Naturally Occurring Acute Coliform Mastitis in Holstein Cattle

Christopher K. Cebra, Franklyn B. Garry, and R. Page Dinsmore

Physical examination and clinicopathologic findings from 44 adult Holstein cows with naturally occurring coliform mastitis were studied. The cattle were grouped for comparison by stage of lactation and survival. Cattle within the first 4 weeks of lactation maintained higher median mature neutrophil counts (1,200 versus 300/μL) in peripheral blood than cattle later in lactation. Nonsurviving cows had higher median creatinine concentration (2.5 versus 1.6 mg/dL) and anion gap (25 versus 20 mEq/L), and lower serum protein (7.1 versus 7.6 gm/dL) and total CO_2 (19.8 versus 25 mEq/L) concentrations than surviving cows ($P < .05$). These findings indicate that cattle with uremia and metabolic acidosis are less likely to survive the infection. Bacteriologic blood cultures were performed on 34 of the 44 cows studied. *Escherichia coli* was isolated from the blood in 11 (32%) cows. Clinical presentation and clinicopathologic data were compared in bacteremic versus nonbacteremic cows to evaluate these data as predictors of bacteremia. Bacteremic cows were sick longer prior to admission (2 versus 1 days), maintained higher median counts of total nucleated cells (6.6 versus 2.4 $\times$ 10^3 cells/μL), myelocytes (0.2 versus 0 $\times$ 10^3 cells/μL), metamyelocytes (0.5 versus 0.02 $\times$ 10^3 cells/μL), band neutrophils (0.7 versus 0.1 $\times$ 10^3 cells/μL), and lymphocytes (2.1 versus 1.4 $\times$ 10^3 cells/μL) than nonbacteremic cows, and had higher plasma fibrinogen concentration (600 versus 500 mg/dL) ($P < .05$). There were no differences between the physical or serum biochemical measurements. Four of 11 bacteremic cows and 5 of 23 nonbacteremic cows died or were euthanized ($P > .05$). The high prevalence of bacteremia seen in cows with coliform mastitis has not been reported previously, and may have been due to the duration of disease, severity of signs, or culture technique. These findings suggest that systemic antibiotic therapy may be beneficial in some severe cases of coliform mastitis.

J Vet Intern Med 1996;252–257.

Peracute and severe acute coliform mastitis (ACM) are combinations of local and systemic inflammatory disease, and result from invasion of the mammary gland by *Escherichia coli* or a similar coliform organism.[1-3] Cattle in early lactation are reported to be more susceptible to the disease.[3-6] This may be attributable to decreased neutrophil function,[7,8] delayed migration of neutrophils into the gland,[6,9] and faster bacterial replication during early lactation.[5] Failure to rapidly clear the organism may allow the bacteria to multiply and release more endotoxin prior to the onset of the inflammatory reaction.

The local and systemic signs resemble the reaction to endotoxin, and may be induced by intramammary administration of endotoxin.[10,11] Furthermore, bacteremia has not been demonstrated in cattle with ACM.[12] Because of this, current therapeutic recommendations are centered around removal and inhibition of the toxin and not elimination of the bacteria with antimicrobial agents.[11,13] Lack of attachment to epithelial cells and maintenance of basement membrane integrity have been seen ultrastructurally after experimental infection, and were presumed to prevent systemic invasion by the organism.[14] In contrast, some destruction of the basement membrane of mammary tissue has been seen in early lactation cows with naturally occurring ACM. Bacteria were isolated from regional lymph nodes of these cattle.[9]

Clinicopathologic studies of cows with experimental ACM have revealed transient abnormalities including leukopenia[10,11,15,16] consisting of lymphopenia and neutropenia with left shift,[10,11] hypocalcemia,[11,16] and metabolic acidosis.[11] These reports usually deal with a cow's response to a single dose of endotoxin,[10,11,16] and the relevance of these abnormalities to the course of the disease in animals with naturally occurring disease has not been tested. Although leukopenia,[3,17-19] high aspartate aminotransferase activity,[3,18,19] uremia,[17-20] hypoalbuminemia,[17,18] and hypocalcemia[17-20] have been reported for small numbers of both surviving and nonsurviving cows with natural infections, the importance of these findings relative to disease severity has not been determined.

In the present study, physical examination and clinicopathologic findings from cows with naturally occurring severe ACM that were admitted to the Colorado State University Veterinary Teaching Hospital (CSU-VTH) were compared at different stages of lactation and between survivors and nonsurvivors. This report also establishes the existence of bacteremic ACM, and demonstrates that this clinical syndrome is very similar to nonbacteremic ACM.

Materials and Methods

Case records were reviewed for 44 adult Holstein cows with ACM admitted to the CSU-VTH between January 1, 1990 and February 28, 1995. Only cattle with a record of historical information, physical examination findings, milk culture positive for *E coli*, and a CBC performed at admission were selected. Cows with chronic disease and those not demonstrating systemic signs were excluded. Serum chemistry values, blood gas analyses, and noncontaminated blood bacteriologic culture results were used when available. These 44 cows represent 61% of the Holstein cattle that were admitted for mastitis with systemic signs. Reasons for exclusion of the other 28 animals included no milk culture (n = 15), isolation of exclusively Gram-positive organisms (n = 4), or isolation of Gram-negative organisms without *E coli* (n = 9). Other Gram-negative isolates included *Klebsiella sp.* (n = 3), *Pseudomonas sp.* (n = 2), *Serratia sp.* (n = 2), *Salmonella sp.* (n = 1), and *Pasteurella sp.* (n = 1). The decision whether to obtain a milk sample for culture was more

From the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Accepted December 29, 1995.

An abstract of this study was presented at the 12th Annual ACVIM Forum.

Supported in part by Colorado State University Agricultural Experiment Station Funds.

Reprint requests: Christopher K. Cebra, VMD, Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, CO 80523.

0891-6640/96/1004-0009$3.00/0

dependent on the attending clinician than the status of the animals. The 9 cows with gram-negative isolates other than *E coli* were excluded because they increased the heterogeneity of the study population.

Blood samples for clinical pathology testing were collected aseptically from either the jugular or tail veins. Automated cell counts (Coulter S+ IV; Coulter Corp, Hialeah, FL), serum chemistry profiles (Hitachi 911 Automatic Analyzer; Boehringer Mannheim, Indianapolis, IN), and blood gas analysis (ABL 300 Acid-Base Analyzer; Radiometer, West Lake, OH) were performed. Milk culture samples were collected into sterile tubes after the teat end was cleaned with isopropyl alcohol, and streaked onto Columbia and MacConkey agar plates. The plates were incubated at 35° to 37°C in a 10% CO_2 atmosphere. Colonies were identified by standard laboratory methods. Sensitivity to antimicrobial agents was determined by minimum inhibitory concentration using commercial plates (Sensititre CBV6LG; Radiometer, West Lake, OH).

For blood culture, the hair was clipped from a site over the jugular vein. The site was then disinfected by alternating applications of povidone iodine and isopropyl alcohol. Twenty milliliters of blood were removed aseptically from the vein and injected aseptically through a new needle into a 50-mL blood culture vial of Brain Heart infusion containing 0.6% sodium polyanetholsulfonate (BBL; Becton Dickinson Co, Cockeysville, MD). The sample was aerated through the needle after blood inoculation, incubated, and subcultured as described above on days 0, 1 and 7.

For comparison, the cattle were stratified in 3 separate groups. For the first comparison, cattle were grouped by outcome: the survivor group (S) consisted of cattle that were discharged and the nonsurvivor group (NS) of cattle that died or were euthanized during their hospital stay. Euthanasia was considered to be an identical outcome to natural death because cattle were only euthanized for humane reasons when their condition failed to improve with therapy. Such cattle were characteristically unable to rise, tachycardic, completely anorectic, and often in respiratory distress.

For the second comparison, the cattle were divided into 3 groups based on their stage of lactation. The 3 stages were early (EL; 1 to 28 days), middle (ML; 29 to 119 days), and late (LL; 120 or more days) lactation.

For the third comparison, a noncontaminated blood culture sample was necessary for inclusion. Thirty-four of the 44 cows fulfilled this criterion. These cattle were classified as bacteremic or nonbacteremic based on the presence or absence of *E coli* in the blood culture.

Parameters used for comparison consisted of historical, physical examination, and clinicopathologic data. Specifically, age in years, days in milk, days sick prior to admission, prior treatment, rectal temperature, heart rate, and respiratory rate were taken from reports of the initial examination. Laboratory samples were taken at admission to the CSU-VTH. PCV and total and differential leukocyte counts were taken from the CBC. Serum urea nitrogen, glucose, creatinine, calcium, phosphorus, total protein, albumin, globulin, albumin-globulin ratio, total bilirubin, creatine kinase (CK) activity, aspartate aminotransferase (AST) activity, sodium, potassium, chloride, total CO_2, and anion gap were taken from the serum chemistry profile. Blood pH and bicarbonate were measured by blood gas analysis.

For statistical analysis of 2-group comparisons, the Wilcoxon rank-sum test[21] was used if the data were continuous and Fischer's exact test[22] was used if the data were ordinal. The data from the 3 groups of cows stratified by days in milk were compared by the Kruskal-Wallis test.[23] The Kruskal-Wallis tests and internal comparisons between groups were performed using SigmaStat statistical software (Jandel Scientific Software, San Rafael, CA). Statistical significance was determined when $P < .05$.

Results

In addition to *E coli,* bacteria isolated from milk samples included *Staphylococcus sp.* (n = 5), *Streptococcus sp.* (n = 1), and *Klebsiella sp.* (n = 1). Two or 3 colony types of *E coli* were isolated from 7 cows. Although sensitivity to tetracycline was inconsistent (54%), bacterial colonies resistant to gentamicin and ceftiofur were isolated only from 1 cow each. Prior to admission, 26 of 44 cows had been treated by the owners or herd veterinarians with an intramammary infusion of either gentamicin (n = 17) or cephapirin sodium (n = 8), or both (n = 1). Two cows treated with intramammary gentamicin were also treated with intramammary polymixin B. Nineteen of the 26 cattle that had received intramammary therapy also had been treated with 1 or more systemic antibiotic agent (oxytetracycline [n = 8], ceftiofur sodium [n = 3], procaine penicillin G [n = 5], gentamicin [n = 2], or sulfa-based antibiotics and macrolides [n = 4]), and 4 additional cows had been treated with systemic antibiotics alone (ceftiofur sodium [n = 2], gentamicin [n = 1], or oxytetracycline [n = 1]). Fourteen cows had no history of antibiotic treatment; 22 cows had been treated with flunixin meglumine (Banamine; Schering-Plough Animal Health, Kenilworth, NJ).

The historical, CBC, serum chemistry, and blood gas analysis values for the study population are listed in Tables 1 and 2. Cattle had been sick for a median of 2 days (range, 1 to 7 days). Lactating cattle of all ages and stages of lactation were affected. Characteristic findings on physical examination were heart rates above 80 beats per minute (n = 39) and respiratory rates above 28 breaths per minute (n = 31). Nine had rectal temperatures above 39.5°C and 9 had temperatures below 37.5°C. The leukogram characteristically showed leukopenia, consisting of lymphopenia (n = 33), neutropenia (n = 26), and a left shift of the neutrophil line (n = 36). The serum chemistry analysis showed increases in blood urea nitrogen (21 of 23), creatinine (19 of 38),

Table 1. Historical, Physical, and Hematologic Findings in 44 Cows With Naturally Occurring Acute Coliform Mastitis

Variable	Median (min–max)	Reference Values*
Age (y)	5 (2–12)	
Days in milk	65 (1–300)	
Days sick	2 (1–7)	
Rectal Temperature (°C)	38.7 (35.3–40.6)	
Heart rate (beat/min)	110 (66–150)	
Respiratory rate (breath/min)	40 (20–88)	
Nucleated Cells ($\times 10^3/\mu L$)	3.6 (0.7–27.5)	4–12
Myelocytes ($\times 10^3/\mu L$)	0 (0–2.8)	0
Metamyelocytes ($\times 10^3/\mu L$)	0.1 (0–6.9)	0
Band neutrophils ($\times 10^3/\mu L$)	0.2 (0–13.8)	0–0.1
Mature neutrophils ($\times 10^3/\mu L$)	0.4 (0–5.0)	0.6–4.0
Lymphocytes ($\times 10^3/\mu L$)	1.7 (0.5–8.7)	2.5–7.5
PCV (%)	36 (27–48)	24–46
Fibrinogen (mg/dL)	600 (200–1,100)	200–400

* Reference values used by the Colorado State University Clinical Pathology Laboratory.

Table 2. Serum Chemistry and Blood Gas Analysis Results in 44 Cows With Naturally Occurring Acute Coliform Mastitis

Variable	Median (min–max)	Reference Values*
Serum urea nitrogen (mg/dL)	36 (13–89)	7–20
Glucose (mg/dL)	84 (38–164)	55–95
Creatinine (mg/dL)	1.7 (0.9–4.0)	0.9–1.7
Calcium (mg/dL)	8.2 (5.1–28.8)	7.6–10.2
Phosphorus (mg/dL)	8.3 (4.1–12.9)	4–8.6
Total protein (g/dL)	6.9 (5.7–8.3)	6.4–9.5
Albumin (g/dL)	3.4 (2.7–4.6)	2.5–4.3
Globulin (g/dL)	3.3 (2.1–5.5)	2.6–6.5
Albumin/globulin	1 (0.5–2.0)	0.5–1.3
Bilirubin (mg/dL)	0.7 (0.2–6.6)	0.1–0.4
Creatine phosphokinase (IU/L)	506 (86–55,580)	57–280
Aspartate aminotransferase (IU/L)	108 (43–873)	40–130
Sodium (mEq/L)	138 (130–152)	136–147
Potassium (mEq/L)	4.0 (2.4–5.7)	4.0–5.0
Chloride (mEq/L)	97 (85–115)	95–105
Total CO_2 (mEq/L)	24.1 (12.7–31.5)	21–27
Anion gap (mEq/L)	21 (12–35)	14–26
Blood pH	7.46 (7.24–7.53)	7.31–7.53
Bicarbonate (mEq/L))	25.8 (9.3–32.4)	25–35

* Reference values used by the Colorado State University Clinical Pathology Laboratory.

phosphorus (16 of 37), CK (28 of 37), AST activity (15 of 37), anion gap (8 of 37), and bilirubin (31 of 37) and decreases in potassium (18 of 39), calcium (10 of 37), and chloride (14 of 38). Measurement of systemic acid-base balance revealed polarized results: total CO_2 >27 mEq/L (9 of 37), pH >7.45 (14 of 27), and bicarbonate >24 mEq/L (17 of 27) were common, but total CO_2 <21 mEq/L (9 of 37), pH < 7.35 (5 of 27), and bicarbonate <18 mEq/L (5 of 27) were also encountered.

All cattle were treated for ACM at the clinic. Standard treatment consisted of IV polyionic replacement fluids (40 to 140 L), flunixin meglumine (0.25 to 0.5 mg/kg, IV, every 6 to 8 hours), and stripping out affected quarters every 2 hours. Additionally, 41 of 44 cows were treated with antibiotics systemically (ceftiofur sodium [n = 36], procaine penicillin G [n = 18], others [n = 4]) or intramammary (gentamicin [n = 15], cephapirin sodium [n = 14], cloxacillin [n = 1]). Three cows were not treated with antibiotics at the owners' request. Twelve of the 44 cows died or were euthanized.

Comparison by Outcome

Comparison of S to NS cows (Table 3) showed nonsurvivors had significantly higher serum creatinine, sodium, potassium, AST activity, and anion gap, and significantly lower serum protein, total CO_2, and blood pH ($P < .05$). However, the protein and anion gaps for both groups were most commonly within normal reference ranges. Sodium, potassium, and chloride concentrations were more likely to be abnormally low among survivors than high among nonsurvivors, and total CO_2, blood pH, and bicarbonate were more likely to be abnormally high in survivors than in nonsurvivors. Sodium was characteristic of the electrolytes, in that NS cows tended toward higher values over a similar range as S cows (Fig 1), while total CO_2 was typical of measurements of metabolic acid-base status, in that S cows rarely were acidemic, whereas NS cows commonly were (Fig 1). Seven of 8 cows with a serum total CO_2 <20 mEq/L died or were euthanized, and 3 of the 4 nonacidemic NS cows were both alkalemic and bacteremic. Prior antibiotic therapy was similar in survivors (24 of 32) and nonsurvivors (8 of 12).

Comparison by Stage of Lactation

There were 12 cows in early lactation (EL), 20 in middle lactation (ML), ans 12 in late lactation (LL). There were significant differences among cattle at different stages of lactation for PCV and peripheral blood counts of mature neutrophils and lymphocytes ($P < .01$), and the difference in peripheral counts of nucleated cells ($P = .12$) and band neutrophils ($P = .08$) approached significance (Table 4). The EL cows maintained higher median numbers of band cells and neutrophils than the other 2 groups. The LL cows maintained a higher PCV than either other group. Five of 12 EL cattle, 3 of 20 in the ML group, and 4 of 12 in the LL group died or were euthanized.

Comparison by Blood Culture Results

Blood culture results were available from single samples on 33 cows, and from paired samples on 1 cow, and were positive for *E coli* from 11 cows (32%). An additional bacterial isolate was found for 2 bacteremic cows (one each of *Staphylococcus* and *Pasteurella*). The pattern of antibiotic sensitivity of the blood isolate was very similar or identical to that for a mammary isolate. Previous antibiotic therapy was more common in bacteremic than nonbacteremic cows (72% versus 61%), but a smaller percentage of bacteremic cows (36% versus 48%) had received systemic antibiotic

Table 3. Comparison of Selected Hematologic and Serum Chemistry Values for Surviving and Nonsurviving Cows With Naturally Occurring Acute Coliform Mastitis

	Survivors (n = 32)		Non-Survivors (n = 12)
Variable	Median (min–max)	*P* Value	Median (min–max)
PCV (%)	35 (27–46)	.13	39 (32–48)
Creatinine (mg/dL)	1.6 (1.0–4.0)	.002	2.5 (1.7–3.9)
Total protein (g/dL)	7.6 (5.7–8.3)	.01	7.1 (5.7–7.0)
Globulin (g/dL)	3.5 (2.4–5.5)	.15	3.3 (2.8–3.6)
Aspartate aminotransferase (IU/L)	105 (48–439)	.005	187 (84–873)
Sodium (mEq/L)	137 (131–148)	.02	145 (134–152)
Potassium (mEq/L)	3.9 (2.4–5.2)	.03	4.7 (2.6–5.7)
Chloride (mEq/L)	96 (86–110)	.08	101 (85–115)
Total CO_2 (mEq/L)	25 (19.1–31.5)	.03	19.8 (12.7–30.4)
Anion gap (mEq/L)	20 (12–28)	.005	25 (20–35)
Blood pH	7.47 (7.26–7.53)	.003	7.35 (7.24–7.41)
Bicarbonate (mEq/L)	25.9 (9.3–30.9)	.05	19.7 (9.7–29.6)

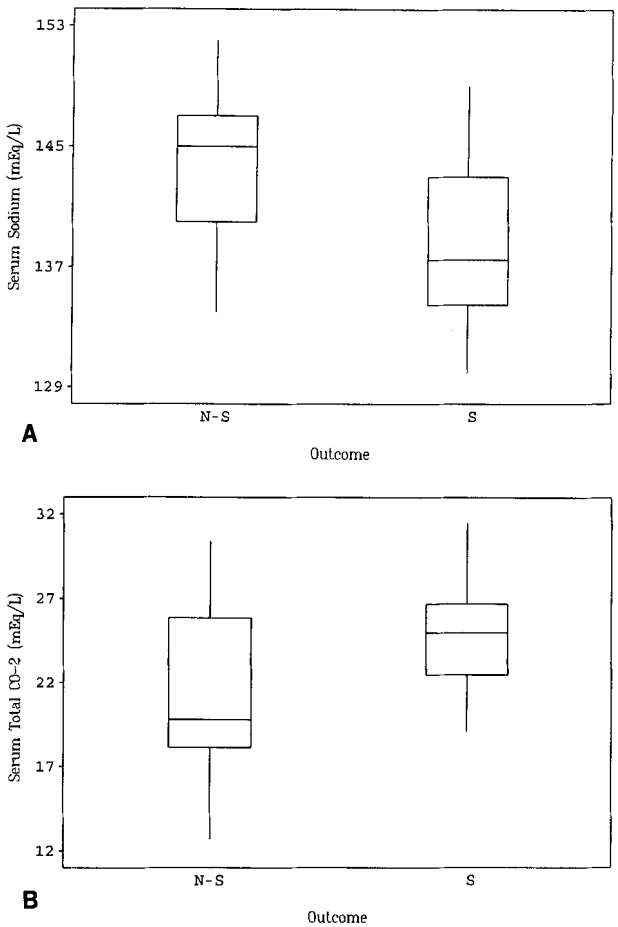

Fig 1. Box and whisker plots representing the serum concentrations of (A) sodium and (B) total carbon dioxide in nonsurviving (N-S) and surviving (S) cows with acute coliform mastitis. The central box contains the interquartile range, with the vertical bars extending to the maximum and minimum values. The median is represented by the horizontal bar, which divides the interquartile range.

therapy. The prior use of antibiotics was not significantly different between bacteremic and nonbacteremic cows ($P > .05$).

Bacteremic cows maintained significantly higher fibrinogen concentrations and counts of all forms of immature neutrophils and lymphocytes than did nonbacteremic cows ($P < .05$) (Table 5). Although a left shift was common in both groups of cows, large numbers of immature neutrophils rarely were found in nonbacteremic animals. Other physical parameters and clinicopathologic data from bacteremic cows were similar to those from nonbacteremic cows. Survival did not appear to relate to stage of lactation and was similar in bacteremic cows (7 of 11) and nonbacteremic cows (18 of 23). The duration of illness prior to admission was longer for the bacteremic versus nonbacteremic cows ($P = .01$).

Discussion

The clinicopathologic abnormalities seen in this study population resembled those reported for cows with both experimental[10,11,15,16] and natural coliform mastitis.[3,17-20] However, although the noted changes were transient (less than 24 hours) with experimental[10,11,15,16] or mild natural infection,[20] they were found several days after the onset of signs in our studied cattle. This may represent a persistence or recurrence of these changes.

Lymphopenia and neutropenia with left shift were fairly ubiquitous changes, and thus of no value in predicting outcome. There was an association between the magnitude of the left shift and bacteremia, such that suspicion of bacteremia should rise if high counts of myelocytes, metamyelocytes, or band neutrophils are detected. It may be that this left shift is part of the normal regenerative response, because bacteremic cows had a longer history of illness prior to admission. Neutropenia was not a consistent finding in early lactation cows. Impaired neutrophil mobilization and delayed diapedesis into the gland also have been described in early postparturient cattle,[6,7,9] with the degree of impairment related to the severity of the disease.[24] The higher neutrophil counts seen in early lactation cows in this study may be additional evidence of decreased responsiveness to coliform mammary infection in early lactation cows, although increased release from bone marrow may not be excluded. In spite of this consideration, nonsurvivors occurred with similar frequency in early and late lactation cows.

In contrast to blood cellular changes, biochemical abnormalities were associated with outcome. Hemoconcentration (high PCV), uremia, high AST activity, and organic acidosis, all evidence of systemic dysfunction, were typical of nonsurviving cows, whereas decreased concentrations of sodium, potassium, and chloride, and metabolic alkalosis were often encountered in surviving cows. The trend towards higher PCV and lower serum protein concentration in nonsurviving cows may relect hemoconcentration in the face of protein loss into the mastitic secretion[10,25] or gastrointestinal tract.

The pattern of acid-base abnormalities was similar to that reported for cows affected with abomasal volvulus.[26,27] More severely affected cows developed organic acidosis with increasing anion gap, due to deteriorating cardiovascular function, whereas survivors had a tendency towards lower serum

Table 4. Comparison of Hematologic Values for 44 Cows With Naturally Occurring Acute Coliform Mastitis at Different Stages of Lactation

	Early (n = 12)	Middle (n = 20)	Late (n = 12)
Variable	Median (min-max)	Median (min-max)	Median (min-max)
Days in Milk	7 (1-27)	65 (30-114)	216 (120-300)
Band cells ($\times 10^3/\mu L$)	0.4 (0-13.8)	0.2 (0-3.0)	0.2 (0-0.7)
Mature neutrophils ($\times 10^3/\mu L$)	1.2[a] (0.1-5.0)	0.5[a] (0-2.5)	0.2[b] (0-0.7)
Lymphocytes ($\times 10^3/\mu L$)	2.2[a] (1.1-4.4)	1.5[b] (0.5-4.3)	3.6[a] (1.3-8.7)
PCV (%)	35[a] (28-40)	34[a] (27-48)	40[b] (34-458)

NOTE. Values marked with different superscripts are significantly different ($P < .05$).

Table 5. Comparison of Selected Hematologic Values for Bacteremic and Nonbacteremic Cows With Naturally Occurring Acute Coliform Mastitis

	Nonbacteremic		Bacteremic
Variable	Median (min–max)	*P* Value	Median (min–max)
Days sick	1 (1–7)	.01	2 (1–4)
Rectal temperature (C°)	38.7 (36.1–40.5)	.18	38.4 (35.3–40.1)
Nucleated cells ($\times 10^3/\mu L$)	2.4 (0.7–9.3)	.01	6.6 (1.6–27.5)
Myelocytes ($\times 10^3/\mu L$)	0 (0–0.1)	.01	0.2 (0–2.8)
Metamyelocytes ($\times 10^3/\mu L$)	0 (0–0.3)	.002	0.5 (0–6.9)
Band neutrophils ($\times 10^3/\mu L$)	0.1 (0–1.1)	.003	0.7 (0.1–13.8)
Mature neutrophils ($\times 10^3/\mu L$)	0.4 (0–3.4)	.46	0.5 (0.1–5.0)
Lymphocytes ($\times 10^3/\mu L$)	1.4 (0.5–8.6)	.03	2.1 (0.7–8.5)
Fibrinogen (mg/dL)	500 (200–1,100)	.008	600 (400–1,100)

chloride concentration and alkalosis, likely due to endotoxin induced gastrointestinal stasis and third-space sequestration of abomasal secretion. In cows with ACM, this trend of association between acidosis and nonsurvival may have a similar basis as in cows with abomasal volvulus. Data from the cows in this study support the conclusion that, in spite of the diagnostic and therapeutic modalities available in the referral hospital setting, cows with ACM and metabolic acidosis and uremia are unlikely to survive.

The high prevalence of bacteremia (32%) in these cows was contrary to previous studies,[12] and was perhaps the most important finding in this study relative to treatment considerations. The study population consisted of cows referred for treatment, often after on-farm therapy had failed. These cows had severe, protracted disease, and are not representative of the overall population of cows with ACM. In contrast, a previous study that did not detect bacteremia was performed on field cases with early signs of the disease.[12] Thus, it appears reasonable to presume that cows with ACM and minimal systemic signs are unlikely to be bacteremic.

Culture technique may have been important in the detection of bacteremia. Culture samples consisted of a minimum of 20 mL of blood, with subcultures plated out on 3 separate occasions, compared with 5-mL blood samples and 1 to 2 subcultures used in another study.[12] It has been shown that success at culturing bacteria from the blood relates to the volume of the sample.[28] Repeated sampling and greater dilution of the sample in culture media, which helps to neutralize antibiotic effects, might have led to even greater yields.

Because these cattle had naturally occurring disease, it is impossible to determine whether uncontrolled factors may have led to the concurrent mastitis and bacteremia. The mammary and blood *E coli* isolates may have been from unrelated foci of infection, the blood-borne bacteria may have seeded the mammary gland, or another disease process in the body may have aided the bacteria's invasion. Evidence of hematogenous coliform mastitis has been reported,[9,29] although it is unlikely to be the cause of most coliform intramammary infections. Because of the similarities in antibiotic sensitivities determined on the milk and blood-borne organisms, it is unlikely that coincidental infections were the cause. Polymicrobial infections are uncommonly reported in cows, yet several of the cows in this study had multiple intramammary pathogens, one also had concurrent *Pasteurella* bacteremia and another *Staphyloccocal* bacteremia. These additional organisms may have aided in the damage to the basement membrane of the mammary gland.

In conclusion, many of the transient clinicopathologic abnormalities noted in cows with experimental ACM were persistent or recurrent in cows with naturally occurring disease. Hematologic data had greater correlation to bacteremia, whereas biochemical data were more related to outcome. Cows with large numbers of immature neutrophils in peripheral blood were often bacteremic, and bacteremic cows as well as those with biochemical evidence of metabolic derangement often did not survive the disease. Although the efficacy of different treatment protocols was not evaluated in this study, the occurrence of bacteremia in some cows with severe or protracted ACM supports a role for systemic antibiotic therapy in the treatment of this disease.

References

1. Schalm OW, Woods GM. Characteristics of coliform mastitis and treatment with dihydrostreptomycin. J Am Vet Med Assoc 1952; 120:385–388.

2. Eberhart RJ. Coliform mastitis. J Am Vet Med Assoc 1977; 170:1160–1163.

3. Radostits OM. The clinical aspects of coliform mastitis in cattle. Proceedings of the VI International Conference on Cattle Diseases. Stillwater, OK: Heritage Press; 1970:67–74.

4. Hill AW, Shears AL, Hibbitt KG. The elimination of serum-resistant *Escherichia coli* from experimentally infected single mammary glands of healthy cows. Res Vet Sci 1978;25:89–93.

5. Shuster DE, Kehrli ME. Comparison of *Escherichia coli* mastitis in early lactation and mid-lactation dairy cows. J Dairy Sci 1993; 76:159 (suppl 1).

6. Hill AW, Shears AL, Hibbitt KG. The pathogenesis of experimental *Escherichia coli* mastitis in newly calved dairy cows. Res Vet Sci 1979;26:97–101.

7. Kehrli ME, Nonnecke BJ, Roth JA. Alteration in bovine neutrophil function during the periparturient period. Am J Vet Res 1989; 50:207–214.

8. Cai T, Weston PG, Lund LA, et al. Association between neutrophil functions and periparturient disorders in cows. Am J Vet Res 1994;55:934–943.

9. Frost AJ, Brooker BE. Hyperacute *Escherichia coli* mastitis of cattle in the immediate post-partum period. Aust Vet J 1986;63: 327–331.

10. Carroll EJ, Schalm OW, Lasmanis J. Experimental coliform (*Aerobacter aerogenes*) mastitis: Characteristics of the endotoxin and its role in pathogenesis. Am J Vet Res 1964;25:720–726.

11. DeGraves FJ, Anderson KL. Ibuprofen treatment of endotoxin-induced mastitis in cows. Am J Vet Res 1993;54:1128–1132.

12. Powers MS, White ME, Dinsmore P, et al. Aerobic blood culturing in cows with coliform mastitis. J Am Vet Med Assoc 1986; 189:440–441.

13. Erskine RJ, Tyler JW, Riddell MG, et al. Theory, use, and

realities of efficacy and food safety of antimicrobial treatment of acute coliform mastitis. J Am Vet Med Assoc 1991;198:980–984.

14. Frost AJ, Hill AW, Brooker BE. Pathogenesis of experimental bovine mastitis following a small inoculum of *Escherichia coli.* Res Vet Sci 1982;33:105–112.

15. Schalm OW, Lasmanis J, Carroll EJ. Pathogenesis of experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. Am J Vet Res 1964;25:75–82.

16. Griel LC, Zarkower A, Eberhart RJ. Clinical and Clinicopathological effects of *Escherichia coli* endotoxin in mature cattle. Can J Comp Med 1975;39:1–6.

17. Vestweber JGE, Kruckenberg SM, Spencer H, et al. Disseminated intravascular coagulation in a cow with coliform mastitis. Comp Cont Educ Pract Vet 1983;5:S185–S190.

18. DeJong GA. Acute mastitis in cows—Part I: Fatal cases. Mod Vet Pract 1987;68:430–435.

19. Oetzel GR. Coliform mastitis and hypocalcemia in two dairy cows in mid lactation. Comp Cont Educ Pract Vet 1985;7:S237–S242,S244.

20. Katholm J, Haubro Andersen P. Acute coliform mastitis in dairy cows: Endotoxin and biochemical changes in plasma and colony-forming units in milk. Vet Rec 1992;131:513–514.

21. Milton JS, Tsokos JO. Statistical Methods in the Biological and Health Sciences. New York, NY: MacGraw-Hill; 1983:429–433.

22. Matthews DE, Farewell VT. Using and Understanding Medical Statistics. Basel, Switzerland: Karger; 1985;20–38.

23. Milton JS, Tsokos JO. Statistical Methods in the Biological and Health Sciences. New York, NY: MacGraw-Hill; 1983:433–438.

24. Kremer WDJ, Noordhuizen-Stassen EN, Grommers FJ, et al. Preinfection chemotactic response of blood polymorphonuclear leukocytes to predict severity of *Escherichia coli* mastitis. J Dairy Sci 1993;76:1568–1574.

25. Shuster DE, Kehrli ME. Pathophysiology, host defense, and cytokine production during coliform mastitis in midlactation dairy cows. J Dairy Sci 1992;75:160 (suppl 1).

26. Garry FB, Hull BL, Rings DM, et al. Prognostic value of anion gap calculation in cattle with abomasal volvulus: 58 cases (1980-1985). J Am Vet Med Assoc 1988;192:1107–1112.

27. Simpson DF, Erb HN, Smith DF. Base excess as a prognostic and diagnostic indicator in cows with abomasal volvulus or right displacement of the abomasum. Am J Vet Res 1985;46:796–797.

28. Washington JA II. Conventional approaches to blood culture. In: Washington JA II, ed. The Detection of Septicemia. West Palm Beach, FL: CRC Press; 1978;44–46.

29. Heidrich HJ, Renk W. Diseases of the Mammary Glands of Domestic Animals. Philadelphia, PA: WB Saunders; 1967;213.