ABSTRACT

Gastrointestinal disease is the most common cause of mortality in dairy calves. Septicemia is an important sequela of diarrhea, and the possibility of bacteremia is the primary justification for empirical antimicrobial therapy. Prior reports estimate that approximately one-third of diarrheic calves are bacteremic; however, those estimates may not be representative of routine cases in heifer calves on commercial dairy operations early in the course of disease. We hypothesized that the prevalence of bacteremia in calves with diarrhea and systemic signs of illness is less than prior estimates (~31%), and that clinical signs or hematological values would be associated with the presence or absence of bacteremia. Female calves less than 21 d of age with and without diarrhea were enrolled from 2 commercial dairy farms over a 10-wk period. Diarrheic calves were enrolled if they were newly diagnosed, had loose to watery stool, had either dehydration (assessed by skin tent and eye position) or depression (assessed by suckle reflex and standing ability), and had no prior antimicrobial treatments. Complete health assessments were conducted at 0, 7, and 14 d following enrollment. An aseptic jugular venous sample was collected and cultured using aerobic and anaerobic methods, and bacterial species were identified using mass spectrometry. Poisson regression models were used to identify associations with bacteremia and compute adjusted prevalence ratios. The prevalence of bacteremia in diarrheic and healthy calves was 9.26% (10/108, 95% confidence interval: 4.5–16%) and 14.8% (4/27, 95% confidence interval: 1.4–28.2%), respectively. Among calves with diarrhea, those with a fever (>39.7°C) or depression were 4.8 and 6.5 times more likely, respectively, to have bacteremia. Only 1 of 47 calves (2%) without signs of depression was bacteremic. The prevalence of bacteremia in diarrheic calves with signs of systemic illness (depression or dehydration) was significantly lower than previous estimates, and bacteremia was rare among calves without observed depression. Antimicrobial therapy targeting bacteremia is not currently justified in routine cases of diarrhea in preweaning calves without signs of depression. These results suggest a substantial opportunity for more targeted antimicrobial therapy to improve antimicrobial stewardship.

Key words: heifer calf, diarrhea, bacteremia prevalence, antimicrobial stewardship

INTRODUCTION

Calfhood gastrointestinal disease is the most common cause of mortality in preweaning dairy calves (USDA, 2018). Diarrhea causes dehydration and depression, and though there is minimal peer-reviewed evidence, it is a common belief that disease progression can lead to septicemia and death if not treated appropriately. Mortality and production losses caused by diarrhea result in a large economic burden in the dairy industry (Constable et al., 2004). Additionally, calf diarrhea and the perceived risk of septicemia are the most common reasons for the use of antimicrobials (Constable et al., 2004). The overuse of antimicrobials should be avoided to mitigate the development and spread of antimicrobial resistance and assuage societal concerns about the use of antimicrobials in animal agriculture. Judicious antimicrobial use requires evidence-based treatment protocols to support routine treatment decisions by farm personnel responsible for initiating antimicrobial therapy.

Judicious use of medically important antimicrobials, particularly critically important antimicrobials (e.g., ceftiofur), is imperative to public health (WHO AGISAR, 2018). For instance, third generation cephalosporins are routinely used to combat foodborne infections in humans, especially children (WHO AGISAR, 2018). Similarly, calf producers routinely use ceftiofur in cases of neonatal calf diarrhea when septicemia is
suspected (Constable et al., 2004). The use of ceftiofur increases the risk of carrying resistant *Escherichia coli* isolates in calves (Pereira et al., 2014), and may contribute to the foodborne transmission of resistant bacteria or the genetic mediators of resistance. Therefore, antimicrobial use should be targeted toward cases where use is necessary to preserve animal health and welfare.

In general, producers are responsible for making the routine, on-farm decision to initiate therapy. The criteria used to initiate antimicrobial therapy in diarrheic calves vary widely, but this decision made by the producer is often based on treatment recommendation protocols from the herd veterinarian based on a pre-existing veterinarian-client-patient relationship. For instance, up to 40% of producers reported that they routinely administer antimicrobials for mild to moderate diarrhea cases with no systemic signs of illness (Habing et al., 2016). The appropriateness of antimicrobial therapy for diarrheal illnesses of varying levels of severity is not clear. The American Association of Bovine Practitioners (AABP, 2017), the American College of Veterinary Internal Medicine (Weese et al., 2015), and the American Veterinary Medical Association (Smith et al., 2019) all have antimicrobial stewardship recommendations published, but more specific consensus recommendations for when antimicrobials are indicated as well as timing of the initiation and duration of antimicrobial therapy have not been developed.

Bouts of transient bacteremia (i.e., bacteria present in the blood stream) can occur in young animals with incomplete development of their gastrointestinal luminal barrier (Wood et al., 2015). However, in combination with diarrhea and systemic clinical signs, likely caused by a compromised immune system due to failure of passive transfer and inadequate immunoglobulin levels (Besser and Gay, 1994; Tyler et al., 1999; Pardon et al., 2015), bacteremia can lead to a systemic inflammatory response (i.e., septicemia). Septicemia, in contrast to bacteremia, is a systemic, multiorgan, inflammatory response to blood-borne infection and comorbidities such as diarrhea (Christaki and Giamarellos-Bourboulis, 2014). In cases of diarrhea, the perceived likelihood of bacteremia when interpreted in the presence of systemic clinical signs has been advocated as sufficient justification for antimicrobial therapy (Constable, 2004). Therefore, though prevalence and clinical outcomes of bacteremia are not completely understood, prevalence estimates of bacteremia from calves diagnosed early in the course of routine cases of diarrhea are necessary to support decisions in this common context. Similarly, efforts to change antimicrobial-use behaviors and reduce unnecessary usage are dependent on clear criteria that distinguish unnecessary from justifiable treatments.

Expert recommendations for antimicrobial administration in calves with diarrhea often point to 2 studies that estimate the prevalence of bacteremia and septicemia in calves. Fecteau et al. (1997b) found that 31% of primarily male calves on a single farm were bacteremic. Similarly, Lofstedt et al. (1999) found 31% of male and female calves presenting to a veterinary teaching hospital were septicemic. Male calf rearing farms often have high rates of failure of passive transfer of immunity (Wilson et al., 2000), and veterinary teaching hospitals typically see a referred population of calves with severe or advanced stages of disease. Consequently, these study populations may not appropriately represent the context for antimicrobial decision-making on commercial dairy operations, if at all. Currently, there is no consensus to guide producers on the appropriate point for initiating antimicrobial therapy for diarrheic calves. Previous studies have created scoring systems for clinicians to assist them in predicting bacteremia in calves (Fecteau et al., 1997a; Lofstedt et al., 1999), foals (Brewer and Koterba, 1988), and humans (Töllner, 1982). Commercial dairy-farm workers make routine decisions on the initiation of antimicrobial therapy based on visible signs of illness; therefore, there is a need to define the set of clinical signs most associated with bacteremia.

The objectives of this study were to determine the prevalence of bacteremia in diarrheic dairy calves with signs of systemic illness (dehydration or depression), and to identify clinical signs and hematologic values associated with bacteremia. We hypothesized that the prevalence of bacteremia in commercial dairy calves with diarrhea on the first day of diagnosis is significantly less than prior estimates. Additionally, we hypothesized that clinical signs and hematologic values would be associated with the presence or absence of bacteremia in diarrheic dairy calves.

**MATERIALS AND METHODS**

**Study Design and Location**

This prospective cohort study was conducted on 2 commercial dairy farms in central Ohio, with approval by The Ohio State University Institutional Animal Care and Use Committee (Animal Use Protocol: 2015A0000131). The 2 farms were selected because they were commercial dairy farms raising replacement heifers and were within 70 miles of The Ohio State University. Farms were visited 3 to 5 times per week for 10 wk between May and August, 2018. Personnel walked through the calf pens and visually assessed the health of all calves ≤21 d of age to identify calves that met enrollment criteria. Each farm used antimicrobials as
treatment for disease in calves and not for prophylactic or metaphylactic use.

The sample size calculation was based on the ability to demonstrate that the prevalence of bacteremia was significantly less than prior estimates (~0.30). Assuming that the true prevalence is 0.15, enrolling 50 calves would provide sufficient power (β = 0.20) for the one-sided hypothesis test. Additional calves beyond the required sample size were enrolled, as allowed by time and budget, to maximize the ability to identify factors associated with bacteremia.

**Animals, Housing, and Feeding**

Calves located on farm 1, a 2,000-head milking herd, were separated from dams at birth. After separation from the dams, calves were housed completely indoors with mechanical tube ventilation in individual pens made with wire fencing on straw bedding for the duration of the study. Calves were fed 3 L of colostrum as soon as possible after birth with a second feeding of 3 L within the first 12 h of birth. After the first 24 h, calves were fed 7.6 L of milk replacer per day. Cow vaccinations included Scourguard (Zoetis Inc.) 3 wk before expected calving. Preweaning calf vaccinations included Inforce 3 (Zoetis Inc.) at 2 d of age, Once PMH (Merck) at 2 d of age, and One Shot (Zoetis Inc.) at 3 to 4 wk of age. Calves with diarrhea were not treated with antibiotics; these calves were treated with kaolin pectin (Kao-Pec Agri Laboratories, Ltd.). Treatment was based on fecal consistency only.

Farm 2, a 1,164-head milking herd, separated calves at birth. After separation from the cow, calves were housed in individual hutches outside with natural ventilation and straw bedding for the duration of the study. Calves were fed 4 L of colostrum as soon as possible after birth and fed a second feeding of 2 L within 12 h. After the first 24 h, they were fed 4 L of UV-pasteurized whole milk per day. Cow vaccinations included Enviracor J-5 and Salmonella Newport Bacterial Extract SRP (Zoetis Inc.) during the dry period. Preweaning calf vaccinations included a multivalent vaccine against clostridial diseases (manufacturer unknown) administered between 2 and 9 d of age, and Bovi-Shield Gold (Zoetis Inc.) administered at 5 wk of age. Calves with diarrhea were treated with sulfamethazine (Sustain III, Bimeda), and calves with diarrhea and depression were treated with sulfamethazine and ceftiofur (Excenel, Zoetis Inc.).

**Calf Enrollment Criteria**

Calves were enrolled into either the diarrheic or nondiarrheic group. Calves eligible for enrollment in the diarrheic group were between 1 and 21 d of age with a newly (less than 24 h) observed case of diarrhea (fecal score of 2 or 3), presence of dehydration (score of 1, 2, or 3) or depression (score of 1 or 2), and no prior treatment with antimicrobials. Calves with a depression score of 3 (unable to rise) were not enrolled so that treatment could be initiated immediately. For calves in the diarrheic group, health assessments were additionally performed at 7 and 14 d postenrollment. Calves were enrolled in the nondiarrheic group if they were between 1 and 21 d of age with a fecal score of 0 or 1 and no visible clinical signs of illness. We enrolled 1 or 2 calves in the nondiarrheic group from each farm during each week of the study. Longitudinal data were not collected for the nondiarrheic group.

**Health Assessment Scoring**

Research personnel responsible for health assessments received 1 wk of training by a large animal clinical veterinarian at The Ohio State University, College of Veterinary Medicine. As part of training, individuals responsible for data collection independently conducted health assessments on several calves and compared scores for each component of the assessment until 100% interobserver agreement was reached. The health assessments included scoring criteria for fecal consistency, dehydration, depression, respiratory disease signs, joint inflammation, navel inflammation, temperature, heart rate, and respiratory rate (Table 1; Pempek et al., 2019). Pyrexia was defined as rectal temperature over 103.5°F (39.7°C). Heart and respiratory rates were recorded in beats per minute and breaths per minute, respectively. Postenrollment health assessments included the same criteria excluding heart and respiratory rate.

**Sample Collection and Analysis**

After health assessments were completed, a venous blood sample was aseptically collected from the jugular vein. Each calf was restrained, and the hair in the midcervical region over the jugular vein was clipped. Researchers put on a new pair of nonsterile nitrile gloves immediately before blood sampling and scrubbed the area with povidone iodine medical scrub (titratable 0.75% iodine; Covetrus) beginning in the middle of the shaved area and progressively working outward in a circular motion. This procedure was repeated 3 times (Caldeira et al., 2011). After a minimum of 3 min of contact time, the scrub was removed by gauze soaked in 70% isopropyl alcohol. The vein was occluded below the aseptic area, and 20 mL of blood was collected (18-gauge, 2.5-cm needle) from each calf into a 20-mL
Joint inflammation
No inflammation present
Slight swelling; not warm or painful
Swelling with pain or heat; slight lameness
Swelling with severe pain, heat and lameness

Navel inflammation
Normal (pencil size); no heat, swelling, or discharge
Bigger than normal (width of the pointer finger); no heat, swelling, or discharge
Bigger than normal (width of 2 fingers combined); slight pain or moisture
Bigger than normal (width of 3 fingers combined); heat, pain, or malodorous discharge

The scoring system was adapted from previously validated studies (Fecteau et al., 1997a; Pempek, 2019). Temperature, heart rate, and respiratory rate were recorded as measured quantitative values.

Laboratory Methods

To determine serum total protein, clotted whole blood was centrifuged at a relative centrifugal force of 2,305 × g for 10 min at 20°C (room temperature). A hand-held refractometer was used to determine the serum total protein, measured in grams per deciliter. Complete blood counts were performed on anticoagulated whole blood at the Ohio State Veterinary Teaching Hospital Diagnostic Hematology Laboratory. Erythrograms, platelet counts, and total leukocyte counts were recorded by an automated machine (Advia 2120i Hematology System), and leukocyte differential counts were recorded manually by clinical pathology laboratory technicians.

Blood-culture bottles were incubated at 35°C for 1 h. After 1 h, the growth indicator chambers were inserted into the blood-culture bottles, which were then returned to the incubator for up to 7 d of incubation and observation. A positive bottle (i.e., bacterial growth) was identified if culture broth rose from the bottle into the indicator top from an increase in pressure from bacterial growth or if evident turbidity was observed. Medium culture from positive bottles was aseptically plated onto growth medium immediately following observation and incubated under anaerobic, aerobic, and microaerophilic conditions.
tericin plates (Becton, Dickinson and Co.) were placed into pouches with a microaerophilic gas-generating system (Pouch-MicroAero, Mitsubishi Gas Chemical Co. Inc.) and incubated at 42°C. Mycoplasma broth (Hardy Diagnostic) and thioglycollate broth (Becton, Dickinson and Co.) were aerobically incubated in 35°C CO₂ conditions. We used MALDI-TOF MS (Bruker Daltonics Inc.) to identify the bacteria genus and species isolated from bacteriological culture.

**Statistical Analysis**

The prevalence of bacteremia among calves was calculated as the number of positive cases divided by the total number enrolled. Skin-associated bacteria, including coagulase-negative *Staphylococcus* species and *Bacillus* species, are commonly identified as contaminants of blood draws (Nagase et al., 2002; Gopal et al., 2015). Samples with only the recovery of these putative contaminants were categorized as negative. Exact confidence intervals were calculated for each prevalence calculation using the EXACT statement within the FREQ procedure in SAS (version 9.4; SAS Institute). All analyses were performed using SAS software (version 9.4).

Clinical health assessment scores were dichotomized for analysis. Health assessment scores were considered clinically “normal” if dehydration score = 0; fecal score = 0 or 1, depression score = 0, temperature <39.7°C, navel score = 0, joint score = 0, eye or nose score = 0, ear score = 0, and cough score = 0. Calves were considered to have “abnormal” health outcomes if dehydration score = 1, 2, or 3; fecal score = 2 or 3; depression score = 1, 2, or 3; temperature ≥39.7°C; navel score = 1, 2, or 3; joint score = 1, 2, or 3; eye or nose score = 1, 2, or 3; ear score = 1, 2, or 3; or cough score = 1, 2, or 3. Levels of total serum protein (TSP) reflect a combination of maternal antibodies, hemoconcentration, and systemic inflammatory response; therefore, the typical cut-off for failure of passive transfer was not used, and the value was instead dichotomized using the median value (6.2 g/dL). Total white blood cell count was dichotomized at 5 × 10³ cells/µL. The neutrophil-to-lymphocyte ratio was dichotomized at 0.6 (Wood and Quiroz-Rocha, 2010), and the number of bands were dichotomized as present or absent.

Univariable Poisson regression models (PROC GENMOD, SAS v. 9.4) with robust standard errors were constructed to identify variables that were associated with the bacteremia while controlling for farm as a fixed effect (Zou, 2004). This model framework was used to compute the adjusted prevalence ratios, which are more interpretable in this context relative to odds ratios. (Knol et al., 2012). Significance was set at *P* < 0.05, and *P* < 0.10 was considered a trend. We additionally used Poisson regression models to determine the association between bacteremia and death within 21 d of enrollment, and between bacteremia at d 0 and the subsequent diagnosis of depression at d 7 or 14 after enrollment. A multivariable Poisson regression model (PROC GENMOD, SAS, v. 9.4) was constructed, where 3 separate models used the d-7 depression score, d-14 depression score, or the 21-d mortality as dependent variables. The blood culture result was evaluated within the model while controlling for farm as a fixed effect.

**RESULTS**

**Prevalence of Bacteremia**

Compared with the 50 calves calculated for a sample size necessary to provide sufficient power for the 1-sided hypothesis test, 108 calves were enrolled (Table 2). The prevalence of bacteremia was 9.26% (10/108 calves; 95% CI: 4.58–16.4%) and 14.8% (4/27 calves; 95% CI: 1.4–28.2%) in diarrheic and healthy calves, respectively. The difference between the 2 groups was not significant (*P* = 0.37). The prevalence of bacteremia in diarrheic calves was not different by farm (*P* = 0.38); 5.6% (2/36) and 11.1% (8/72) of calves from farms 1 and 2, respectively, were positive. Of 10 diarrheic calves that were blood-culture positive, bacterial species cultured included *Salmonella* species (3), *E. coli* (2), *Enterobacter aerogenes* (1), *Campylobacter fetus* (1), *Klebsiella aerogenes* (1), *Streptococcus dysgalactiae* (1), and *Trueperella pyogenes* (1). From the 4 nondiarrheic calves that were blood-culture positive, bacterial species cultured included *Bacteroides pyogenes* (2), *C. fetus* (1), and *Strep. dysgalactiae* (1). Seven calves were blood-culture positive but were categorized as nonbacteremic because the bacteria cultured, coagulase-negative *Staphylococcus* and *Bacillus* species, have been previously reported as common integument contaminants. This approach is consistent with other literature addressing the prevalence of bacteremia (Nagase et al., 2002; Gopal et al., 2015).

**Univariable Predictors of Bacteremia in Diarrheic Calves**

In general, the univariable associations among diarrheic calves reflected clinical or hematological changes expected for calves with more severe enteric disease. Controlling for the effect of farm within the Poisson models, calves with signs of depression were found...
to have 6.46 times the prevalence of bacteremia ($P = 0.08$). Only 1 of 47 diarrheic calves with no signs of depression had a positive blood culture (Table 3). Twenty-three percent of calves with a fever (>39.7°C) had positive blood-culture results, compared with only 6.6% of calves without a fever (prevalence ratio = 4.72, $P = 0.02$). Additionally, samples from calves <12 d of age were 3.79 times more likely (prevalence ratio = 3.79, $P = 0.04$) to be bacteremic than those from calves ≥12 d; 15.6% (7/45) of calves <12 d of age were bacteremic compared with 4.8% (3/63) of calves that were ≥12 d (Table 3; Figure 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Bacteremic, % (no./total)</th>
<th>Model coefficient (SE)</th>
<th>Adjusted prevalence ratio (95% CI)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>10.7 (8/75)</td>
<td>0.54 (0.79)</td>
<td>1.71 (0.36–8.06)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.5 (2/31)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Dehydration score</td>
<td>0</td>
<td>3 (1/33)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1, 2, 3</td>
<td>12 (9/75)</td>
<td>1.26 (1.08)</td>
<td>3.52 (0.43–28.9)</td>
<td>0.24</td>
</tr>
<tr>
<td>Depression score</td>
<td>0</td>
<td>2.1 (1/47)</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1, 2, 3</td>
<td>14.8 (9/61)</td>
<td>1.87 (1.06)</td>
<td>6.46 (0.80–51.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Nasal discharge</td>
<td>0</td>
<td>8.4 (8/95)</td>
<td>0.86 (0.82)</td>
<td>ref</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1, 2, 3</td>
<td>15.4 (2/13)</td>
<td></td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Eye/Ear</td>
<td>0</td>
<td>7.7 (1/13)</td>
<td>0.07 (1.10)</td>
<td>1.07 (0.13–9.16)</td>
<td>0.95</td>
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<tr>
<td></td>
<td>1, 2, 3</td>
<td>10.1 (10/99)</td>
<td>NE†</td>
<td>NE†</td>
<td>0.97</td>
</tr>
<tr>
<td>Cough</td>
<td>0</td>
<td>0 (0/9)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Joint inflammation</td>
<td>0</td>
<td>9.6 (10/104)</td>
<td>NE</td>
<td>NE†</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>1, 2, 3</td>
<td>0 (0/4)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>&lt;39.7°C</td>
<td>6.6 (6/91)</td>
<td></td>
<td>Referent</td>
<td>0.02</td>
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<tr>
<td></td>
<td>≥39.7°C</td>
<td>23.5 (4/17)</td>
<td>1.56 (0.67)</td>
<td>4.71 (1.2–18.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total serum protein</td>
<td>≥6.2 g/dL</td>
<td>5.5 (3/55)</td>
<td></td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;6.2 g/dL</td>
<td>12.2 (6/49)</td>
<td>0.75 (0.71)</td>
<td>2.12 (0.52–8.57)</td>
<td>0.29</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;12 d</td>
<td>15.6 (7/45)</td>
<td>1.33 (0.70)</td>
<td>3.79 (1.0 – 14.9)</td>
<td>0.04</td>
</tr>
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<td></td>
<td>≥12 d</td>
<td>4.8 (3/63)</td>
<td></td>
<td>ref</td>
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<tr>
<td>White blood cells</td>
<td>≥5 (×10⁶ cells/μL)</td>
<td>9.8 (10/102)</td>
<td>NE</td>
<td>NE†</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>&lt;5 (×10⁶ cells/μL)</td>
<td>0 (0/1)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Segmented neutrophil:lymphocyte</td>
<td>≥0.6</td>
<td>10.1 (9/89)</td>
<td>0.34 (1.06)</td>
<td>1.41 (0.17–11.2)</td>
<td>0.73</td>
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<tr>
<td></td>
<td>&lt;0.6</td>
<td>7.7 (1/13)</td>
<td></td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Bands</td>
<td>≥0 (×10³ cells/μL)</td>
<td>20.0 (1/5)</td>
<td>0.87 (1.05)</td>
<td>2.39 (0.30–18.9)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>&lt;0 (×10³ cells/μL)</td>
<td>9.2 (9/98)</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
</tbody>
</table>

$†$Not estimated.
Clinical signs and hematologic parameters were tested for collinearity before modeling (PROC CORR, SAS v. 9.4), and correlations between all variables were small (r < 0.30). We found 33% (3/9) of bacteremic calves had TSP >6.2 g/dL, while 66% (6/9) had TSP <6.2 g/dL. Calves with an increased segmented neutrophil count ($P = 0.08$) tended to have a positive blood culture; however, the hematologic parameters selected for analysis (Tables 2 and 3), were not significantly different between bacteremic and nonbacteremic calves.

**Long-Term Health Outcomes of Calves with Bacteremia**

The analysis focused on the incidence risk of depression and mortality in calves initially categorized as bacteremic or nonbacteremic. Calves that had bacteremia were numerically but not significantly more likely to have depression on the follow-up assessments. Seventy percent (7/10) of calves that were bacteremic had depression at the 7-d follow-up assessment, compared with 37.8% (37/98) of nonbacteremic calves [$P = 0.21$; model coefficient (SE) = 0.51 (0.41)]. Likewise, 40% (4/10) and 18.4% (18/98) of the calves with and without bacteremia, respectively, had depression at the 14-d follow-up visit [$P = 0.25$; model coefficient (SE) = 0.63 (0.55)]. Among the calves enrolled in the study, 0% (0/10) of the bacteremic and 4.08% (4/98) of the nonbacteremic calves died during the 21-d follow-up period.

**DISCUSSION**

This study is the first to estimate the prevalence of bacteremia in replacement dairy heifer calves early in the course of diarrhea, when antimicrobial-use decisions are typically made. The estimated prevalence is 9.26% (10/108), substantially lower than previously published studies. All diarrheic calves in this study had systemic signs, yet a smaller proportion had bacteremia. These results suggested that antimicrobial therapy targeting bacteremia may not be justified in most cases. Furthermore, the prevalence of bacteremia in nondiarrheic calves was 14.8% (4/27), and not significantly different than calves with diarrhea. This study was not designed to provide a precise estimate in nondiarrheic calves; nonetheless, the result suggests the recovery of bacteria may not be associated with diarrheal illness. Additional research to characterize the incidence and health consequences in calves is necessary.

![Figure 1](image-url). Age frequency distribution of calves with diarrhea that were bacteremia positive (black) and negative (gray) based on blood-culture results. 15.6% (7/45) of calves <12 d of age were bacteremic, compared with 4.8% (3/63) of that were >12 d; calves <12 d of age were 3.79 times more likely (prevalence ratio = 3.79, $P = 0.04$) to be bacteremic than those >12 d.
Still, among calves with diarrhea, bacteremia was significantly associated with more severe clinical signs. Depression tended to be associated with bacteremia, and fever (>39.7°C) and young age (<12 d) were significantly associated with bacteremia. Routine collection of these data will be useful to guide antimicrobial therapy. For instance, calves with diarrhea and without depression seemed to be unlikely to be bacteremic. Only 1 of 47 diarrheic calves without signs of depression (score of 0) had a positive blood-culture result.

Calves with bacteremia had a numerically, but not significantly higher risk of depression in subsequent health assessments, suggesting an important consequence of bacteremia. A study with the same prevalence of bacteremia in depressed (0.02) and nondepressed (0.15) calves would require 141 enrolled calves for sufficient power (0.80) to find this association. It is possible that bacteremia is simply an indicator of more severe initial disease and gut permeability, rather than a direct cause of the subsequent depression diagnosis. The relatively low number of positive blood cultures (n = 10) among diarrheic calves and the exclusion of severely depressed calves from enrollment makes identification of the health outcomes difficult. Likely, the consequences are different between bacteremic and nonbacteremic calves. This is in contrast to the expected hemoconcentration associated with bacteremia. A study with the same prevalence of bacteremia in depressed (0.02) and nondepressed (0.15) calves would require 141 enrolled calves for sufficient power (0.80) to find this association. It is possible that bacteremia is simply an indicator of more severe initial disease and gut permeability, rather than a direct cause of the subsequent depression diagnosis. The relatively low number of positive blood cultures (n = 10) among diarrheic calves and the exclusion of severely depressed calves from enrollment makes identification of the health outcomes difficult. Likely, the consequences are different between bacteremic and nonbacteremic calves.

In other cases, however, recovery of bacteria may represent short-lived and low-consequence episodes associated with increased gut permeability. Larger studies are required to identify medium- or long-term consequences of bacteremia in routine cases of enteric disease.

None of the blood values were significantly associated with bacteremia. This study suggests that the parameters under evaluation were much less useful than clinical signs for predicting bacteremia. Nonetheless, increased segmented neutrophil counts tended to be higher in bacteremic calves. This is in contrast to the expected response for septicemia, where the peripheral migration of neutrophils results in a decreased neutrophil count (Tennant et al., 1975). The TSP levels were not different between bacteremic and nonbacteremic calves. A lower TSP among may indicate failure of transfer of passive immunity, which often leads to increased morbidity and mortality in neonatal calves (Peek et al., 2018). A lower TSP may also reflect the consumption of acute phase proteins as a component of the inflammatory response or a protein losing enteropathy suggestive ongoing gastrointestinal disease (Ettinger et al., 2017). It is possible that magnitude of the association between TSP and bacteremia is lower than anticipated because of the expected hemoconcentration associated with fluid loss falsely elevating the TSP due to dehydration caused by diarrhea (Ettinger et al., 2017); however, this study does not provide evidence of an association between TSP and bacteremia.

Predictive models have previously been published to help clinicians predict when diarrheic calves are septicemic (Fecteau et al., 1997a; Lofstedt et al., 1999). Lofstedt et al. (1999) created 2 models, a laboratory model and a clinical model, whereas Fecteau et al. (1997a) created 1 clinical model. The models constructed by Lofsted (1999) and Fecteau et al. (1997a) included clinical and laboratory variables such as serum creatinine, toxic changes in neutrophils, failure of passive transfer, poor suckle reflex, appearance of scleral vessels, and presence of a focal infection. To be widely adopted by producers, models should be rapid and affordable (Garcia et al., 2020), as animal caretakers generally rely on easily observable signs, such as depression, attitude, and dehydration to make routine antimicrobial-use decisions (Olson et al., 2019). If a decision tool were applied to calves with diarrhea and systemic signs, the use of a model could result in more targeted therapy, depending on current farm treatment practices. Temperature, depression, and age, all associated with bacteremia in this study, are easily attainable information and are often routinely assessed by farm personnel.

Our study identified Salmonella spp. followed by E. coli and Strep. dysgalactiae to be the most frequent bacteria recovered from calves with diarrhea. This aligns with previous studies that recognized Salmonella spp., Streptococcus spp., and E. coli isolates from blood in calves with diarrhea and diagnosed with septicemia (Fecteau et al., 2009). In this study, there were 7 calves that were blood-culture positive that were categorized as nonbacteremic. The bacteria cultured from these calves, including coagulase-negative Staphylococcus (Nagase et al., 2002; Bierowiec et al., 2019) and Bacillus species (Gopal et al., 2015), have been identified in previous animal and human medicine studies as common integumentary contaminants. Excluding these 7 calves in our analysis avoided misclassification of bacteremia in diarrheic calves, which may have obscured meaningful associations with clinical signs and biased the prevalence estimate of bacteremia.

The prevalence of bacteremia in diarrheic and healthy calves was not statistically different. The study was not designed to provide a precise estimate of the prevalence of bacteremia in nondiarrheic calves; nonetheless, the unexpectedly high prevalence in nondiarrheic calves (14.8%) warrants additional investigation, and this finding suggested the presence of bacteremia in diarrheic calves may not be as clinically important as previously thought. Blood cultures were positive from 4 calves (2 from each farm) from the nondiarrheic group with normal health assessments. Possible explanations include contamination of the field samples.
despite aseptic technique, intermittent bouts of bacteremia, and undiagnosed disease. Despite strict aseptic technique, contamination of blood-culture specimens is common and has been shown to occur in up to 12% of samples in human hospitals (Hughes et al., 2018). Bacteria in the environment are often the same as those inhabiting the gut, and thus sample contamination is difficult to differentiate from infection based on the species of bacteria. Additionally, apparently healthy neonatal calves may still have sufficient permeability in the gut epithelium to allow gut bacteria to translocate from the lumen of intestines to the blood stream. Wood et al. (2015) examined the relationship between aging and gastrointestinal tract (GIT) permeability in Holstein bull calves and suggested that permeability decreases as age increases. This concept has also been documented in studies of other species’ gastrointestinal permeability changes including piglets (Moесer and Blilkslager, 2007), rat pups (Henning, 1981), and rabbit kits (Udall et al., 1981). Meale et al. (2017) evaluated published research on how calf GIT develops physically and physiologically from pre- to postweaning. They demonstrated that GIT permeability changes with age and that Bacteroides species are dominant in the lower gut of preweaning calves. These factors may explain the recovery of Bacteroides in 2 apparently healthy preweaning calves; however, no prior studies have evaluated the prevalence of bacteremia as associated with changes in age and gut permeability. This study was designed to estimate the prevalence of bacteremia among diarrheic calves, but studies with larger sample sizes are necessary to determine if calves routinely experience bouts of bacteremia without apparent clinical signs. The current study used apparently healthy control calves to assess aseptic techniques and, as such, took 1 venous sample from each calf. To investigate the prevalence of bacteremia in healthy calves, future studies should include multiple samples over a period of time from different venous sites.

Most cases of neonatal calf diarrhea are attributed to several factors including infectious etiologies (Bartels et al., 2010), husbandry practices (Barrington et al., 2002), and environmental stressors (Klein-Jöbstl et al., 2014), all of which must be considered in the evaluation of data presented here. The 2 commercial dairy farms enrolled in this study had differing management practices, including housing location (indoor vs. outdoor), housing system (hutch vs. no hutch), bedding (straw on concrete vs. straw on natural ground), ventilation, and proximity to other calves. The variation in farm husbandry and small farm sample size may explain the numerical difference in prevalence of bacteremia among calves with diarrhea, which potentially reflects differences in disease course or management between the populations. Including this variability enables the data collected to encompass multiple types of husbandry that exist in dairy calf rearing. However, larger studies will be necessary to estimate the between-farm variation in the incidence of bacteremia.

Contaminated cultures are difficult to avoid (even in hospital settings), and the false positives likely resulted in misclassification of some number of calves as bacteremic (Dawson, 2014; Hughes et al., 2018). Nonetheless, this study demonstrated that the prevalence of bacteremia in replacement calves with diarrhea and systemic signs of illness is lower than prior estimates generated at male calf rearing farms or a veterinary teaching hospital (Fecteau et al., 1997b; Lofstedt et al., 1999). Ensuring appropriate use of antimicrobials in dairy calves is crucial to safeguarding the effectiveness of their future use in the dairy industry. Further investigation and larger studies are warranted to generate simple, accurate decision-making algorithms to support on-farm antimicrobial-use decisions. Furthermore, additional studies are necessary to investigate neonatal gut permeability and its relationship to bacteremia in apparently healthy dairy calves.

CONCLUSIONS

The prevalence of bacteremia in replacement heifer calves with diarrhea and systemic signs of illness was 9.26% (10/108), significantly lower than previously reported estimates. Observable clinical signs including fever, depression, and age were associated with bacteremia in heifer dairy calves with diarrhea. Bacteremia was rare among calves without signs of depression; therefore, antimicrobial therapy targeting potential bacteremia is not currently justified in routine cases of diarrhea in preweaning calves without signs of depression. These data are an important step toward creating tangible and evidence-based guidelines for antimicrobial-use decisions on commercial dairy farms.

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Garcia et al.: BACTEREMIA IN DIARRHEIC DAIRY CALVES


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