

# Prevalence of Bacteremia in Dairy Cattle with Acute Puerperal Metritis

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**Background:** Acute puerperal metritis (APM) affects 30% of postpartum dairy cattle. Bacteremia negatively impacts survival in cattle with coliform mastitis. However, the prevalence of bacteremia in dairy cattle with APM is unknown.

**Hypothesis:** Bacteremia is detectable in a large proportion of cattle with APM.

**Animals:** Seventeen dairy cows with APM and 17 healthy dairy cattle.

**Methods:** Prospective case-control study. Cases were identified by daily monitoring of cattle in the first 10 days after calving. Controls were matched to cases by parity and days in milk. Cows were examined at the time of identification of APM. A complete blood count, serum biochemical analysis, and bacteriologic culture of blood and lochial fluid were performed on each animal at the time of diagnosis. The same samples were collected from healthy herdmates of a similar parity and days in milk. Blood culture results and clinicopathologic variables were compared between groups. Conditional logistic regression was used to evaluate factors associated with APM, whereas multivariate logistic regression was used to evaluate factors associated with bacteremia.

**Results:** Bacteremia occurred in 53% (9/17) of cattle with APM and 53% (8/15) controls. *Bacillus* spp. was the organism most commonly isolated from the bloodstream in cattle of both groups. Bacteremic cattle in both groups were significantly less likely to have basophils in the peripheral circulation ( $P = .02$ ) and more likely to have higher serum globulin concentrations ( $P = .02$ ).

**Conclusions and Clinical Importance:** Bacteremia is a common occurrence in postpartum dairy cattle. Further study is warranted to investigate the modes by which bacteria colonize the bloodstream in this population of animals and the importance of bacteremia on health and productivity of affected animals.

**Key words:** Dairy cattle; Inflammation; Postpartum; Sepsis; Uterine.

Dairy cattle are susceptible to numerous disorders in the immediate postpartum period. Acute puerperal metritis (APM), defined as the presence of a fetid, watery uterine discharge, an enlarged, flaccid uterus, and overt signs of systemic illness that might include fever, dehydration, depression, and toxemia is one of the most commonly encountered infectious diseases in modern dairy practice.<sup>1,2</sup> APM occurs within the first 21 days of lactation and typically affects 20–30% of all cattle.<sup>3</sup> While a variety of microorganisms might be isolated from the reproductive tract of both healthy postparturient cattle and cattle with APM, *Escherichia coli* and *Trueperella pyogenes* represent the bacteria most commonly associated with clinical disease.<sup>2</sup>

Bacteremia is defined as the presence of bacteria within the bloodstream and, until recently, was thought to be an uncommon occurrence in adult large animal veterinary patients.<sup>4</sup> Bacteremia has been documented in approximately 32% of adult dairy cattle

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## Abbreviations:

APM	acute puerperal metritis
DIM	days in milk
LR	likelihood ratio
ROC	receiver operating characteristic curve
TMR	total mixed ration

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with coliform mastitis.<sup>5</sup> In cows with acute coliform mastitis, the presence of bacteremia, particularly bloodborne infection with organisms such as *E. coli*, *Pasteurella multocida*, and *Mannheimia hemolytica*, had a significant impact on cow survival.<sup>5</sup>

The purposes of the study reported here were to investigate the prevalence of bacteremia in dairy cattle with naturally occurring APM, determine if an association exists between the bacteria cultured from the bloodstream and those present in uterus and identify factors that might be of use in predicting occurrence of bacteremia in cattle with APM.

## Materials and Methods

### Animals

Cows at 3 dairies in northeast and southwest Georgia that developed APM between September 2011 and November 2013 were eligible for inclusion in the study. Cows were fed a corn-silage based total mixed ration (TMR) formulated to meet the requirements of dairy cattle in early lactation as set forth by the National Research Council. Milk production at the 3 dairies averaged 60–90 lb per cow per day. All cattle were housed in groups based on production and were milked in a parlor 3 times daily. All cows were housed in drylot pens or freestall barns.

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### ***Inclusion Criteria and Data Collection***

Fresh cows at each dairy were examined daily by one of the authors (BCC). For inclusion in the study, cattle had to be enrolled in a fresh cow monitoring program. The rectal temperature of each animal was checked daily with a digital thermometer<sup>a</sup> for the first 10 days in milk and monitoring for the development of APM began on day 1 postpartum. A rectal examination was performed on any animal with a temperature >103°F and lochial fluid was collected by transrectal massage of the uterus. For the purposes of this study, APM was diagnosed if a cow was less than 10 days in milk, had a fetid, watery, reddish-brown uterine discharge, and systemic signs of illness that included at least one of the following: fever (rectal temperature >103°F), obtundation, toxemia (injected mucous membranes), tachycardia (heart rate >84 beats/min), or tachypnea (respiratory rate >36 breaths/min). Any animals with evidence of other concurrent disease processes (mastitis, abomasal displacement, respiratory disease, diarrhea) were excluded from the study. Cows diagnosed with APM were treated according to on-farm protocols after evaluation and sample collection by study personnel. Treatment varied between and within farms and included systemic antimicrobials, anti-inflammatories, prostaglandin, and oral electrolyte solutions. Healthy herd mates, as determined by a complete physical examination and of similar parity and days in milk (DIM) as cases, were enrolled as controls. Control cattle were monitored for the development of APM daily until day 10 after parturition. Any control that developed APM was removed from the study.

### ***Hematologic Testing***

Blood samples were collected from the jugular vein at the time of diagnosis from both cattle with APM and controls. Samples anti coagulated in EDTA were used for complete blood count (CBC) analysis. Samples collected into plain tubes without anticoagulant were used for serum chemistry analysis.

### ***Bacteriologic Culture***

A swab of lochial fluid was collected at the time of diagnosis. The tail was held to the side and the external genitalia were cleaned with 3 alternating applications of 4% chlorhexidine scrub and 70% isopropyl alcohol. A double-guarded culture swab<sup>b</sup> was passed into the uterus, a sample obtained, and the culture swab removed. The swab was capped and transported to the laboratory for processing on the day of collection. Swabs were cultured aerobically at 37°C for 48 hours on sheep blood agar and MacConkey agar, and anaerobically for up to 7 days on pre-equilibrated sheep blood agar. Plates were evaluated for growth and findings recorded at each observation. Bacteria were identified on the basis of characteristics of the colony, morphology, Gram stain, hemolysis, and biochemical profile.

Blood was collected from the jugular vein of each cow with APM and each control cow on the day of diagnosis. The hair over the jugular vein was shaved using a battery-operated clipper with a #40 blade. The skin was disinfected with at least 3 alternating applications of a 4% chlorhexidine surgical scrub and 70% isopropyl alcohol. Thirty-five milliliters of blood was aseptically drawn from the vein into a 35-mL syringe through a 16-ga, 1-½ inch needle. Ten milliliters of blood was injected aseptically through a new 16-ga, 1-½ inch needle into a 30 mL culture vial of 3.0% soybean-casein digest broth containing 0.05% sodium polyanetholsulfonate (BD Bactec Plus Aerobic/F<sup>c</sup>) and a 40 mL culture vial of 2.75% soybean-casein digest broth containing 0.035% sodium polyanetholsulfonate (BD Bactec Lytic/10/Anaer-

obic/F<sup>c</sup>) and submitted for aerobic and anaerobic culture. Both the aerobic and anaerobic samples were subcultured onto blood agar on days 0, 1, and 7. Plates were examined for growth and findings recorded at each observation.

### ***Statistical Analysis***

Cattle were grouped by disease (control or APM) and blood culture (positive or negative) status. The proportion of cattle with bacteremia and the frequency with which specific bacteria were isolated from the uterus was compared between cattle with and without APM using McNemar's test for paired proportions. Clinicopathologic data were compared between cattle with APM and healthy controls using a Wilcoxon signed-rank test, whereas clinicopathologic data were compared between bacteremic and non-bacteremic cattle using a Wilcoxon rank sum test. Data were reported as median and 10th and 90th percentiles. Likelihood ratios were calculated to investigate the role of farm in blood culture status. The association of clinicopathologic data with disease status was investigated using conditional logistic regression, whereas the association of clinicopathologic data with blood culture status was investigated using backward stepwise logistic regression. All variables were first screened using univariate logistic regression and all variables with a  $P < .2$  were allowed to enter in the final logistic regression model. For the multivariate model, all variables with a  $P < .05$  remained in the model. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. An OR greater than 1 corresponds to a positive association with APM or bacteremia and an OR of less than 1 corresponds to a negative association. The final logistic regression model fit was evaluated using the Hosmer-Lemeshow Goodness-of-Fit test. The ability of the model to predict a given outcome was assessed by use of receiver operating characteristic (ROC) curve analysis. Statistical analysis was performed using commercially available statistical software.<sup>d</sup>

## **Results**

### ***Uterine Bacteriology***

A total of 34 cows were enrolled in the study, 17 cases with APM and 17 controls. *Escherichia coli* and *T. pyogenes* were the organisms most frequently isolated from the uterus of cattle with APM (Table 1). Other isolates included *Clostridium* spp., and combinations of Gram positive and Gram negative aerobes and anaerobes (Table 1). Cattle with APM were significantly more likely to have *E. coli*, *T. pyogenes*, Gram positive aerobes, and Gram positive anaerobes isolated from the uterus than were control cattle (Table 1).

### ***Clinicopathologic Values***

Cattle with APM had significantly lower serum albumin concentration, albumin/globulin ratio, serum sodium, and total serum calcium concentration than control cattle, whereas plasma fibrinogen concentration, monocyte count, serum globulin concentration, and serum glucose concentration were significantly higher in cattle with APM than in controls (Table 2). No variables were significantly associated with APM in the final conditional logistic regression model.

**Table 1.** Bacterial isolates obtained from the uterus of clinically healthy cattle (n = 15) and cattle with APM (n = 17).

Organism	Group		P-value
	Control	APM	
<i>Escherichia coli</i>	2	9	.03
<i>Trueperella pyogenes</i>	6	15	.01
<i>Clostridium</i> spp.	0	4	.10
Gram positive aerobes <sup>a</sup>	4	12	.03
Gram positive anaerobes <sup>b</sup>	4	11	.04
Gram negative aerobes <sup>c</sup>	1	3	.60
Gram negative anaerobes <sup>d</sup>	8	10	1.00

<sup>a</sup>*Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp.

<sup>b</sup>*Peptostreptococcus* spp.

<sup>c</sup>*Pasteurella* spp., *Proteus* spp., *Actinobacillus* spp.

<sup>d</sup>*Fusobacterium* spp., *Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., *Prevotella* spp.

### Blood Culture

Bacteria were isolated from the bloodstream of 53% (9/17) of cattle with APM and 53% (8/15) of control cattle. No difference in the prevalence of bacteremia between groups was detected ( $P = .724$ ). *Bacillus* spp. was the organism most frequently isolated from the bloodstream of both bacteremic control cattle (5/8, 63%) and bacteremic cattle with APM (5/9, 56%) (Table 3). *Bacillus* spp. were isolated in combination with *T. pyogenes* in 1 cow with APM and in combination *Clostridium* spp. in another cow with APM.

Because of the lack of a statistically different prevalence of bacteremia between the 2 groups of cattle, the groups were combined for further analysis. There was a significant effect of farm on blood culture status. Cattle from farms 1 and 3 were significantly more likely to have a positive blood culture than cattle from farm 2 (LR = 7.7,  $P = .02$ ). Cattle with bacteremia had significantly greater DIM and serum globulin concentration than non-bacteremic cattle, whereas basophil count and albumin/globulin ratio was significantly lower in bacteremic than non bacteremic cattle (Table 4).

The only variables significantly associated with bacteremia in a multivariate logistic regression model (overall significance of the model  $P = .0005$ ) were the absence of basophils and serum globulin concentration (Table 5). The model correctly predicted 81% of cases. The area under the receiver operating characteristic curve for the ability of serum globulin concentration and absence of basophils to predict bacteremia was 0.850 (95% confidence interval, 0.679–0.951).

### Discussion

The findings of this study suggest that bacteremia is a common occurrence in both healthy postpartum dairy cattle and dairy cattle with APM, occurring in 53% of cattle in each group. The process of uterine involution is not sterile and, in 1 study, 93% of all uteri sampled within 2 weeks of calving were culture positive for various bacteria.<sup>6</sup> In addition to bacterial contamination of the uterine lumen after parturition, the surface cells of the endometrium slough and expose

**Table 2.** Comparison of hematologic and biochemical findings (median, 10th, and 90th percentile) in clinically healthy cattle (n = 15) and cattle with APM (n = 17).

Variable	Reference Range	Group		P-value
		Control	APM	
Hct (%)	24–46	29.3 (25.7–33.3)	27.6 (23.3–32)	.19
Platelets ( $\times 10^3/\mu\text{L}$ )	100–800	261 (156–561)	315 (183–535)	.33
Fibrinogen (mg/dL)	100–600	300 (300–800)	800 (500–1100)	.01
WBC ( $\times 10^3/\mu\text{L}$ )	4–12	10.9 (7.20–17.9)	8.4 (6.2–12.5)	.11
Segs ( $\times 10^3/\mu\text{L}$ )	0.6–4	4.35 (1.11–10.5)	2.4 (1.4–7.8)	.18
Bands ( $\times 10^3/\mu\text{L}$ )	0–0.1	0 (0–0.65)	0.3 (0–0.6)	.79
Lymphs ( $\times 10^3/\mu\text{L}$ )	2.5–7.5	5 (2.57–10)	3.9 (1.8–7.3)	.20
Monocytes ( $\times 10^3/\mu\text{L}$ )	0–0.9	0.3 (0–1.44)	0.8 (0.2–1.6)	.03
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0–2.4	0.12 (0–0.26)	0.1 (0–0.5)	.56
Basophils ( $\times 10^3/\mu\text{L}$ )	0–0.2	0 (0–0.12)	0.7 (0–0.1)	.06
Creatinine (mg/dL)	1–1.8	0.6 (0.12–0.7)	0.6 (0.4–1)	.04
Total protein (g/dL)	6.4–9.5	6.6 (5.8–7.4)	6.7 (5.7–7.7)	.32
Albumin (g/dL)	2.5–4.5	3.4 (2.8–3.8)	2.8 (2.3–3.5)	.01
Globulin (g/dL)	2.6–6.5	3 (2.4–4.4)	3.7 (2.8–5.2)	.01
A/G ratio	N/A	1.2 (0.67–1.2)	0.7 (0.4–1.3)	.002
Glucose (mg/dL)	55–95	59 (48–70)	63 (31–81)	.03
Sodium (mEq/L)	136–147	142 (139–147)	141 (136–145)	.01
Potassium (mEq/L)	4–5	3.9 (3.3–4.4)	3.7 (3.4–4.2)	.33
Chloride (mEq/L)	95–105	101 (96.4–103)	100 (94–104)	.41
Bicarbonate (mEq/L)	20–30	27 (23.1–30)	28 (25–31)	.24
Anion gap (mEq/L)	13–20	20 (13–22.4)	18 (13–19)	.06
Total calcium (mg/dL)	7.6–10.2	9.2 (7.9–10.1)	8.5 (7.1–9.3)	.003

**Table 3.** Bacterial isolates obtained from the bloodstream of clinically healthy cattle (n = 15) and cattle with acute puerperal metritis (n = 17).

Organism	Group	
	Control	APM
<i>Bacillus</i> spp.	5	5
<i>Trueperella pyogenes</i>	0	1
<i>Clostridium</i> spp.	0	1
<i>Kytococcus sedentarius</i>	1	0
<i>Staphylococcus equorum</i>	1	0
Other <sup>a</sup>	1	2

<sup>a</sup>Multiple organisms present or organism unable to be identified.

the deeper uterine layers. Indeed, the remnants of the maternal caruncle are necrotic by day 5 after calving and, by day 12 postpartum, a denuded endometrial surface with exposed blood vessels can be found.<sup>7</sup> It is well accepted that the epithelial barriers of the body's mucosal surfaces serve as a barrier to bacterial invasion of the deeper tissues and systemic circulation.<sup>8</sup> Bacteremic cattle were significantly later in lactation than non bacteremic cattle (8 days versus 4.5 days, respectively). The longer period of time from parturition to diagnosis in these animals might put them at greater risk of bacteremia simply because of prolonged contact of a denuded endometrium with contaminating bacteria. Thus, it is possible that sloughing of the

endometrial epithelium allows the bacteria that normally colonize the uterine lumen after calving to gain access to the systemic circulation before the beginning of re-epithelialization.

Cattle diagnosed with APM had significantly lower serum albumin concentrations and significantly higher serum globulin concentrations than healthy controls. Similarly, bacteremic cattle in this study had significantly higher serum globulin concentrations and a trend toward lower serum albumin concentrations. A decline in plasma protein has been demonstrated in dairy cattle immediately after calving.<sup>9</sup> Here, decreasing plasma globulin concentrations, likely resulting from uptake of IgG<sub>1</sub> by the mammary gland, rather than a decline in albumin caused the decrease in plasma protein. Other studies have found a decrease in serum albumin concentration in periparturient cattle and it has been suggested that expansion of plasma volume, impaired hepatic function secondary to lipid accumulation, or inflammatory disorders that downregulate hepatic albumin production might be responsible for these findings.<sup>10-12</sup> It is also possible that the decrease in serum albumin concentration in cattle with APM reflects loss of serum proteins into the uterine lumen because of tissue compromise. The increase in globulin concentration seen in both cattle with APM and bacteremic cattle in this study is likely because of an increase in antigenic stimulation. Cattle with APM had significantly lower serum total calcium concentrations than controls and studies have found that the population risk to develop APM attributable to

**Table 4.** Comparison of hematologic and biochemical findings (median, 10th and 90th percentile) in bacteremic (n = 17) and nonbacteremic cattle (n = 15).

Variable	Reference Range	Group		P-value
		Bacteremic	Nonbacteremic	
Parity	N/A	1 (1-3)	2 (1-5)	.25
DIM	N/A	8 (2.4-10)	4.5 (2-8)	.01
Hct (%)	24-46	28.4 (24-31)	28 (24-35)	.91
Platelets ( $\times 10^3/\mu\text{L}$ )	100-800	270 (170-577)	341 (175-492)	.55
Fibrinogen (mg/dL)	100-600	600 (370-1100)	650 (370-930)	.63
WBC ( $\times 10^3/\mu\text{L}$ )	4-12	10.5 (6.8-12.5)	9.6 (6-17.5)	.78
Segs ( $\times 10^3/\mu\text{L}$ )	0.6-4	3.7 (1.1-7.9)	3.9 (1.4-10.5)	.09
Bands ( $\times 10^3/\mu\text{L}$ )	0-0.1	0 (0-0.7)	0 (0-0.28)	.06
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	2.5-7.5	4.4 (2.4-7.7)	4.6 (2-10.3)	.64
Monocytes ( $\times 10^3/\mu\text{L}$ )	0-0.9	0.5 (0.1-1.4)	0.66 (0.1-1.6)	.30
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0-2.4	0 (0-0.4)	0.1 (0-0.33)	.47
Basophils ( $\times 10^3/\mu\text{L}$ )	0-0.2	0 (0-0.1)	0.1 (0-0.2)	.01
Creatinine (mg/dL)	1-1.8	0.7 (0.5-1)	0.7 (0.4-1)	.62
Total Protein (g/dL)	6.4-9.5	6.9 (5.9-7.8)	6.5 (5.6-7.1)	.01
Albumin (g/dL)	2.5-4.5	2.8 (2.3-3.6)	3.3 (2.7-3.8)	.08
Globulin (g/dL)	2.6-6.5	4 (2.9-5.2)	3 (2.4-4.3)	.01
A/G ratio	N/A	0.7 (0.4-1.2)	1.1 (0.6-1.5)	.02
Glucose (mg/dL)	55-95	59 (23-66)	61 (31-82)	.43
Sodium (mEq/L)	136-147	141 (137-146)	143 (137-147)	.06
Potassium (mEq/L)	4-5	3.8 (3.4-4.4)	3.9 (3.4-4.2)	.88
Chloride (mEq/L)	95-105	101 (96-104)	102 (94-104)	.30
Bicarbonate (mEq/L)	20-30	27 (22-31)	29 (26-30)	.25
Anion gap (mEq/L)	13-20	19 (12-22)	18 (14-20)	.35
Total calcium (mg/dL)	7.6-10.2	8.9 (7.9-10.1)	8.5 (7.0-9.6)	.09



**Table 5.** Result of multivariate logistic regression analysis of the variables associated with bacteremia in postpartum dairy cows.

Variable	Coefficient	SE	P-value	OR	(95% CI)
Intercept	-4.790	0.074	.001	N/A	N/A
Basophils	-2.581	1.142	.023	0.076	0.008–0.711
Globulin	1.563	0.658	.018	4.771	1.314–17.33

OR, odds ratio; CI, confidence interval; SE, standard error.

subclinical hypocalcemia was 91.3%.<sup>13</sup> Thus, our findings are in line with previously reported data.

Farm played a role in the prevalence of bacteremia in this study. Cattle from 2 of the 3 farms enrolled in the study were significantly more likely to be bacteremic than cattle from the other farm. The reasons for this are not clear. The distance from these farms to the diagnostic lab were similar to the other. In addition, similar techniques were used to collect the blood cultures on each farm. One factor might be that cattle on the 2 farms from which bacteremic cattle were more frequently identified often diagnosed cattle with APM at a later time during the 10-day monitoring period.

The significance of identifying bacteremia in over half of all cattle sampled in this study is unknown. However, studies in humans and mice have shown that bacterial translocation from various organ systems to the mesenteric lymph nodes and mammary gland occurs during late pregnancy and early lactation.<sup>14,15</sup> It is believed that these bacteria might serve as a means to program the neonatal immune system to bacterial molecular patterns and ensure appropriate responses to pathogens and commensal organisms.<sup>14,15</sup> Clearly, more work needs to be done in this area before definitive conclusions can be reached.

Numerous studies have shown that periparturient dairy cattle experience varying degrees of immunocompromise.<sup>16–18</sup> Cells of the innate and adaptive immune systems, particularly members of the neutrophil, monocyte/macrophages, and circulating lymphocytes, have long been thought to be responsible for clearance of bacteria from the bloodstream. For example, Reggiardo and Kaeberle identified bacteremia in 85% of cattle experimentally infected with bovine viral diarrhea virus.<sup>19</sup> Here, bacteremia was closely associated with the number of circulating leukocytes, particularly the total lymphocyte population. In humans, neutropenia has been found to be a significant risk factor for bacteremia associated with Gram negative bacilli.<sup>20</sup> In this study, patients with neutropenia (<500 neutrophils/ $\mu$ L) were 8.1 times more likely to be bacteremic than patients with >500 neutrophils/ $\mu$ L.<sup>20</sup> Work from cattle with coliform mastitis would suggest that neutropenia is a contributor to bacteremia in that population of animals.<sup>21</sup> In this study, cattle with basophils present in the circulation were approximately 13 times less likely to be bacteremic than cattle without circulating basophils. Traditionally, basophils have been seen as contributors to allergic reactions and anti parasitic defense.<sup>8</sup> However, recent evidence suggests that

basophils might play a role in enhancement of immunologic memory responses by enhancing B-cell proliferation and immunoglobulin production.<sup>22</sup> In addition, mice depleted of basophils and experimentally infected with *Streptococcus pneumoniae* were more likely to die than mice that were basophil replete.<sup>22</sup> It is clear, therefore, that cellular immune responses are important for defense against blood borne bacterial infection.

*Bacillus* spp. was the organism most frequently isolated from the bloodstream of cattle in both groups in this study. Traditionally, *Bacillus* spp. has been thought to have a ubiquitous distribution in the environment and is often viewed as a contaminant of blood cultures from both humans and animals.<sup>23–26</sup> Nevertheless, *Bacillus* spp. was identified as the organism most frequently isolated from dairy cattle with coliform mastitis, even though it was not routinely isolated from milk of affected animals.<sup>5</sup> In fact, *Bacillus* spp. could be isolated from the bloodstream with approximately 8 times greater frequency in cattle with coliform mastitis than in controls. However, unlike cattle in which *E. coli*, *Salmonella* spp., or *Klebsiella pneumoniae* could be isolated, animals with *Bacillus* spp. bacteremia were not at increased risk of mortality.<sup>5</sup> In addition, the frequency with which *Bacillus* spp. could be isolated was similar across disease severity groups. Members of the *Bacillus* genus, particularly *B. licheniformis*, can frequently be isolated from the uterine lumen of cattle with and without uterine disease.<sup>27</sup> In fact, Williams et al. showed that cattle from which *B. licheniformis* could be isolated had greater acute phase protein responses than cattle from which this organism was not cultured.<sup>27</sup> Thus, *Bacillus* spp., under the right circumstances, can be a pathogen and stimulate significant inflammatory responses.

In addition to originating from the uterine lumen, the bacteria isolated from the bloodstream in the cattle in this study might have originated from the gastrointestinal tract. Liver abscesses have been identified in 23.4% of Holstein cattle at slaughter.<sup>28</sup> It is well accepted that the bacteria found in liver abscesses originate from the rumen and gain access to the circulation via a compromised rumen mucosa.<sup>29,30</sup> Thus, it is possible that the bacteria identified in the bloodstream of certain cattle in this study, particularly high-producing cattle, originated from the rumen and these animals were bacteremic as a result of subclinical rumen acidosis secondary to feeding a high concentrate ration in the early postpartum period.

Studies from sheep and in vitro studies of bovine pulmonary endothelial cells have shown that endotoxin and inflammatory mediators can cause cellular damage severe enough to result in increased permeability and hydraulic conductance.<sup>31,32</sup> In addition, studies in mice have shown displacement of proteins associated with intercellular tight junctions during experimentally induced sepsis.<sup>33</sup> This altered expression and a disrupted mucosal barrier, as measured by radioactively labeled biotin permeability, accompanies disruption of tight junction proteins.<sup>33</sup> Furthermore, studies have

also shown that cattle with APM have increased levels of LPS in the circulation when compared to health controls.<sup>34</sup> Therefore, bacteremia with *Bacillus* spp. might reflect translocation of the bacteria from the uterine lumen or distant sites through compromised cellular barriers resulting from systemic inflammation or, as previously mentioned, a disrupted uterine epithelial barrier.

The results of this study demonstrate that bacteremia occurs in a large proportion of postparturient dairy cattle, both healthy and with APM. While the cause of the high risk of bacteremia is unclear, bacterial colonization of the involuting uterus, periparturient immunosuppression, and systemic inflammation can all play a role. In addition, novel data from humans and mice would suggest that bacteremia in the mother might serve to inoculate the neonatal gastrointestinal tract with bacteria. On the basis of the results of this study, bacteremia should be considered a common occurrence in postparturient dairy cow. Future studies with larger numbers of animals should be performed to further identify other differences between healthy and diseased cattle.

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## Footnotes

<sup>a</sup> GLA M750 Series Thermometer; GLA Agricultural Electronics, San Luis Obispo, GA

<sup>b</sup> Double guarded culture swab; Jorgensen Laboratories, Loveland, CO

<sup>c</sup> BD Laboratories, Franklin Lakes, NJ

<sup>d</sup> Stata, Version 12.1; StataCorp, LP, College Station, TX

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*Conflict of Interest Declaration:* Authors disclose no conflict of interest.

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