diagnosis of an infected joint is supportive of septicemia and warrants a guarded prognosis. Evidence of abnormal gait dictates the need for a detailed orthopedic examination. Importantly, signs of swelling and pain may only become apparent on palpation; therefore, joints should be closely evaluated in all sick neonatal calves, including those without obvious lameness. Besides flexural deformities and traumatic injuries, septic polyarthritis is the most frequent cause of lameness in calves 1 week of age and older. Characteristic signs include joint distension and pain. In a retrospective study of infectious arthritis in cattle of varying ages, incidence of septic arthritis was higher in large high-motion joints, such as the stifle, than in the smaller, low-motion joints, such as the tarsus. Arthrocentesis is indicated in calves with visible joint distension and/or lameness, and should also be performed in suspect as well as easily accessible joints. Techniques for sample collection and processing have been reviewed by Bohn et al. Gross assessment of the color and viscosity of joint fluid offers an advantage. Clear, highly viscous fluids are presumptively classified as normal (Figure 3), while turbidity and low viscosity are

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**Table 3.** Score sheet for determining the likelihood of sepsis and prediction of bacteremia in diarrheic calves.

<table>
<thead>
<tr>
<th>CRITERIA EVALUATED</th>
<th>RESULT OF OBSERVATION</th>
<th>POINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal site of infection</td>
<td>NO</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>YES</td>
<td>1.5</td>
</tr>
<tr>
<td>Age in days</td>
<td>&lt; 7 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>≥ 7 days</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Total C.S. = __________

**Table 4.** Guidelines for assessment of hydration status in calves with diarrhea.

<table>
<thead>
<tr>
<th>Dehydration</th>
<th>Demeanor</th>
<th>Eyeball recession</th>
<th>Skin tent duration(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5%</td>
<td>Normal</td>
<td>None</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6% - 8% (mild)</td>
<td>Slightly depressed</td>
<td>2 - 4 mm</td>
<td>1 - 2</td>
</tr>
<tr>
<td>8% - 10% (moderate)</td>
<td>Depressed</td>
<td>4 - 6 mm</td>
<td>2 - 5</td>
</tr>
<tr>
<td>10% - 12% (severe)</td>
<td>Comatose</td>
<td>6 - 8 mm</td>
<td>5 - 10</td>
</tr>
<tr>
<td>&gt; 12%</td>
<td>Comatose/dead</td>
<td>8 - 12 mm</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

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reveal the inciting pathogen in up to 25% of cases which would likely have yielded a negative culture result. Correct classification of the etiological diagnosis will result in cost savings and substantially impact case prognosis over the long-term. The practitioner should therefore weigh the benefits of arthrocentesis, fluid analysis, and culture on a case-by-case basis. In some cases, infected joints will be observed concurrent with umbilical infections, the umbilical site having served as the source of bacteria through hematogenous spread.

Figure 1. Alteration of scleral vasculature in septicemic patients. Compared with Image A (normal sclera of a calf), both images B and C demonstrate increased prominence of scleral vessels. Image B denotes scleral injection while the ruptured vessels in Image C demonstrate blood seepage.

Figure 2. The stilted stance and hunched posture observed in this < 2 week-old calf indicates pain associated with the lameness observed in cases of septic polyarthritis.

Figure 3. Normal joint fluid demonstrating high viscosity.

deemed hallmarks of septic joint fluid. Test criteria established by Rohde et al for total nucleated cell count, neutrophil number, and total protein concentration in septic joint fluid were > 25,000 cells/µL, 20,000 cells/µL, and 4.5 g/dL, respectively. Although a positive culture confirms the presence of infection, typically only 60% of septic-joint-fluid cultures yield a positive result. Gram staining, also easily performed by the practitioner, may reveal the inciting pathogen in up to 25% of cases which would likely have yielded a negative culture result. Correct classification of the etiological diagnosis will result in cost savings and substantially impact case prognosis over the long-term. The practitioner should therefore weigh the benefits of arthrocentesis, fluid analysis, and culture on a case-by-case basis. In some cases, infected joints will be observed concurrent with umbilical infections, the umbilical site having served as the source of bacteria through hematogenous spread.
Umbilical Evaluation

External examination of the umbilicus is not sufficient to fully assess its normalcy, since a moist, purulent and/or obviously enlarged umbilical stalk is not always present in animals with significant umbilical infection. As a result, deep palpation of internal umbilical structures is warranted in all calves. Digital palpation of umbilical structures is best performed with the calf in lateral recumbency. However, dependent on the degree of abdominal distension, examination in the standing calf may prove more rewarding. Once the area of the umbilicus has been identified, the 3 middle fingers of each hand should be used to isolate and evaluate the umbilical vein as it courses cranially towards the liver, and finally, the umbilical arteries and urachus as they course caudally towards the bladder, for any evidence of enlargement or pain. This is best achieved by rolling the fingers ventrally off the abdominal musculature to eliciting an obvious “blip” (Figure 4). Palpation should be performed in a manner such that one is able to determine whether discharge or evidence of infection can be elicited externally. Infection of the urachus, the most commonly infected umbilical remnant, may result in accompanying cystitis, pyuria, and abnormal urination, while omphalophlebitis may extend to and involve the liver, resulting in unthriftness and evidence of localized infections elsewhere.

Evaluation of Acid-Base Status

It is important to note that while the mechanism of each differs, both the calf with primary diarrhea and the septicemic patient may present with metabolic acidosis (Table 1). Having been historically restricted to referral institutions, determination of acid-base status in medical management of livestock patients became feasible in general practice settings with the advent of demeanor scores. Affordable portable serum chemistry analyzers such as the i-Stat, and the IRMA TruPoint blood analysis system, are now in routine use. Acid-base status describes the ability of the body’s bicarbonate/carbon dioxide buffering system to neutralize hydrogen ions (H⁺) which circulate freely within the extracellular fluid compartment. Normally, the concentration of H⁺ is maintained at extremely low levels in body fluids.

Assessment of calf demeanor or attitude is a reliable indicator of acid-base status in calves with primary diarrhea, and has proven invaluable in guiding treatment decisions (Table 5). Depressed mentation and weakness are commonly observed in diarrheic calves, with animals less than a week old quickly developing weakness as a result of rapid dehydration. The profound state of depressed mentation and weakness observed in calves 8 days and older has been attributed to the rapid development of severe metabolic acidosis. The movement of potassium from intracellular to extracellular compartments and whole-body depletion of this ion during diarrheic episodes also potentiates weakness in these patients.

It must be emphasized that while primary diarrhea and septicemic calves may display similar changes in behavior and appearance, demeanor scores cannot be used to assess acid-base status in septicemic patients. Not only is acid-base status highly variable in calves with septicemia, but this variability is attributable to the presence of systemic illness, ongoing infection, and inflammation. Consequently, it is best to use a portable chemical analyzer to assess blood pH and bicarbonate ion (HCO₃⁻) concentration in the potentially septicemic calf. A negative base-excess value is indicative of an increased base deficit, and is therefore supportive of metabolic acidosis. Practitioners may consider the use of a portable pH meter as an alternative. Portable pH meters are easy to use and are relatively inexpensive. Since this device does not directly measure serum bicarbonate concentration, the value for base excess/deficit must be estimated using the following formula, where pHₘ represents the patient’s measured serum pH:

$$BE = -301.158 + (39.617 \times pH_m)$$

It must be noted, however, that the pH meter proved to be more accurate at measuring ruminal and urine pH than that of blood. Since circulating bicarbonate ions are the major contributors to total carbon dioxide concentration (TCO₂), the patient’s acid-base status can also be evaluated by
recommended in overtly normal calves (5.2 g/dL). The discrepancy noted between the 2 values (5.5 and 5.2 g/dL) represents the effect of dehydration which falsely elevates serum protein concentration. The sodium sulfite test is less susceptible to changes in circulating fluid volume. Using this test procedure, 0.1 ml of serum is added to 1.9 ml of test solution (18%). Presence or absence of turbidity is assessed after incubation for a period of 15 minutes at room temperature. Absence of turbidity is suggestive of failure of passive transfer (Figure 5, Image B).

A portable clinical MBC QTII™ analyzer is available for evaluation of bovine serum/plasma analytes, including IgG concentration. Preliminary data indicated improved identification of true positives for APT over refractometry assessing either the serum or plasma TCO₂ concentration in millimoles/Liter (mmol/L) using a portable TCO₂ apparatus the accuracy of which has been validated. In the case of an acidic patient, the measured carbon dioxide concentration may be used in Equation I to calculate the patient’s estimated bicarbonate requirement in millimoles (mmol).

Equation I⁵⁶: bicarbonate (HCO₃⁻) requirement = 
\[(30 - \text{TCO}_2) \times \text{BW}_{kg} \times 0.6^b\]

Alternatively, using the demeanor scoring system (in calves with primary diarrhea), the bicarbonate requirement or total base deficit in millimoles (mmol) may be determined by inserting the estimated base deficit into Equation II.

Equation II⁶⁰: total body base deficit (mmol) = 
\[\text{BW}_{kg} \times \text{base deficit/excess}' \times 0.6^b\]

### Ancillary Diagnostic Procedures

**Assessment of Passive Transfer**

Passive transfer status should be assessed on all sick calves. Adequate passive transfer (APT), is reflected by a serum IgG concentration of at least 1000 mg/dL (1g/dL), and may be determined as early as 24 to 48 hours post-colostrum intake. It is critical to note that a minimum of 24 hours is required to allow for immunoglobulin passage from the gastrointestinal system into the bloodstream. Likewise, evaluating blood from calves older than 14 days, the putative mean half-life of serum IgG, may result in the erroneous classification of calves as hypogammaglobulinemic.

Test procedures most readily adapted to practice settings include refractometry, and the sodium sulfite turbidity test. In the case of refractometry, clinically ill calves with serum protein concentrations less than 5.5g/dL should be considered to have inadequate transfer of colostral immunoglobulins. It is important to note that this endpoint (5.5 g/dL) is substantially higher than that recommended in overtly normal calves (5.2 g/dL). The discrepancy noted between the 2 values (5.5 and 5.2 g/dL) represents the effect of dehydration which falsely elevates serum protein concentration. The sodium sulfite test is less susceptible to changes in circulating fluid volume. Using this test procedure, 0.1 ml of serum is added to 1.9 ml of test solution (18%). Presence or absence of turbidity is assessed after incubation for a period of 15 minutes at room temperature. Absence of turbidity is suggestive of failure of passive transfer (Figure 5, Image B).

A portable clinical MBC QTII™ analyzer is available for evaluation of bovine serum/plasma analytes, including IgG concentration. Preliminary data indicated improved identification of true positives for APT over refractometry.

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**Table 5. Estimating acid-base status of non-septic diarrheic calves of varying ages using demeanor scores.**

<table>
<thead>
<tr>
<th>Demeanor score</th>
<th>Description</th>
<th>Base deficit of calves ≤ 8 days</th>
<th>Base deficit of calves &gt; 8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Alert, active, normal</td>
<td>0 mmol/L</td>
<td>7 mmol/L</td>
</tr>
<tr>
<td>II</td>
<td>Depressed, slow lethargic</td>
<td>5 mmol/L</td>
<td>11 mmol/L</td>
</tr>
<tr>
<td>III</td>
<td>Sternal recumbency, sucking reflex absent</td>
<td>12 mmol/L</td>
<td>16 mmol/L</td>
</tr>
<tr>
<td>IV</td>
<td>Lateral recumbency, sucking reflex absent</td>
<td>13 mmol/L</td>
<td>20 mmol/L</td>
</tr>
</tbody>
</table>


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**Figure 5.** Results of the sodium sulfite test using sera from 2 calves. The increased turbidity observed in tube A demonstrates adequacy of passive transfer in the sampled calf. Alternatively, the ability of the reader to discern the script through the solution in tube B supports a diagnosis of failure of passive transfer in the calf from which the blood was sampled.
Hematology and Pathogen Detection

Detailed antemortem laboratory testing is expensive and is generally not indicated in calves with primary diarrhea except in the case of dysentery (diarrhea containing mucus and blood) and/or tenesmus. Common pathogens associated with bloody diarrhea in calves up to 2 weeks of age include *Clostridia* spp and *Salmonella* serovars, 2 bacterial pathogens commonly incriminated in cases of septicemia. Indicated diagnostic tests include polymerase chain reaction (PCR) analysis for clostridial enterotoxins, and culture for salmonella organisms. Although not pathognomonic, an inflammatory leukogram (hyperfibrinogenemia, neutrophilia or neutropenia with a degenerative left shift and toxic neutrophils) is supportive of a diagnosis of septicemia. *E. coli*, an environmental opportunist, is often the most frequent isolate in clinically-ill, depressed, septicemic calves, with diarrhea, ranging from 51% to 65% in separate studies. While false-negative results are not uncommon, confirmation of bacteremia, a likely precursor of septicemia, through bacteriological blood culturing is indicated in the treatment of valuable calves. Since results are usually reported within 48 to 72 hours, empiric therapy must be instituted during the waiting period, with the institution of specific therapy subsequent to the availability of test findings. Even if case prognosis is deemed to be grave, test results may provide beneficial farm-specific prevalence data and/or prove useful in directing therapy in similar cases in the future. Additionally, with the continued emergence of antimicrobial resistance, increased diligence in testing may prove useful in clarifying resistance patterns both within and across species.

Needs Assessment for Supportive Care

Supportive care may be required in calves with either primary diarrhea or diarrhea secondary to septicemia. In addition to correctly identifying the acid-base status of the calf, the ability to assess the need for intravenous versus oral fluid therapy, and/or make the decision for referral, is critical to the delivery of optimal care. In conjunction with recommended physical examination procedures, practitioners should utilize the ability of the calf to suckle, severity of central nervous system depression, degree of weakness (the calf’s ability to stand), and presence or absence of ileus to facilitate the decision-making process. Evidence of sustained tachycardia and tachypnea in septic calves is valuable in determining prognosis.

**Treatment**

**Fluid and Electrolyte Therapy**

Patients with diarrhea may lose up to 16% of their original body weight through the fluid feces. Therefore, fluid replacement therapy should be an integral component of the therapeutic protocol in cases of both primary diarrhea and diarrhea secondary to septicemia. While the re-establishment of extracellular fluid and circulating volume are major goals of fluid therapy, decreasing the resulting D-lactatemia, restoring metabolic acid-base homeostasis (venous blood pH 7.35-7.45), and correcting electrolyte abnormalities are also critical. Since negative energy balance is a common feature, strategies aimed at addressing the energy deficit and/or facilitating repair of the damaged intestinal mucosal surface should be implemented. Intravenous fluid therapy is indicated in moderately to more severely dehydrated calves (fluid deficits ≥ 8%), as well as in animals with depressed mentation and absent suckle reflex. Conversely, early recognition of clinical signs (fluid deficit less than 8%) generally ensures the efficacy of oral electrolyte solutions. Oral hydration therapy in a calf with ileus will, however, result in bloat and potentially rumen acidosis.

**Correcting Metabolic Acidosis**

Sodium bicarbonate (NaHCO₃) solution is the alkalinizing treatment of choice for severely acidic calves, and can be safely administered intravenously in its isotonic form (1.3%; 13g NaHCO₃/L). As portrayed in Example 1, evaluation of the patient’s base deficit allows the practitioner to determine the volume of isotonic bicarbonate needed to replenish the patient’s extracellular fluid compartment. It is important to note that 1 gram of sodium bicarbonate salt (pure baking soda) contains 12 mmol of bicarbonate ions (HCO₃⁻).

If large volumes of sterile water are unavailable, fluids should be prepared to order, using either distilled or deionized water. While administration of preparations of both isotonic sodium bicarbonate and sodium chloride may be required, at least initially, in severe cases, it is critical that the practitioner seeks to obtain history regarding treatments which may have been previously administered by the calf attendant. Given the wide variety of oral electrolyte solutions that are commercially available, it is not impossible to be presented with a calf that is mildly dehydrated, yet severely acidic.

Additional recommendations regarding fluid protocol design are extensively detailed by Radostits et al and others.
criteria while facilitating intestinal absorption include acetate and propionate. Glucose, citrate or glycine may be utilized to facilitate sodium and water reabsorption while bicarbonate will purely serve to neutralize acids.

In summary, the electrolyte content, osmolality, glucose:sodium ratio, and alkalinizing ability should be considered during the selection of oral fluids.

It is noteworthy that suspension of milk feedings in diarrheic patients is considered sub-optimal care. Milk favors both weight gain and mucosal healing. Calves on an electrolyte-only solution diet could develop profound negative energy balance. Therefore, if an animal refuses to voluntarily ingest milk, this should not be allowed to persist for more than 12 hours. Calf attendants should therefore be advised that veterinary intervention must be sought.

Use of bicarbonate as an alkalinizing agent in oral fluids bears an inherent risk. Unlike other alkalinizing agents, when administered orally, bicarbonate-containing fluids are likely to permit bacterial proliferation within the abomasum (a risk factor for developing septicemia), as well as inhibit the formation of the ‘milk clot’ (mediated by the abomasal enzyme chymosin or “rennin”). ‘Milk clot’ formation (coagulation of milk fat and proteins) occurs within 10 minutes of ingesting a whole-milk diet, and facilitates the gradual release of nutrients into the small intestines within 24 hours.

Example 1. Approach to correcting metabolic acidosis in primary diarrhea calves using isotonic (1.3%) sodium bicarbonate salt (pure baking soda) solution.

Step 1: Note the animal's signalment and clinically assess the patient based on the parameters in Table 5:

A 121 lb (55 kg), 8-day-old Holstein calf. It is laterally-recumbent and does not have a suckle reflex. The patient's assessed demeanor score is IV.

Diagnostic interpretation: metabolic acidosis with a base deficit of -20 mmol/L

Step 2: Determine the patient's total body base deficit (insert the relevant data into Equation II – “Evaluation of acid-base status”):

\[
\text{Total body base deficit (mmol) = BW_{kg} \times \text{measured (or estimated in this case) base deficit/excess} \times 0.6}
\]

Total body base deficit = 55 x 20 mmol/L x 0.6 = 660 mmol

Step 3: Correction of base-deficit-grams of pure baking soda needed to correct the calculated total base deficit:

660 mmol/12 mmol/G = 55 grams

Step 4: Calculate the total yield of isotonic (13g/L or 1.3%) bicarbonate solution obtainable, using 55 grams of the salt:

Total volume of isotonic bicarbonate solution needed to correct the calf’s total base deficit = 55g ÷ 13g/L = 4.2L

Since correction of acidosis facilitates the intracellular redistribution of potassium (K+), supplementation of this cation may be beneficial in restoring total body stores. Since patients sometimes require glucose supplementation, it is important to note that K+ redistribution is also induced by glucose-stimulated insulin release. To avoid cardiotoxic effects, the practitioner is advised to adhere to the recommended maximal intravenous rate of 0.5 milliequivalents of potassium chloride/kg/hour.

Following resolution of life-threatening acid-base and electrolyte abnormalities using intravenous therapy, it is possible to successfully transition the calf with primary diarrhea onto oral therapy. While this strategy will minimize treatment costs, to ensure the successful therapy of the patient with profuse diarrhea, practitioners must impress upon calf attendants that oral rehydration therapy must be aggressive, appropriate, and strategic. Since oral fluids do not require sterilization, are inexpensive, and can be administered by the calf attendant, rehydration therapy via this route is the mainstay of treatment protocols.

Given that most diarrheic calves tend to be in a state of negative energy balance, oral fluid supplementation must serve as a source of energy and simultaneously supply sufficient sodium to normalize extracellular fluid volume. Alkalizing agents which satisfy these criteria while facilitating intestinal absorption include acetate and propionate. Glucose, citrate or glycine may be utilized to facilitate sodium and water reabsorption while bicarbonate will purely serve to neutralize acids. In summary, the electrolyte content, osmolality, glucose:sodium ratio, and alkalinizing ability should be considered during the selection of oral fluids.

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Clot formation reduces the likelihood of spikes in the concentration of circulating amino acids and urea. If the use of bicarbonate-containing fluids is either indicated or cannot be avoided in whole-milk-fed calves, particularly during the very early postnatal period, a 2- to 4-hour delay is recommended prior to, or after, administering milk feedings. Acetate and propionate are very effective at neutralizing acidity, and are easily metabolized in fed and fasted calves. These products are recommended for use in the treatment of the mildly acidic calf. In the presence of rennin, the commonly used soy-based and whey milk replacers either fail to form a milk clot or only form a ‘soft’ clot. As a result, avoiding the administration of bicarbonate to calves fed these types of milk replacers may not be relevant.

**Antimicrobial use in Diarrheic Calves**

Correction of dehydration, inhibition of intestinal microbial overgrowth, and restoration of the absorptive capacity of the gastrointestinal tract remain the mainstay of therapy. However, antimicrobial use must be considered in individual cases. Evidence suggests that bacteremia is likely to develop in 8% to 18% of systemically ill calves, even when adequate passive transfer of immunity has occurred. In calves with primary diarrhea, a predominance of *E. coli* and other coliforms has been detected in the distal small intestines, even after elimination of the inciting enteric pathogen. While the administration of antimicrobials is warranted in FPT calves which are at risk of developing bacteremia, administration of antimicrobials early in the course of illness is particularly indicated in diarrhea resulting from infection with salmonella and clostridial organisms. While animals with salmonellosis are more likely to have a favorable outcome, patients with clostridial infections often succumb despite aggressive therapy.

To minimize the potential development of antimicrobial resistance, antimicrobial use in large animal patients should be guided by principles which include the welfare of the animal, ease and frequency of administration, tissue residue and withholding periods. To confirm the latter, calf attendants must be closely guided in the use of established treatment protocols and encouraged to maintain adequate treatment records. Since efficacy of amoxicillin (oral formulation), 1 of the few antibiotics labeled by the US Food and Drug Administration for treatment of diarrhea in food animals, was demonstrated solely under experimental conditions, the use of alternatives must be guided by the 1994 Animal Medicinal Drug Use Act (AMDUCA). Basic therapeutic intervention for patients diagnosed with meningitis, pneumonia, septic arthritis, omphalitis/omphalophlebitis, and overwhelming enteritis are outlined in Table 6.

**Conclusions**

Determining whether diarrhea in calves is primary or secondary to septicemia is important. Physical examination is the single most important diagnostic and prognostic tool. Historical data and results of selected diagnostic tests will also be helpful in differentiating the 2 syndromes, determining prognosis, and guiding therapeutic intervention. Compared with their septicemic counterparts, calves with primary diarrhea will respond quickly to therapy and at minimal cost. Septicemic patients, which most consistently demonstrate scleral injection and localized infections, usually require hospitalization along with pharmacological and physiological supportive care over the long term, and these animals often die. The initiation of aggressive therapy should be dependent on the value of the calf, the financial resources of the client, and patient prognosis.

Whether diarrhea is primary or occurs secondary to septicemia, fluid therapy and correction of electrolyte and acid-base abnormalities are crucial. Route of administration is determined by the degree of dehydration and severity of illness. Also of significance is the use of antimicrobial agents, which must be instituted under strict guidelines in select cases.

Preventive strategies which contribute to the decreased incidence of diarrhea in neonatal calves include the incorporation of biosecurity measures such as the care of animals in a sanitary and thermo-neutral environment, administration of a quality colostrum product with the appropriate mass of immunoglobulins, and establishment of a tightly-confined calving season in cow-calf operations. The informed practitioner is critical in the fight against diarrhea-related calf deaths.

**Endnotes**

1 The Calf Health Scoring Chart was developed by large animal internist Dr. Sheila McGuirk of the University of Wisconsin, CVM. Available at: http://www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm.
2 Abbott Point of Care Inc., 400 College Road East, Princeton, NJ 08540.
3 International Technidyne Corporation (ITC), Piscataway, NJ 08854.
4 The Cardy Twin pH meter, Spectrum Technologies Inc., 12360 S. Industrial Drive E., Plainfield, IL 60585.
5 The correlation (r²) between pH and calculated base excess was 0.911; p<0.005.
<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Clinical signs</th>
<th>Oxygen therapy (humidified)</th>
<th>Anti-inflammatory medication</th>
<th>Anticonvulsant medication</th>
<th>Fluid therapy</th>
<th>Surgical/medical intervention</th>
<th>Antibiotic therapy</th>
</tr>
</thead>
</table>
| Meningitis        | Fever, depressed mentation, convulsions, opisthotonus, hyperesthesia, hypopyon, hypoxia +/-hypercapnia | 5 to 10 L/hr; in the absence of ventilatory support for hypercapnic patients, respiratory stimulants (caffeine [NoDoz, 200 mg tablet - loading dose: 4.54 mg/lb (10 mg/kg) followed by 1.13 to 1.36 mg/lb/24 hr (2.5 to 3 mg/kg/24 hr)]) or in the case of emergency, doxapram hydrochloride 0.23 mg/lb (0.5 mg/kg) IV, or 2.27 to 4.54 mg/lb (5 to 10 mg/kg) at the base of the tongue. | Flunixin meglumine - 0.11 to 0.15 mg/lb (0.25 to 0.33 mg/kg) IV TID<sup>22</sup> | Diazepam - 0.005 to 0.009 mg/lb (0.01 to 0.02 mg/kg), IV q 30 min. to effect<sup>22,46</sup> | Plasma - 1 to 2 L from disease-free adult<sup>22</sup> balanced electrolyte crystalloids (2.5 to 5% dextrose + 0.9% NaCl) IV - 18.1 to 36.3 mL/lb/day (40 to 80 mL/kg/day) to replace insensible losses and fluid deficit; correction of base deficit if >-10 mmol/L<sup>22</sup> | N/A | Ampicillin sodium - 4.54 to 9.07 mg/lb (10 to 20 mg/kg), IV TID; *ceftiofur - 0.5 to 1.0 mg/lb (1.1 to 2.2 mg/kg) IM; 'trimethoprim-sulfonamide (TMS) - 2.27 mg/lb (5 mg/kg), IV<sup>22,47</sup>

<p>| Pneumonia         | Respiratory distress, hypoxia fever, depressed mentation, wheezes, crackles, pleural friction rubs | 2 to 10 L/hr&lt;sup&gt;22&lt;/sup&gt; | Flunixin meglumine 0.11 to 0.15 mg/lb (0.25 to 0.33 mg/kg) IV TID&lt;sup&gt;22&lt;/sup&gt; | N/A | +/- intravenous plasma and balanced electrolyte crystalloids to replace insensible losses and fluid deficit administered in 4.54 to 9.07 mL/lb (10 to 20 mL/kg) boluses&lt;sup&gt;22&lt;/sup&gt; | N/A | *Naxel® (ceftiofur sodium) - 0.5 to 1.0 mg/lb (1.1 to 2.2 mg/kg) IM or SQ SID per label&lt;sup&gt;22&lt;/sup&gt;; **Excenel® RTU EZ (ceftiofur hydrochloride) - 0.5 to 1 mg/lb (1.1 to 2.2 mg/kg) IM or SQ SID at 24 hr intervals&lt;sup&gt;22&lt;/sup&gt; per label, OR 1 mg/lb (2.2 mg/kg) on day 1 and again on day 3, per label; ***Enrofloxacin (Baytril® 100) - 1.1 to 2.3 mg/lb (2.5 to 5 mg/kg) SQ SID, or 3.4 to 5.7 mg/lb (7.5 to 12.5 mg/kg) SQ, ONCE, per label; *Trimethoprim sulfanilamide (TMS) - loading dose of 18.1 mg/lb (40 mg/kg) PO, then 9.07 mg/lb (20 mg/kg) PO BID for &lt;2-week-old calves. Similar dose TID for calves, 2 to 3 wk of age&lt;sup&gt;22&lt;/sup&gt; Nuflor® (florfenicol) - 9.07 mg/lb (20 mg/kg) IM in the neck Q 48 hr for a total of 3 doses, or a single dose of 18.1 mg/lb (40 mg/kg), SQ in the neck&lt;sup&gt;22&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical Signs</th>
<th>Treatment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic arthritis</td>
<td>Pyrexia, depressed mentation, joint distension, warmth, pain on palpation and reluctance to stand and feed</td>
<td>Flunixin meglumine - 0.1 mg/lb (2.2 mg/kg) IV or IM SID for 2 to 3 days²⁵</td>
<td>Joint lavage using an 1&quot; (2 cm), 16 gauge needle with a “through and through” (preferred) or an “in and out” technique using 500 mL to 1 liter of 98.6°F (37°C) lactated Ringer’s solution. Repeat in 48 hrs if needed. An arthroscopy or arthrotomy should be reserved for severe lesions in valuable animals²⁵</td>
</tr>
<tr>
<td>Omphalitis</td>
<td>Enlarged umbilicus, +/-pyuria, incontinence</td>
<td>Flunixin meglumine - 0.1 mg/lb (2.2 mg/kg) IV or IM SID for 2 to 3 days²⁵</td>
<td>Supportive fluid therapy, Umbilical resection or marsupialization procedure in severe cases</td>
</tr>
<tr>
<td>Enteritis</td>
<td>Fever, dull mentation, blood-tinged diarrhea, anorexia</td>
<td>Flunixin meglumine - 0.1 mg/lb (2.2 mg/kg) IV bid²⁵</td>
<td>Plasma - 1 to 2 L in animals with total protein &lt; 5.5 g/dl, from a disease-free adult;²² balanced electrolyte crystalloids - (2.5 to 5% Dextrose + 0.9% NaCl) IV - 18.1 to 36.3 mL/lb/day (40 to 80 mL/kg/day) to replace insensible losses and fluid deficit; correction of base deficit if &gt;10 mmol/L²²</td>
</tr>
</tbody>
</table>

²²As of April 12, 2013, US Federal law prohibits the extralabel use of all ceftiofur medications at unapproved doses, frequency, duration or route of administration in cattle and other major food-producing animals (21CFR Part 530 Federal Register Vol 77, No.4).  
²³Duration of therapy is often empiric. Combination therapy (ampicillin + ceftiofur) or (ampicillin + TMS) may improve spectrum of activity.²²  
²⁴The extralabel use of fluoroquinolones is prohibited in cattle. Use is limited to the treatment of respiratory disease.  
²⁵Key considerations for the use of NSAIDs include evidence of a left shift and toxic neutrophils. Limiting use to 2 to 3 days of consecutive therapy will eliminate the risk of gastrointestinal ulceration.  
²⁶Trade name, Tribissen²²  
²⁷Cited references are denoted by numbered superscripts. The reader is referred to the reference list in the manuscript for the complete reference.
Serum TCO₂ concentration (mmol/L) in healthy calves.

The ‘bicarbonate space’ in the extracellular fluid of neonates. Occasionally this value is substituted with 0.5. This corresponds to 0.3 or 0.4, the accepted values for adults. Naylor JM. Therapeutic approach to the diarrheic calf. Proceedings. 22nd Annu Conf Am Assoc Bov Pract 1989; 143-145.

The estimated (see Table 5) or measured (on a blood gas analyzer) base deficit/excess value (mmol/L).


On rare occasions, this figure is replaced by 1.26%; 12.6 g NaHCO₃/L.

Acknowledgement

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References


