Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows

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Objective—To determine the incidence of bacteremia in dairy cows with naturally occurring acute coliform mastitis (ACM) with a wide range of disease severity.

Design—Cohort study.

Animals—144 dairy cows with ACM from 6 herds.

Procedure—Cows were examined at time of identification of ACM (time 0) and classified as having mild, moderate, or severe mastitis on the basis of rectal temperature, hydration status, rumen contraction rate, and attitude. Cows were reexamined at 24 or 48 hours. Bacteriologic culturing of milk and blood (30 ml), CBC, and serum biochemical analysis were performed at each time point. Appropriate samples were obtained at a single point from herdmates without mastitis (controls) that were closely matched for lactation number and days since parturition. Blood culture results were compared among severity groups and controls by use of χ^2 tests, as was outcome of an ACM episode for cows grouped by blood bacterial isolates.

Results—Bacteria were isolated from 52 blood samples from 46 of 144 (32%) cows with ACM, which was significantly more than control cows (11/156; 7.1%). Group-1 isolates (*Escherichia coli, Pasteurella multocida, Mannheimia haemolytica, Klebsiella pneumoniae, Enterobacter agglomerans,* and *Salmonella enterica* serotype Typhimurium) were identified in 20 of 144 (14%) cows with ACM and 0 of 156 control cows. Group-1 isolates were identified in 4.3, 9.1, and 42% of cows classified as having mild, moderate, and severe ACM, respectively. *Escherichia coli* and *K pneumoniae* milk and blood isolates obtained from the same cow were of the same genotype. *Bacillus* spp were identified in 21 of 144 (15%) cows with ACM, which was significantly more than control cows (3/156; 1.9%). Thirty-five percent of cows with a group-1 isolate died during the mastitis episode.

Conclusions and Clinical Relevance—Results suggest that bacteremia develops in a substantial proportion of cows with ACM. Classification of severity of disease is important for establishment of effective treatment protocols; parenteral antimicrobial treatment may be indicated in cows with ACM. (*J Am Vet Med Assoc* 2001;219:976–981)

RUMINANTS

Acute coliform mastitis (ACM), typified by *Escherichia coli* intramammary infection, has become the predominant form of mastitis in herds in which contagious mastitis has been effectively controlled.[1-3] Despite decades of research focused on ACM, wholly effective control measures have yet to be established, and proper treatment of the disease remains controversial. Our understanding of ACM pathophysiology is based largely on studies of the experimental disease model. These studies typically involved inoculation of a healthy mammary gland with coliform organisms (*Aerobacter aerogenes, E coli, and Klebsiella* spp) or with purified endotoxin. Results of these experiments suggest coliform bacteria are noninvasive, and bacteremia (the presence of bacteria in the circulating blood) is not considered a significant sequela to ACM.[4-6] Furthermore, disease manifestation has been primarily attributed to the effects of endotoxin. Consequently, it has been widely held that antibiotic therapy is not warranted in cases of ACM. We feel that studies of naturally occurring ACM will help resolve discrepancies between knowledge based on the experimental disease model and practitioner experience in the field.

In 1 study[7] of 20 cases of naturally occurring ACM, only *Bacillus* spp were isolated from a few cases, a finding that was attributed to skin contamination. The severity of disease was not clearly defined, only one 5-ml blood sample was evaluated, and controls were not included in the study. In our previous work,[8] the existence of bacteremia associated with naturally occurring ACM was established, in which bacteremia associated with *E coli* was identified in 32% (11/34) of cows with severe protracted coliform mastitis. That study, however, lacked controls and was limited to a select population of cows with severe protracted disease. Furthermore, it has been suggested that bacteremia observed in these cows may have been the result of protracted disease rather than specific to the ACM episode.[6] Recently, we have reported important pathophysiologic differences among cows with ACM grouped by severity of systemic disease signs.[9]

The purposes of the study reported here were to determine the incidence of bacteremia in dairy cows with naturally occurring ACM and a wide range of disease severity, determine whether the affected mammary gland was the source of bacteremia, and identify factors that may be useful in predicting the occurrence of bacteremia in dairy cows with ACM.

Materials and Methods

Herds—Cows at 6 dairies surrounding Fort Collins, Colo, that developed clinical mastitis between July 1997 and January 1999 were eligible for inclusion in the study. During the study, there were approximately 4,000 Holstein cows in

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lactation at the 6 dairies. Cows were fed a **total mixed ration (TMR)** in groups based on production level and were milked in a parlor 3 times daily. All cows were housed in drylot pens or freestalls. Mean somatic cell count of bulk tank milk was < 300,000 cells/ml throughout the study period for all dairies. All cows were vaccinated with a bacterin containing the J-5 strain of *E coli*[a] at the start of the nonlactating period and 4 weeks before and within 2 weeks after parturition. Heifers received the J-5 bacterin 2 weeks before and within 2 weeks after parturition. All dairies practiced effective contagious mastitis control programs and participated in a program for monthly bacterial culture of bulk tank milk samples.

Inclusion criteria and data collection—Farm personnel identified cows as possibly having coliform mastitis if 1 or more of the following signs were present: reduced milk production, abnormal milk, and 1 or more abnormal mammary quarters. Initial examination and sample collection (time 0) was performed by 1 of the authors (JRW, KM), usually within an hour after notification from the farm. Cows identified with mastitis during the evening or night milking were examined the following morning. Farm personnel treated cows according to established farm protocols after initial examination and sample collection by study personnel. Treatment was not controlled in order to maximize producer participation in the study and determine the incidence of bacteremia associated with ACM given current varied farm treatment protocols. Treatment among farms was consistent in the use of anti-inflammatory drugs (flunixin meglumine, phenylbutazone), 7.2% sodium chloride solution (administered IV), and electrolyte solutions (administered PO). Antimicrobial therapy varied among and within farms and included commercially available antibiotic preparations (intramammary), gentamicin (intramammary), dexamethasone (intramammary), ceftiofur (IV and IM), and oxytetracycline (IV). Secretions were removed from affected mammary glands 3 times a day at the time of milking and an additional 2 times a day during treatments. Cows for which bacterial culture of a milk sample yielded *E coli* or *Klebsiella* spp were included in the study. Healthy herdmates, closely matched to infected cows by lactation number and days since parturition, were enrolled as controls.

Cows were evaluated at the time of initial examination (time 0) and again 24 hours later or at time 0 and again 48 hours later. Control cows were examined at 1 of the time points. Age, lactation number, days since parturition, rectal temperature, heart rate, respiratory rate, and rumen contraction rate were recorded. Hydration status was estimated on the basis of degree of enophthalmos and scored as 0 (none), 1 (mild enophthalmos), 2 (moderate enophthalmos), or 3 (severe enophthalmos). Attitude was classified on the basis of signs of depression as none, mild, or severe.

Severity classification—Cows with ACM were classified as having mild, moderate, or severe disease on the basis of rectal temperature, hydration status, rumen contraction rate, and attitude at time 0 (**Appendix**).

Bacteriologic culture—Secretions from affected mammary glands were collected at time 0 and 24 hours later or time 0 and 48 hours later and submitted for bacterial culture. A composite sample of milk from all quarters of control cows was collected at a single time point. Teat ends were disinfected with 70% ethanol prior to sample collection, and samples were collected in sterile vials. Samples were stored on ice for transport to the laboratory and processed the day of collection, except that those submitted to the laboratory after 5:00 PM were frozen at –4 C and processed the following day. Samples were mixed gently by inverting the tube, and 100 µl of each sample was plated on blood agar and MacConkey agar plates. Plates were incubated at 37 C in an atmosphere containing 10% CO_2 and examined after 24 and 48 hours. Bacterial colonies were identified in accordance with National Mastitis Council guidelines.[10] Coliform intramammary infection was diagnosed if samples contained ≥ 10 **colony-forming units (cfu)** of at least 1 coliform organism/ml. Plates with ≥ 3 organisms were considered contaminated. Bacterial numbers obtained following culture were reported as < 10,000, 10,000 to 100,000, and > 100,000 cfu/ml of milk.

Blood was collected from the jugular vein of cows with ACM at time 0 and 24 hours later or at time 0 and 48 hours later and submitted for bacterial culture. Blood from control cows was collected at a single time point. Hair was shaved, using a disposable razor, and the skin was disinfected with at least 3 alternating applications of povidone iodine scrub and 70% ethanol at a site over the jugular vein. Thirty milliliters of blood was aseptically drawn from the vein into a 35-ml syringe through an 18-ga needle. Fifteen milliliters of blood was injected aseptically through a new 18-ga needle into each of two 50-ml blood culture vials of brain-heart infusion broth containing 0.6% sodium polyanetholsulfonate.[b] Samples were aerated through a filtered needle and incubated at 37 C in an atmosphere containing 10% CO_2. Samples were subcultured onto blood agar on days 0, 1, and 7 and incubated at 37 C in an atmosphere containing 10% CO_2. Plates were examined for growth and recorded at 24 and 48 hours. *Escherichia coli, K pneumoniae, Pasteurella multocida*, *Mannheimia haemolytica*, *Enterobacter agglomerans,* and *Salmonella* serotype Typhimurium isolates were considered to be important pathogenic bacteria and were classified as group-1 isolates. Environmental streptococci, coagulase-negative staphylococci, and *Acinetobacter* spp were considered to be coincidental findings and classified as group-2 isolates. *Bacillus* spp were grouped separately.

Coliform isolate genotyping—Genotyping of *E coli* and *K pneumoniae* was performed on isolates obtained from milk and blood of the same cow as described previously.[11] Enterogenic repetitive intergenic consensus sequence primers were used to perform **polymerase chain reaction (PCR)** on the genomic DNA of *E coli* and *K pneumoniae* isolates. The presence and size (base pairs) of PCR products were compared between milk and blood coliform isolates obtained from the same cow.

Hematologic testing—Blood samples were collected from the tail vein at time 0 and 24 hours later or time 0 and 48 hours later. Testing of blood from control cows was not performed, because laboratory reference ranges were already established. Samples anticoagulated with EDTA were used for CBC and determination of plasma protein and fibrinogen concentrations. Samples collected in plain tubes without anticoagulant were used to harvest serum, and serum glucose, creatinine, total protein, albumin, globulin, total bilirubin, phosphorus, calcium, magnesium, sodium, potassium, chloride, and bicarbonate concentrations; aspartate transaminase, γ-glutamyltransferase, sorbitol dehydrogenase, and creatine kinase activities; and anion gap were determined. Hematologic values were compared between cows with a group-1 bacteremia and those in which blood culture results were negative or a non–group-1 bacteremia within each severity group.

Outcome—Survival and retention in the herd following the episode of mastitis were assessed to determine outcome. Cows that died during the episode of mastitis were classified as nonsurvivors; all others were considered survivors. Cows leaving the herd as a direct result of mastitis (ie, because of mastitis or low production) within 30 days after the episode of mastitis were classified as culled. Cows still present in the

herd 30 days after the episode of mastitis were classified as retained.

Data analyses—Continuous variables (days since parturition, age, lactation, and results of clinicopathologic tests) were tested for normality by use of the Kolmogorov D statistic. Normally distributed continuous variables were compared among groups (mild, moderate, or severe disease) by use of ANOVA. Continuous variables that were not normally distributed were compared among groups by use of Wilcoxon rank sum or Kruskal-Wallis tests. Categorical variables (milk bacterial count, proportion of bacteremic cows) were compared among groups by use of χ^2 tests, except that Fisher exact tests were used when the expected count in > 25% of the categories was < 5. Influence of farm on blood culture results and outcome data was evaluated by use of a maximum-likelihood ANOVA procedure.[c] Statistical calculations were performed by use of commercially available software.[c] Relative risk was calculated as an odds ratio; the odds ratio was not considered significantly different from 1 if its 95% confidence interval included 1. For all other tests, values of $P < 0.05$ were considered significant.

Results

Samples were collected from 178 cows with suspected ACM. Cows were excluded if samples yielded no growth on 0-hour milk culture (n = 13), a noncoliform organism was isolated (n = 15), or the 0-hour milk sample was contaminated (n = 2). Four cows were excluded because data necessary for severity classification were missing.

Bacteriologic testing—Coliform organisms were isolated from the time-0 milk culture of 144 cows that were subsequently enrolled in the study. Organisms included *E coli* (n = 122), *K pneumoniae* (n = 19), and 3 cases in which both *E coli* and *K pneumoniae* were isolated.

Bacteria were isolated from 52 blood samples in 46 of 144 (32%) cows with ACM, which was significantly ($P < 0.001$) more than those isolated from 11 of 156 (7.1%) control cows. Group-1 isolates (*E coli, K pneumoniae, Pasteurella multocida, Mannheimia haemolytica, E agglomerans,* and *Salmonella* Typhimurium), group-2 isolates (environmental streptococci, coagulase negative staphylococci, and *Acinetobacter* spp), and *Bacillus* spp isolates were evaluated independently. Group-1 isolates alone were identified in a single blood sample from 14 cows, in combination with a group-2 isolate in 2 cows, and in combination with *Bacillus* spp in 4 cows with ACM. Group-2 isolates alone were identified in a single blood sample from 8 cows and in combination with *Bacillus* spp in 4 cows with ACM. *Bacillus* spp alone were identified in a single blood sample of 14 cows with ACM. Bacteria were isolated from 30, 8, and 14 blood samples of cows with ACM taken at 0, 24, and 48 hours, respectively. Bacteria were isolated from the blood of cows with ACM at both 0 and 24 hours from 3 cows and at both 0 and 48 hours from 3 cows. Group-1 isolates were identified at time 0 in 11 cows, at 24 hours in 3 cows, at 48 hours in 4 cows, at 0 and 24 hours in 1 cow, and at 0 and 48 hours in 1 cow. Cows were classified as bacteremic if bacteria were isolated from at least 1 blood sample at any time point. Group-1 bacteremia was identified in a significantly ($P < 0.001$) greater percentage of cows with ACM (14%; 20/144), compared with control cows (0%; 0/156). Fifteen percent (21/144) of cows with ACM had a *Bacillus* spp bacteremia, which was significantly ($P < 0.001$) more than control cows (1.9%; 3/156). The percentage of cows with group-2 isolates was not significantly ($P = 0.2$) different between cows with ACM and controls.

When evaluated on the basis of severity of ACM, a significantly greater percentage of cows in the severe group had a group-1 bacteremia (42%), compared with moderate (9.1%) and mild (4.3%) groups (**Table 1**). In contrast, significant differences were not detected in the percentage of cows with group-2 or *Bacillus* spp isolates among severity groups. The odds of a group-1 bacteremia in cows in the severe group were 15 (95% **confidence interval [CI]**, 4.1 to 61.9) and 7.2 (95% CI, 2.1 to 25.2) times greater than in cows in the mild and moderate groups, respectively.

Group-1 bacteremia was identified in a significantly greater percentage of cows with > 100,000 cfu/ml in milk from the infected gland at time 0 (23%; 15/64) than in cows with < 100,000 cfu/ml (5.3%; 4/76). Significant differences in cfu/ml bacteria in milk from the infected gland were not detected in cows with group-2 or *Bacillus* spp isolates alone.

Coliform isolate genotyping—Genotyping was performed on coliform organisms of the same genus isolated from the milk and blood of the same cow. *Escherichia coli* was isolated from the milk and blood of 10 cows, and *K pneumoniae* was isolated from the milk and blood of 1 cow. The DNA fingerprint of *E coli* milk and blood isolates, obtained from the same cow, had the same banding pattern, indicating they were the

Table 1—Number and percentage of positive blood culture results by bacterial isolate from cows with acute coliform mastitis classified by severity of disease and control cows

	All isolates		Group-1 isolates		Group-2 isolates		*Bacillus* spp	
Severity (n)	No.	%	No.	%	No.	%	No.	%
Control (156)	11	7.1[a]	0	0[a]	9	5.8[a]	3	1.9[a]
Mild (69)	21	30[b]	3	4.3[b]	10	15[b,c]	11	16[b]
Moderate (44)	10	23[b]	4	9.1[b]	3	6.8[a,c]	5	11[b]
Severe (31)	15	48[c]	13	42[c]	1	3.2[a,c]	5	16[b]

Values within a column with different superscripts are significantly ($P < 0.05$) different. Blood bacterial isolates were evaluated independently; therefore, cows with more than 1 isolate type were counted more than once.

Group-1 isolates = *Escherichia coli, Pasteurella multocida, Mannheimia hemolytica, Klebsiella pneumoniae, Enterobacter agglomerans, Salmonella* Typhimurium. Group-2 isolates = Environmental streptococci, coagulase-negative staphylococci, and *Acinetobacter* spp.

Table 2—Survival and retention rates in a group of 144 cows with acute coliform mastitis

	Negative culture result		Group-1 isolates		Group-2 isolates		*Bacillus* spp	
Outcome	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Lived	98	100*	13	65*	8	100	14	100
Died	0	0	7	35	0	0	0	0
Retained	78	80	10	77	8	100	12	86
Culled	20	20	3	23	0	0	2	14

*Values were significantly ($P < 0.001$) different. Cows were grouped according to results of bacteriologic culture of blood. Comparisons were only made between groups of isolates and cows with negative culture results; comparisons were not made between bacteremic groups.

Died = No. of cows that died during the mastitic episode. Retained = No. of cows still present in the herd 30 days after the mastitic episode. Culled = No. of cows removed from the herd as a direct result of mastitis.

See Table 1 for remainder of key.

same genotype. The *K pneumoniae* milk and blood isolates from the same cow were also of the same genotype.

Differentiation of group-1 bacteremic cows and cows with negative blood culture results or non–group-1 bacteremia—Signalment and hematologic data were compared between cows with group-1 bacteremia and those with negative blood culture results or non–group-1 bacteremia within each severity group. Significant differences were not detected in mean number of days since parturition, age, and lactation number of cows with group-1 bacteremia versus those without by severity group.[9] Complete hematologic data were available for 141 cows. Median values of CBC and serum biochemical analysis by severity group have been reported.[9] There were no significant differences in hematologic values of cows with group-1 bacteremia versus those without by severity group. Significant and clinically important differences did exist in the severity classification of cows with group-1 bacteremia versus those without (Appendix). Sixty-five percent (13/20) of cows with group-1 bacteremia were classified as severe, compared with only 15% (18/122) of those without group-1 bacteremia.

Outcome—Outcome was evaluated based on survival through the mastitic episode and retention on farm for 30 days following the episode (**Table 2**). No significant differences in the percentage of cows that died or were culled were observed between cows with a group-2 isolate or *Bacillus* spp isolate versus cows with negative blood culture results. A significantly ($P < 0.001$) greater percentage (35%) of cows with a group-1 isolate died, compared with cows with negative blood culture results or a non-group 1 isolate (0%). Significant differences in culling were not observed among cows grouped by blood culture results.

Discussion

Results of this study indicated bacteremia develops in a substantial proportion of cows with naturally occurring ACM. Furthermore, bacteremia caused by group-1 pathogens (*E coli, P multocida, M haemolytica, K pneumoniae, E agglomerans,* and *Salmonella* Typhimurium) develops in a significant proportion of cows with ACM and is associated with severity of systemic disease signs. Our results also revealed that a significantly greater percentage of cows in the mild and moderate groups had group-1 bacteremia, compared with controls from which no group-1 isolates were cultured (Table 1). Antimicrobial use was variable among and within farms and may have had an impact on results of this study. Therefore, the proportion of bacteremic cows may be higher in cases of ACM that are not treated with antibiotics.

Intermittent bacteremia is commonly the result of bacterial infiltration of the blood through the lymphatics from a site of localized infection.[12] It has been suggested that the *E coli* bacteremia observed by Cebra et al[8] may have been nonspecific and attributable to prolonged severe disease. Indeed, *E coli* isolated from the blood samples in that study could have come from the large pool in the gastrointestinal tract. However, antibiotic sensitivity patterns of *E coli* isolated from milk and blood of the same cow were similar, which suggests a common source. Furthermore, in the present study, genotyping by use of PCR indicated that *E coli* and *Klebsiella* spp isolated from blood were the same as those isolated from the mammary gland of the same cow, indicating the infected mammary gland was the likely source of bacteremia.

Resident macrophages in the liver and spleen are primarily responsible for bacterial clearance of the blood; however, bacterial opsonization and subsequent phagocytosis by circulating leukocytes play a role as well.[13] Leukopenia, specifically neutropenia, has been well documented in cows with ACM. Furthermore, results from our previous work revealed that cows with more severe systemic disease signs have more profound neutropenia,[9] which may increase the probability of obtaining positive blood culture results in cows with more severe ACM. Indeed, cohort studies of immunocompromised humans have demonstrated a positive relationship between neutropenia and bacteremia attributable to gram-negative bacilli such as *E coli* and *Klebsiella* spp.[14] However, although it was reported that a significant difference was not detected in median neutrophil count in cows with moderate versus severe systemic disease signs,[9] results of the present study indicated there were significantly more positive blood culture results obtained from cows with severe ACM (48%), compared with cows with moderate ACM (23%). Furthermore, despite the profound neutropenia observed in cows with moderate ACM, compared with those with mild ACM,[9] there was no difference in positive blood culture results between the 2 groups (Table 1). The same was true when only group-1 blood isolates were considered. Our results suggest that a factor other than neutropenia may be

involved in the pathophysiology of bacteremia associated with ACM.

Many factors may have been responsible for failure to identify bacteremia associated with ACM in previous studies.[5-7] Most used experimental disease models in which the mammary glands of healthy cows were inoculated with a specified uniform number of coliform bacteria. Disease manifestation is thought to be the result of interaction of host, pathogen, and environmental factors. Epidemiologic studies[4,15,16] of ACM have indicated that *E coli* is an accidental pathogen with no specific virulence factors involved in establishing infection of the mammary gland. Furthermore, despite the consistency of inoculum size and control of environmental factors afforded by the experimental model, pathophysiologic response varies widely among individual cows.[16] Taken together, these results suggest cow factors (ie, immune status, presence of concurrent disease, teat-end condition) may be most important in determining the pathogenesis of ACM. Therefore, considerable differences in disease manifestation may exist in the experimental disease of healthy cows that arguably have intact host defenses versus naturally occurring disease in cows that are likely to have compromised host defenses.

Recently we reported that severity classification based on systemic disease signs effectively distinguished significant pathophysiologic differences among cows with ACM.[9] Previous studies that failed to identify bacteremia did not evaluate or clearly define severity of ACM cases. Results of the present study indicated that group-1 bacteremia develops in a significantly smaller proportion of cows with mild and moderate disease signs, compared to cows with severe disease signs (Table 1). Consequently, a predominance of more mildly affected cows combined with a small sample size may have resulted in failure to identify bacteremia in previous studies.

Culture technique may account for failure to identify bacteremia associated with ACM. Previous studies typically performed blood culture on 5 ml of blood collected at a single time point. Often < 10 bacteria are present per milliliter of blood in intermittently bacteremic human patients.[17] The chances of obtaining a positive culture are directly related to the volume of blood collected.[18] Increasing the blood volume collected from 2 ml to 20 ml resulted in an increase in positive cultures from 30% to 50% in human patients,[18-20] whereas volumes > 30 ml did not result in a significant increase in positive yield.[20] When sequential blood cultures were performed in humans that did not have endocarditis, 80% were positive on first culture, 90% after second culture, and 99% after third culture.[18] In the present study, 30-ml blood samples were collected at 2 time points in an attempt to optimize identification of bacteremia.

Bacillus spp have been isolated from blood in previous studies but were presumed to be skin contaminants.[6-8] Inclusion of controls in the present study indicates *Bacillus* spp bacteremia is 8 times more likely in cows with ACM, compared with controls. The significance and source of *Bacillus* spp bacteremia in cows with ACM is unclear. The incidence was similar among severity groups, all cows with *Bacillus* spp bacteremia survived, and there was no difference in cull rate, compared to cows with negative blood culture results.

Group-2 isolates (ie, environmental streptococci, coagulase-negative staphylococci, and *Acinetobacter* spp) were considered inconsequential, as there was no difference between controls and cows with ACM. Furthermore, there was no difference in outcome between cows with a group-2 isolate alone and those with negative blood culture results. Group-2 isolates may have been contaminants or present in the blood of all cows periodically.

Blood cultures were performed at 2 time points to increase the chance of identifying cows with bacteremia. The second time point was 24 hours after initial examination (time 0) at the start of the study; however, it was later changed to 48 hours. The 48-hour time point allowed for better evaluation of changes in hematologic data; furthermore, results of our previous study identified bacteremia in cows that were ill for a median of 48 hours.[8] Interestingly, 65% (13/20) of cows with group-1 positive blood culture results were identified at time 0, and 35% (7/20) were in the mild or moderate group. This is further evidence that the observed bacteremia was not merely the result of severe protracted disease.

The design of this study did not allow for discrimination of the cause-and-effect relationship between severity of disease and bacteremia. However, evaluation of the data collected suggests that bacteremia caused by group-1 pathogens had a negative impact on cows with ACM. Death during the mastitis episode only occurred in cows with group-1 bacteremia. Six of 7 cows that died were in the severe group, suggesting severity of an ACM episode is an important factor. Severe disease alone, however, does not appear to determine survival. Seven of 13 severely affected cows with group-1 bacteremia survived, compared with 18 of 18 severely affected cows without group-1 bacteremia. Together, these data suggest group-1 bacteremia was an added insult to severe disease that resulted in a poorer outcome. Severe disease, however, is not a prerequisite for development of group-1 bacteremia, as 35% of group-1 bacteremic cows were in the mild and moderate groups. However, of cows with mild and moderate disease severity, only 1 of 7 (a cow in the moderate group) with group-1 bacteremia died.

The culling rate of surviving cows was the same in cows with ACM regardless of blood culture results (Table 2). Likewise, of the cows with severe disease that survived, there was no difference in culling between cows with group-1 bacteremia (57%; 4/7) and those without (61%; 11/18). Therefore, it appears that group-1 bacteremia has a negative impact on survival but not culling during the first 30 days following a mastitis episode.

Pasteurella multocida or *Mannheimia haemolytica* was isolated from the blood of 7 cows, *E agglomerans* from 1 cow, and *Salmonella* Typhimurium from 1 cow with ACM. These bacteria were not, however, isolated on milk culture of the same cow. These findings suggest some cases of bacteremia associated with coliform mastitis may be the result of bacterial translocation

from other organ systems. Cows with *Pasteurella* or *Mannheimia* spp bacteremia had more severe disease signs, and their prognosis was grave. These bacteria are normal flora of the upper portion of the respiratory tract of cattle and are involved in the bovine respiratory disease complex. Studies in sheep and in vitro studies of bovine pulmonary endothelial monolayers indicate that endotoxin can cause direct dose-dependent damage resulting in an increase in permeability and hydraulic conductance across pulmonary endothelium.[21] Such damage may develop in cows with more severe systemic disease signs and result in bacteremia. It has been proposed that cows with ACM are not endotoxemic.[22] However, endotoxin was identified in the milk vein blood of 5 of 9 cows with naturally occurring ACM.[23] Together with the results of the present study, these data suggest cows with more severe systemic disease signs may be endotoxemic. Further study is needed to determine whether cows with ACM are endotoxemic and identify the relationship between possible endotoxemia, bacteremia, and severity of an ACM episode.

Signalment and hematologic data were not useful in identifying a cow with group-1 bacteremia. Classification of disease severity based on systemic disease signs was the best indicator of a cow likely to be bacteremic.

The results of this study clearly demonstrate that bacteremia develops in a substantial proportion of cows with ACM and has an impact on the outcome of an ACM episode. We also demonstrated the importance of accurate classification of disease severity based on systemic disease signs. Classification of disease severity should play an important role in establishing rational effective treatment protocols for cows with ACM. On the basis of results of this study, there should be a high index of suspicion of bacteremia in cows with severe systemic disease signs; parenteral antimicrobial therapy may be indicated in such cases of ACM.

[a]J-5 Bacterin, Pharmacia and Upjohn Animal Health, Kalamazoo, Mich.
[b]BBL, Becton Dickinson Co, Cockeysville, Md.
[c]SAS/STAT, release 6.12, SAS Institute Inc, Cary, NC.

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Appendix

Scheme based on systemic disease signs for classifying severity of acute coliform mastitis in dairy cows*

Variable	Criteria	Score
Rectal temperature (C [F])	37.8 (100)–39.27 (102.7)	0
	39.33 (102.8)–39.8 (103.7)	1
	> 39.8 (103.7) or < 37.8 (100)	2
Hydration status (degree of enophthalmos)	None	0
	Mild	1
	Moderate	2
	Severe	3
Rumen contraction rate (contractions/min)	≥ 2	0
	1	1
	0	2
Attitude (signs of depression)	None	0
	Mild	1
	Severe	2

*Cows with total score of 0 to 2 were classified as having mild disease, cows with total score of 3 to 5 were classified as having moderate disease, and cows with total score of 6 to 9 were classified as having severe disease.

RUMINANTS