Clinical findings and diagnostic test results for calves with septic arthritis: 64 cases (2009–2014)

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OBJECTIVE
To describe clinical findings and diagnostic test results and identify potential prognostic indicators for calves with septic arthritis.

DESIGN
Retrospective case series.

ANIMALS
64 calves with septic arthritis.

PROCEDURES
The medical record database for a veterinary teaching hospital was searched to identify calves ≤6 months old that were treated for septic arthritis between 2009 and 2014. Data evaluated included signalment, history, physical examination and diagnostic test results, treatment, and outcome. Descriptive data were generated, and calves were assigned to 2 groups (neonatal [≤28 days old] or postneonatal [29 to 180 days old]) on the basis of age at hospital admission for comparison purposes.

RESULTS
64 calves had 92 infected joints; 17 calves had polyarthritis. Carpal joints were most frequently affected followed by the stifles and tarsal joints. Forty-nine bacterial isolates were identified from synovial specimens for 38 calves, and the most commonly identified isolates were catalase-negative Streptococcus spp (n = 14) and Mycoplasma bovis (9). Calves in the neonatal group had a shorter interval between onset of clinical signs and hospitalization and were more likely to have an infected carpal joint than calves in the postneonatal group. Outcome was positive for 35 calves. Synovial fluid total nucleated cell count was positively associated with a positive outcome.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that empirical antimicrobial treatment for calves with septic arthritis should target gram-positive catalase-negative cocci and M bovis and that synovial fluid total nucleated cell count might be a useful prognostic indicator. (J Am Vet Med Assoc 2018;252:995–1005)

Lameness is a common problem in cattle and is responsible for substantial economic losses in the beef and dairy industries.1,2 It is one of the most common reasons for culling dairy cattle.3 The most common causes of lameness in cattle are pathological lesions involving the digits followed by diseases of the articular synovial structures. In fact, diseases of synovial structures are responsible for clinical signs of lameness in 47% of all cattle that become lame because of limb abnormalities localized proximal to the foot.4

Septic arthritis is the most common joint disease of cattle.5 The incidence of septic arthritis was 0.11 cases/1,000 calf-days at risk in a longitudinal study6 of veal calves in Belgium and 0.002 cases/calf-month in a study7 of dairy calves in Sweden. Data regarding the incidence of septic arthritis in calves in North America are lacking.

The severity of articular infection is dependent on several factors including size of the inoculum, virulence of the infecting pathogen or pathogens, host immune system, and local joint factors.6,8 Bacteria can invade a joint by direct trauma (primary infection), extension of a periarticular infection (secondary infection), or hematogenous dissemination (tertiary infection).8 Bacteria that colonize joints by the hematogenous route frequently originate from infected umbilical structures, lungs, or gastrointestinal tract. Tertiary infection is the most frequent cause of septic arthritis in calves and is often associated with polyarthritis.9 Failure of passive transfer of immunity is an important risk factor for septic arthritis in calves because it increases the likelihood of septicemia and bacteremia.8,9

Clinical signs of septic arthritis include acute and severe lameness and detectable distension, heat, redness, and signs of pain during palpation of affected joints. A presumptive diagnosis of septic arthritis is confirmed on the basis of findings of arthrocentesis (bacterial culture results and cytologic evaluation and biomarker quantification of synovial fluid) and ultrasonographic and radiographic evaluation of affected joints.8,10 In a retrospective study8...
of 172 adult cattle and calves with septic arthritis, 103 (60%) had growth of 1 or more pathogens during bacterial culture of the infected joint. For calves < 6 months old, the most frequently isolated bacterial species from infected joints were Trueperella pyogenes, Streptococcus spp, Pasteurellaceae, and Enterobacteriaceae.6 That study6 was conducted before Mycoplasma cultures were routinely performed on infected joint specimens; therefore, detection of Mycoplasma was likely underreported. Among cattle with septic arthritis for which bacterial culture of the infected joint is performed, approximately 47% will have 1 pathogen isolated, 9% will have 2 pathogens isolated, and 4% will have ≥ 3 pathogens isolated.3

The prognosis for calves with septic arthritis is dependent on many factors, such as age, duration of clinical signs, joint or joints affected, number of joints affected, severity of radiographic lesions, pathogen or pathogens isolated from affected joint or joints, concomitant diseases, and value of the animal. However, those factors were extrapolated from case reports or case series that focused on treatment rather than the identification of prognostic indicators. To our knowledge, prognostic indicators have not been evaluated for a large number of calves with septic arthritis.

The objectives of the study reported here were to describe physical examination findings, hematologic and cytologic results, and bacterial culture and Mycoplasma bovis–specific PCR assay results for the infected joints of calves with septic arthritis < 28 days old and 29 to 180 days old and to identify potential prognostic indicators. We hypothesized that, regardless of age, the long-term prognosis would be poor for calves with polyarthritis or from which M bovis was identified or isolated.

Materials and Methods

Case selection criteria

The medical record database of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Université de Montréal was reviewed to identify the records of calves that were examined from January 2009 through January 2014 for which septic arthritis was diagnosed at ≤ 180 days old. To be included in the study, each calf had to have at least one of the following: bacterial growth on culture or positive M bovis–specific PCR assay result for a synovial fluid, fibrin, or membrane specimen; a synovial fluid sample with a TNCC > 25,000 cells/µL, polymorphonuclear cell count > 20,000 cells/µL, TNCC consisting of > 80% polymorphonuclear cells, or total protein concentration 4.5 g/dL;11, or the presence of suppurrative or fibrinous inflammation within a joint detected during lavage or postmortem examination.

Medical records review

Data retrieved from each medical record included breed, age, sex, history, physical examination findings, number of affected joints, concomitant systemic diseases, and antimicrobials administered. When available, results of CBCs and serum biochemical analyses performed at the teaching hospital were reviewed; hematologic results were used to obtain the WBC count, segmented and band neutrophil counts, and plasma fibrinogen concentration as determined by heat precipitation. When available, radiographs of the affected joint or joints were reviewed by a board-certified veterinary radiologist. Joint space reduction was the only feature evaluated because results from a preliminary study by our laboratory group (unpublished data) indicate that it is the only radiographic feature significantly associated with the outcome for calves with septic arthritis. The arthritis was considered a tertiary infection in the absence of a wound in the periarticular area of the affected joint as determined on the basis of the history and physical examination results. That theory was supported by a confirmed or presumptive diagnosis of septicemia or bacteremia as determined on the basis of bacterial culture results for a blood sample, isolation of the same bacterial agent from at least 2 different body fluids, effusion of multiple joints,12 or concomitant systemic diseases present either at the farm of origin or at the time of examination at the teaching hospital.

Bacterial culture and pathogen identification

Bacterial cultures were performed at the Complexe de Diagnostic et d’Épidémiosurveillance Vétérinaire du Québec of the Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec. Bacterial cultures and pathogen identification were performed in accordance with the Clinical and Laboratory Standards Institute standards and guidelines. Specimens most frequently cultured included synovial fluid, fibrin, and membranes obtained during diagnostic procedures (eg, necropsy), arthrocentesis following standard surgical preparation, or surgical procedures. The specimens were most often collected in sterile centrifuge or evacuated glass blood collection tubes. Specimens were submitted as soon as possible after collection or placed in appropriate transport medium and refrigerated. Two types of transport media8,9 and 1 brand of blood culture bottle10 were used for collection and transport of synovial specimens during the course of the study.

Samples collected in blood culture bottles were incubated at 35°C and cultured in accordance with the manufacturer’s recommendations. All synovial fluid, fibrin, and membrane specimens were inoculated onto Columbia agar with 5% sheep blood for routine aerobic bacterial culture, in brain-heart infusion broth as enrichment, and onto blood agar supplemented with gentamicin for anaerobic bacterial culture. Specimens plated on Columbia agar plates were incubated at 35 ± 2°C in an atmosphere with 5% CO2 for 20 to 48 hours, and specimens in brain-heart infusion broth were incubated at 35 ± 2°C in normal
atmosphere for 24 to 48 hours. Specimens plated on anaerobic culture plates were incubated at $35 \pm 2^\circ C$ in an anaerobic environmental system for up to 5 days. Specimens submitted for mycoplasmal culture were inoculated onto Hayflick agar, suspended in Hayflick broth, and incubated at $35 \pm 2^\circ C$ in a candle jar for up to 10 days. Identification of suspected *M. bovis* colonies was performed by immunofluorescence techniques as described.\textsuperscript{13,14} The *M. bovis*-specific PCR assay was performed at the Laboratory of Molecular Diagnostics, Faculty of Veterinary Medicine, Université de Montréal. Following DNA extraction from synovial specimens,\textsuperscript{15} the *M. bovis*-specific PCR assay was performed by use of a fast-cycling PCR kit in accordance with the manufacturer’s instructions\textsuperscript{8} and primers and probes described in another study.\textsuperscript{17} For the purpose of statistical analyses, bacterial isolates were grouped into 7 categories: gram-positive catalase-negative cocci, gram-positive catalase-positive cocci, mycoplasmas, gram-positive rods, Enterobacteriaceae, Pasteurellaceae, and anaerobic bacteria.

When performed, blood samples for bacterial culture were obtained by venipuncture of a jugular vein after standard surgical preparation. The blood sample was added to a blood culture bottle.\textsuperscript{a} The bottle was then incubated at $35^\circ C$, and culture was performed in accordance with the bottle manufacturer’s recommendations.

**Synovial fluid analysis**

Synovial fluid samples were typically obtained during diagnostic procedures or prior to surgery and were placed in standard evacuated glass blood collection tubes without any additives or with EDTA.\textsuperscript{b} Samples were cytologically analyzed as soon as possible after collection or after a period of refrigeration when obtained outside normal laboratory operating hours. For each sample, the total protein concentration was determined by refractometry,\textsuperscript{1} and the TNCC was determined by means of a standard hemacytometer technique.

**Treatments**

Joint lavage was performed by use of needles, teat cannulas, and arthroscopes; during arthroscopic surgery; or some combination thereof. Antimicrobial administration was continued for at least 2 weeks after clinical improvement of lameness. The antimicrobials most frequently used were ampicillin sodium (10 mg/kg [4.5 mg/lb], IV, q 8 h), ceftiofur sodium (2.2 mg/kg [1 mg/lb], IV, q 12 h), trimethoprim–sulfadoxine (3 mL/45 kg [3 mL/100 lb], IV, q 12 h), and enrofloxacin (10 mg/kg, IV, q 24 h). Analgesia was provided during the acute phase of the disease and as needed thereafter by use of NSAIDs, most commonly flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, q 24 h) or meloxicam (0.5 mg/kg [0.25 mg/lb], IV or SC, q 48 h).

**Outcome**

Long-term outcome was defined as positive if the calf was performing in accordance with the owner’s expectations at least 1 year after being discharged from the hospital. That information was acquired by administration of a standardized survey to the owner via telephone by 1 of 3 investigators (CC, MB, or AD). Calf performance was assessed on the basis of answers to questions focused on growth, prior and current or residual lameness, physical appearance of the affected joint, current or expected productivity, and use of the animal.

Outcome was defined as negative if the calf died or was euthanized during or after hospitalization or was not meeting the owner’s expectations at the time of the telephone survey. A calf was defined as lost to follow-up when it had been discharged from the hospital but its status at least 1 year after discharge was unknown. Calves that were discharged from the hospital but did not meet their owners’ expectations at the time of the telephone interview for reasons unrelated to health, growth, lameness, physical appearance of the joint, or use and productivity were excluded from all analyses related to outcome and prognosis.

**Statistical analysis**

Descriptive statistics were generated with standard software.\textsuperscript{3} All statistical analyses were performed with a statistical software package,\textsuperscript{1} and values of $P \leq 0.05$ were considered significant.

Quantitative variables were expressed as the mean ± SD with the exception of the number of infected joints, which was expressed as the median (range). Information regarding specific bacterial isolates and the joints affected was expressed as frequencies.

Calves were then categorized into 2 groups on the basis of their age at hospitalization (≤ 28 [neonatal group] and 29 to 180 days old [postneonatal group]). The 28-day cutpoint for age was selected because 0 to 28 days old represents the neonatal period for calves. A Wilcoxon test was used to compare the distribution of data between the 2 groups.

The respective associations between the clinical variables evaluated (age, sex, number of joints affected, specific joints affected, interval between onset of clinical signs and hospital admission, WBC count, segmented and band neutrophil counts, plasma fibrinogen concentration, synovial fluid TNCC and protein concentration, and bacterial isolates) and long-term outcome were initially assessed with univariate analyses. A t test for unequal variances was used for quantitative variables, and an exact $\chi^2$ test was used for qualitative variables. Variables with values of $P \leq 0.15$ were eligible for inclusion in a multivariable logistic regression model. The multivariable model was built by means of a stepwise backward elimination procedure by which nonsignificant variables were sequentially removed. No variables were forced into the model, and only variables with a value of $P \leq 0.05$ were retained in the final model.

The prognostic value of synovial fluid TNCC was evaluated by construction of receiver operating characteristic curves, which depict the relation between true-positive (sensitivity) and false-positive (1 – specificity) test results. Prognostic sensitivity was plotted against 1 – specificity for
every tested threshold, and the area under the curve was calculated by use of a Wilcoxon nonparametric approach (ie, trapezoidal rule). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated for each evaluated threshold. The prognostic threshold was defined as the synovial fluid TNCC at which the sensitivity and specificity were maximized. Results were reported as the area under the curve and associated 95% confidence interval. Synovial fluid TNCC as a prognostic indicator of long-term outcome was validated by assessing the duration of antimicrobial treatment and number of antimicrobials administered collectively as a proxy for response to treatment.

Results

Calves

The study population consisted of 64 calves including 56 females and 8 males. Breeds represented included Holstein (n = 58), Hereford (2), Angus (1), Ayrshire (1), Jersey (1), and Scottish Highland (1). At the time of hospital admission, 35 calves were ≤28 days old (including 2 that were <24 hours old; neonatal group) and 29 calves were between 29 and 180 days old (postneonatal group). The mean ± SD age was 24.5 ± 32.7 days (range, <1 to 161 days) for all calves, 14.7 ± 7.4 days for calves in the neonatal group, and 59.3 ± 34.3 days for calves in the postneonatal group.

History and clinical signs

At the time of hospital admission, 16 of the 64 (25%) calves had an umbilical infection, of which the origin of infection was identified as the umbilical vein for 14, umbilical artery for 1, and urachus for 1. Fifteen (23%) calves had bronchopneumonia, and 12 (19%) calves had diarrhea. The mean ± SD interval between onset of clinical signs and hospital admission was 7.0 ± 6.6 days (range, 0 to 30 days) for all 64 calves; however, the mean ± SD interval between onset of clinical signs and hospital admission for the neonatal group (5.4 ± 5.4 days) was significantly (P = 0.03) less than that for the postneonatal group (9.2 ± 7.2 days). Seven (11%) calves developed septic arthritis while hospitalized for treatment of another disease. The origin of septic arthritis was determined to be hematogenous dissemination of bacteria (tertiary infection) for 60 (94%) calves and trauma (primary infection) for the remaining 4 (6%) calves.

Affected joints

The 64 study calves had a total of 92 infected joints (Table 1). The most frequently affected joints were carpal joints (n = 27), followed by stifle (22) and tarsal (20) joints. The proportion of calves in the neonatal group with an infected carpal joint was significantly (P = 0.02) greater than the proportion of calves in the postneonatal group with an infected carpal joint. Forty-seven (73%) calves had 1 infected joint, 9 (14%) calves had 2 infected joints, 6 (9%) calves had 3 infected joints, 1 (2%) calf had 4 infected joints, and 1 (2%) calf had 5 infected joints. The median number of joints affected per calf was 1 joint (range, 1 to 5 joints) for all 64 calves and did not differ significantly (P = 0.60) between the neonatal (median, 1 joint; range, 1 to 3 joints) and postneonatal (median, 1 joint; range, 1 to 5 joints) groups.

Hematologic results

A CBC was performed for all 64 calves at hospital admission, and the results were summarized (Table 2). The mean WBC and neutrophil counts and plasma fibrinogen were significantly different between the neonatal and postneonatal groups. The mean WBC and neutrophil counts were lower, and the plasma fibrinogen concentration was higher, in the neonatal group than in the postneonatal group. The mean neutrophil count (X 10⁹ cells/µL) was 6.8 ± 5.9 for calves in the neonatal group and 13.9 ± 6.8 for calves in the postneonatal group (P = 0.35). The mean plasma fibrinogen concentration (mg/L) was 700 ± 260 for calves in the neonatal group and 730 ± 240 for calves in the postneonatal group (P = 0.94).

**Table 1**—Frequency distribution of infected joints for 64 calves with septic arthritis that were treated at a veterinary teaching hospital from January 2009 through January 2014.

<table>
<thead>
<tr>
<th>Joint</th>
<th>All calves</th>
<th>Neonatal group</th>
<th>Postneonatal group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpal</td>
<td>27 (29.3)</td>
<td>19 (41.3)</td>
<td>8 (17.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stifle</td>
<td>22 (23.9)</td>
<td>7 (15.2)</td>
<td>15 (32.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Tarsal</td>
<td>20 (21.7)</td>
<td>11 (23.9)</td>
<td>9 (19.6)</td>
<td>0.89</td>
</tr>
<tr>
<td>Elbow</td>
<td>10 (10.9)</td>
<td>4 (8.7)</td>
<td>6 (13.0)</td>
<td>0.74</td>
</tr>
<tr>
<td>Distal interphalangeal</td>
<td>4 (4.3)</td>
<td>2 (4.3)</td>
<td>2 (4.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Metacarpophalangeal or metatarsophalangeal</td>
<td>4 (4.3)</td>
<td>2 (4.3)</td>
<td>2 (4.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hip</td>
<td>3 (3.2)</td>
<td>1 (2.2)</td>
<td>2 (4.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Proximal interphalangeal</td>
<td>2 (2.2)</td>
<td>0 (0)</td>
<td>2 (4.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>46</td>
<td>46</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The neonatal group consisted of 35 calves that were ≤28 days old at hospital admission, and the postneonatal group consisted of 29 calves that were between 29 and 180 days old at hospital admission. Values represent the number (percentage) of infected joints unless otherwise indicated. Values of P ≤ 0.05 were considered significant.

**Table 2**—Mean ± SD values for select CBC and synovial fluid variables for the calves of Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All calves</th>
<th>Neonatal group</th>
<th>Postneonatal group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (X 10⁹ cells/µL)</td>
<td>11.9 ± 6.4</td>
<td>13.9 ± 6.8</td>
<td>12.3 ± 5.6</td>
<td>0.35</td>
</tr>
<tr>
<td>Neutrophil count (X 10⁹ cells/µL)</td>
<td>6.8 ± 5.9</td>
<td>8.7 ± 6.2</td>
<td>6.7 ± 5.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/L)</td>
<td>700 ± 260</td>
<td>730 ± 240</td>
<td>730 ± 270</td>
<td>0.94</td>
</tr>
<tr>
<td>Synovial fluid TNCC (X 10⁹ cells/L)</td>
<td>57.2 ± 78.2</td>
<td>73.3 ± 84.1</td>
<td>96.9 ± 58.5</td>
<td>0.41</td>
</tr>
<tr>
<td>Synovial fluid total protein (g/dL)</td>
<td>5.30 ± 1.43</td>
<td>4.89 ± 1.08</td>
<td>4.88 ± 1.86</td>
<td>0.99</td>
</tr>
</tbody>
</table>

See Table 1 for key.
ogen concentration did not differ significantly between the neonatal and postneonatal groups.

**Radiographic findings**

Radiographic images of the infected joint or joints were available for review for 54 of the 64 (84%) calves. A reduction in the space of the infected joint was identified for 1 calf of the neonatal group and 4 calves of the postneonatal group; however, the proportion of calves with a reduction of space in the infected joint did not differ significantly ($P = 0.61$) between the 2 groups.

**Bacterial analysis**

Forty-eight of the 64 (75%) calves had a complete bacterial analysis of infected synovial specimens performed. That analysis consisted of ≥ 1 synovial specimen submitted for routine aerobic and anaerobic bacterial cultures as well as mycoplasmal investigation by either mycoplasmal culture or an $M$ bovis–specific PCR assay. A more rudimentary bacterial analysis was performed for the other 16 calves (Table 3). Sixty-three (98%) calves had at least 1 synovial specimen submitted for routine aerobic bacterial culture, 52 (81%) calves had at least 1 synovial specimen submitted for anaerobic bacterial culture, 47 (73%) calves had at least 1 synovial specimen submitted for mycoplasmal culture, and 15 (23%) calves had at least 1 synovial specimen submitted for an $M$ bovis–specific PCR assay. The frequency with which the various types of bacterial analyses were performed did not differ significantly between the neonatal and postneonatal groups.

Thirty-eight of 64 (59%) calves had at least 1 bacterial isolate identified during bacterial or mycoplasmal culture or an $M$ bovis–specific PCR assay. Thirty (47%), 5 (8%), and 3 (5%) calves had 1, 2, and ≥ 3 bacterial isolates identified from synovial specimens, respectively. A total of 49 bacterial isolates were identified, of which 20 (41%) were gram-positive catalase-negative cocci, 13 (27%) were mycoplasmas, and 7 (14%) were gram-positive rods (Table 4).

### Table 3—Summary of types of bacterial analyses performed for the calves of Table 1.

<table>
<thead>
<tr>
<th>Type of bacterial analysis performed</th>
<th>All calves</th>
<th>Neonatal group</th>
<th>Postneonatal group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of calves tested</strong></td>
<td><strong>No. of calves with positive results</strong></td>
<td><strong>No. of calves tested</strong></td>
<td><strong>No. of calves with positive results</strong></td>
</tr>
<tr>
<td>Complete analysis*</td>
<td>48</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>Routine aerobic bacterial culture and mycoplasmal culture or $M$ bovis–specific PCR assay</td>
<td>11</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Routine aerobic and anaerobic bacterial cultures</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mycoplasmal culture or $M$ bovis–specific PCR assay</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>64</strong></td>
<td><strong>38</strong></td>
<td><strong>35</strong></td>
</tr>
</tbody>
</table>

*The complete bacterial analysis consisted of ≥ 1 synovial specimen submitted for routine aerobic and anaerobic cultures and mycoplasmal culture or an $M$ bovis–specific PCR assay. See Table 1 for remainder of key.

### Table 4—Frequency distribution of pathogens isolated from synovial specimens for the calves of Table 1.

<table>
<thead>
<tr>
<th>Bacterial category</th>
<th>Isolate</th>
<th>All calves</th>
<th>Neonatal group</th>
<th>Postneonatal group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive catalase-negative cocci</td>
<td>All isolates combined</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>α-Hemolytic Streptococcus spp</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Streptococcus dysgalactiae</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Streptococcus uberis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Streptococcus spp</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Aerococcus spp</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Enterococcus spp</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Gram-positive rods</td>
<td>All isolates combined</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Trueperella pyogenes</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Bacillus spp</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>All isolates combined</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Proteus spp</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Gram-positive catalase-positive cocci</td>
<td>All isolates combined</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus hyicus</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus spp</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Pasteurellaceae</td>
<td>Mannheimia vangena</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>Mycoplasmas</td>
<td>All isolates combined</td>
<td>13</td>
<td>5</td>
<td>8</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma spp</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mycoplasma bovis</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Fusobacterium necrophorum</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>49</strong></td>
<td><strong>25</strong></td>
<td><strong>24</strong></td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

— = Not applicable. See Table 1 for remainder of key.
The mean ± SD number of bacterial isolates per infected joint did not differ significantly (P = 0.41) between the neonatal (0.73 ± 0.85 bacterial isolates/infected joint) and postneonatal (0.83 ± 0.70 bacterial isolates/infected joint) groups.

Blood samples from 6 calves were submitted for bacterial culture. Only one of those calves had a positive culture result and was considered bacteremic. For that particular calf, an α-hemolytic Streptococcus sp was isolated from the blood sample as well as a synovial fluid sample.

**Synovial fluid analysis**

Cytologic evaluation of at least 1 synovial fluid sample was performed for 34 of the 64 (53%) calves. The mean synovial fluid TNCC and protein concentration did not differ significantly between the neonatal and postneonatal groups (Table 1).

**Outcome**

Of the 64 calves, 53 (83%) were discharged from the hospital and 11 (17%) died or were euthanized while hospitalized. The proportion of calves that survived to hospital discharge did not differ significantly between the neonatal and postneonatal groups. The most common reasons for euthanasia prior to hospital discharge were development of polyarthritis or general degeneration of clinical status. Long-term follow-up was available for 45 of the 53 (85%) calves that were discharged from the hospital. Two of those calves were excluded from the analysis because they did not meet the owners’ expectations for reasons unrelated to health, growth, lameness, physical appearance of the affected joint, or use and productivity. Eight calves were lost to follow-up, and the most common reasons for this were sale of the calf, failure to track the calf within the herd, or inability to contact the owner. Thus, outcome was available for 54 of the 64 (84%) calves. Outcome was positive for 32 (59%) calves and negative for 22 (41%) calves. Eleven of the 55 calves that were discharged from the hospital were culled within the subsequent 12 months, and the reason most frequently cited for culling was persistent lameness or poor growth. The proportion of calves with a positive outcome 12 months after hospital discharge did not differ significantly between the neonatal and postneonatal groups.

Results of univariate analyses of the association between select variables and outcome were summarized (Table 5). The probability of a positive outcome was negatively associated with the number of infected joints (P = 0.01), infection of the stifle joint (P = 0.01), neutrophil count (P = 0.045), and plasma fibrinogen concentration (P = 0.02) and positively associated with synovial fluid TNCC (P = 0.001). Reduction in joint space was not associated with outcome.

Multiple bacterial isolates were identified in synovial specimens for 18 of the 32 (56%) calves with a positive outcome and 14 of the 22 (64%) calves with a negative outcome; however, the identification of ≥1 bacterial isolate from synovial specimens was not significantly (P = 0.54) associated with outcome. Likewise, the type of bacterium (ie, bacterial category) isolated from the infected joint was not associated with outcome (Table 6).

The final multivariable logistic regression model included only 1 variable; synovial fluid TNCC was positively associated with a positive outcome (P = 0.005). Results of the receiver operating characteristic curve analysis indicated that diagnostic sensitivity and specificity were optimized at a TNCC threshold of 72,125 cells/μL. That threshold had a diagnostic sensitivity and specificity of 58.8% and 100%, respectively, and positive and negative predictive values of 100% and 61.1%, respectively. For every 1-cell/μL decrease in the synovial fluid TNCC from the 72,125-cell/μL threshold, the probability of a positive out-

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**Table 5**—Summary statistics for the calves of Table 1 that had positive (n = 32) and negative (22) outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calves with a positive outcome</th>
<th>Calves with a negative outcome</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at hospital admission (d)</td>
<td>35.5 ± 32.1</td>
<td>34.8 ± 41.6</td>
<td>0.91</td>
</tr>
<tr>
<td>Interval between onset of clinical signs and hospital admission (d)</td>
<td>5.4 ± 5.4</td>
<td>6 ± 6.5</td>
<td>0.97</td>
</tr>
<tr>
<td>WBC count (X 10³ cells/µL)</td>
<td>12.3 ± 5.2</td>
<td>15.3 ± 7.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Neutrophil count (X 10³ cells/µL)</td>
<td>6.8 ± 5.2</td>
<td>10.5 ± 7.0</td>
<td>0.045</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/dL)</td>
<td>650 ± 200</td>
<td>830 ± 290</td>
<td>0.02</td>
</tr>
<tr>
<td>Synovial fluid TNCC (X 10⁶ cells/L)</td>
<td>120.6 ± 89.2</td>
<td>32.4 ± 25.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Synovial fluid protein (g/dL)</td>
<td>5.17 ± 1.42</td>
<td>4.97 ± 1.47</td>
<td>0.73</td>
</tr>
<tr>
<td>No. (% of calves with a reduction in joint space</td>
<td>0 (0)†</td>
<td>3 (17)†</td>
<td>0.062</td>
</tr>
<tr>
<td>No. of joints affected per calf</td>
<td>1 (1–3)</td>
<td>2 (1–5)</td>
<td>0.01</td>
</tr>
<tr>
<td>No. (% of calves with an infected stifle joint)</td>
<td>12 (38)</td>
<td>10 (45)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD or median (range) unless otherwise specified. Outcome was determined on the basis of owners’ responses to a telephone survey conducted at least 1 year after a calf was discharged from the hospital and was defined as positive if the calf was performing in accordance with the owner’s expectations. Outcome was defined as negative if the calf died or was euthanized during or after hospitalization or was not meeting the owner’s expectations at the time of the telephone survey. Calves with an unknown status 1 year after hospital discharge were considered lost to follow-up (n = 8), whereas those that were not meeting their owners’ expectations at the time of the telephone survey for reasons unrelated to health, growth, lameness, physical appearance of the affected joint, or use and productivity were excluded from the analysis (2).

*Results for univariate analyses; a test for unequal variances was used for quantitative variables, and an exact χ² test was used for qualitative variables. †Reduction in joint space was determined on the basis of radiographic image review by a board-certified veterinary radiologist, and radiographic images of the affected joint or joints were available for only 28 calves with a positive outcome and 18 calves with a negative outcome.

See Table 1 for remainder of key.
Table 6—Frequency distribution of each bacterial category for the calves of Table 5 with positive (n = 32) and negative (22) outcomes.

<table>
<thead>
<tr>
<th>Bacterial category</th>
<th>Calves with a positive outcome</th>
<th>Calves with a negative outcome</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive, catalase-negative cocci</td>
<td>10 (45.5)</td>
<td>6 (18.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Gram-positive rods</td>
<td>2 (9.1)</td>
<td>5 (15.6)</td>
<td>0.10</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0 (0)</td>
<td>3 (9.4)</td>
<td>0.064</td>
</tr>
<tr>
<td>Gram-positive, catalase-positive cocci</td>
<td>1 (4.5)</td>
<td>2 (6.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>Pasteurellaeae</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mycoplasmas</td>
<td>4 (18.2)</td>
<td>5 (15.6)</td>
<td>0.46</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values represent the number (percentage) of calves unless otherwise specified.
— = Data not available.
See Table 5 for remainder of key.

Discussion

In the present study, the clinical findings and outcomes for calves < 6 months old with septic arthritis were described. Sixty of the 64 (94%) calves evaluated in this study developed tertiary septic arthritis as a consequence of the hematogenous dissemination of bacteria, which is consistent with the findings of another study18 regarding the treatment of septic arthritis in calves. In that study,18 the most common sites of origin for bacteria that subsequently caused arthritis were infections of the umbilicus, lungs, and gastrointestinal tract. Likewise, for the calves of the present study, the most frequent concomitant diseases reported at the time of hospitalization were umbilical infection (16/64 [25%]), bronchopneumonia (15/64 [23%]), and diarrhea (12/64 [19%]). The majority (47/64 [73%]) of the calves of the present study had only 1 infected joint at the time of hospitalization, and the proportion of calves with polyarthritis (17/64 [27%]) was lower than the proportion of calves with septic polyarthritis in other studies.11,18,21 Also, the median number of affected joints per animal reported for foals of other studies19,20 is 1 joint; range, 1 to 5 joints) for the calves of the present study was lower than the median number of affected joints per animal reported for foals of other studies.

It is possible that the fairly low prevalence of polyarthritis for the calves of the present study, compared with that for foals in those other studies, reflected inherent differences in the duration or severity of bacteremia between calves and foals. However, only 1 of the 6 calves of the present study for which bacterial culture of a blood sample was performed had positive results and was considered bacteremic, and there was insufficient data to test that hypothesis further. The fairly low prevalence of polyarthritis among the calves of the present study might also have been the result of selection bias associated with the evaluation of animals examined at a referral hospital. Calves with polyarthritis may be more likely than foals with polyarthritis to be euthanized at the farm rather than referred to a veterinary hospital for treatment. Although the median number of affected joints appeared to be negatively associated with a positive outcome, it was not retained in the final multivariable logistic regression model.

For the calves of the present study, carpal joints were the most frequently infected joints followed by the stifle and tarsal joints, which was similar to results reported for other populations of cattle18,21,22,23 and foals.21,22 Anatomically larger joints have a more expansive synovial membrane surface area than small joints, which increases the risk for bacterial colonization.21 Although an infected stifle joint was negatively associated with a positive outcome on univariate analysis, that variable was not retained in the final multivariable model. The large size of the stifle joint and the challenge associated with performing arthrocentesis or arthrotomy might have contributed to the high rate of treatment failure for calves with septic stifle joints.23 Additionally, the stifle joint has a large range of motion, which may exacerbate the risk for septic arthritis sequelae, such as osteoarthritis, for affected animals.

Calves in the neonatal group (≤ 28 days old) had a significantly shorter interval between onset of clinical signs and hospital admission and were more likely to have an infected carpal joint, compared with calves in the postneonatal group (29 to 180 days old). The 2 age groups were defined based on the accepted definition of the neonatal period for calves.21 Sixty of the 64 (94%) calves were from dairy breeds. Management practices common on dairy herds may be partially responsible for the shorter interval between onset of clinical signs and hospital admission for the neonatal group relative to the postneonatal group. Neonatal dairy calves are often housed individually and fed milk replacer twice daily,25,26 practices that
facilitate frequent observation and rapid detection of sick animals. As calves get older, they are generally transitioned to group housing, which is not always conducive to close observation of individual calves on a regular basis.

The isolation of bacteria from synovial fluid, fibrin, or membrane specimens confirms the diagnosis of septic arthritis but has low sensitivity. Bacterial culture results are affected by the virulence, pathogenicity, and number of bacteria present in the specimen cultured, antibacterial properties of synovial fluid, sequestration of bacteria within the synovial membrane, and history of antimicrobial administration prior to collection of the synovial specimen cultured.27,28 At least 1 bacterial pathogen was isolated from the infected joint or joints from 38 of the 64 (59%) calves of the present study, which is consistent with findings of other studies.29,4 However, a complete bacterial analysis (submission of ≥1 synovial specimen for routine aerobic and anaerobic bacterial cultures, and mycoplasmal investigation by either mycoplasmal culture or an M. bovis–specific PCR assay) was performed for only 48 (75%) calves of the present study. It is likely bacterial pathogens would have been isolated from the infected joints for some proportion of the remaining 16 study calves had a complete bacterial analysis been performed for them as well. Also, some calves may have had false-negative culture results owing to less-than-optimum specimen handling and processing prior to culture inoculation. The success rate for pathogen isolation and identification from bacterial cultures of synovial specimens could be improved by use of standardized transport media and protocols for the types of cultures requested and systematic use of blood culture bottles.30

In the present study, gram-positive catalase-negative cocci were the most frequently identified type of bacteria isolated from the septic joints of calves, and Streptococcus was the most commonly isolated genus. Those results differ from findings of other studies of septic arthritis in cattle31,32 and foals.33 In other studies31,32 involving cattle with septic arthritis, T. pyogenes was the most frequently isolated bacterial species from synovial specimens. Truuerella pyogenes is commonly isolated from chronic infections and is considered an opportunistic bacterium.33 The fact that T. pyogenes was isolated from the infected joints of only 4 of the 63 (6%) calves for which routine aerobic bacterial culture of synovial specimens was performed might be at least partially attributable to the fairly short interval between the onset of clinical signs and hospital admission (mean ± SD, 6 ± 6.5 days), which was insufficient for an opportunistic T. pyogenes infection to develop. In foals, Escherichia coli is the most frequently isolated bacterial species from septic joints.31,32 The infected joints for 16 of 64 (25%) calves of the present study were believed to be a sequela of hemogenous dissemination of bacteria from an infected umbilicus. The prevalence of omphalitis in calves (5% to 15%)34,35 is greater than that in foals (0.71%),36 and the prevalence of septic arthritis subsequent to omphalitis is likewise low for foals.37-39 In calves with umbilical infections, the umbilical vein is often involved (omphalophlebitis), and gram-positive catalase-negative cocci, such as Streptococcus spp., are commonly isolated from infected umbilical structures.38-40 Septic arthritis is a frequent complication (28%) for calves with omphalophlebitis, particularly when the infected umbilical vein extends into the liver parenchyma and the infection extends to the portal vein.38

Mycoplasmas were the second most frequently isolated type of bacteria from the infected joints of the calves of the present study, and M. bovis was the most frequently isolated Mycoplasma spp (9/13 mycoplasmal isolates). Although outbreaks of arthritis and polyarthritis in cattle caused by M. bovis have been described,31,41,42 to our knowledge, the prevalence of M. bovis–induced septic arthritis in cattle has not been investigated. Calves with M. bovis–induced septic arthritis generally do not respond well to typical treatment protocols41,43; however, the isolation of M. bovis from synovial specimens was not associated with the outcome for the calves of the present study. Follow-up information was available for 7 of the 9 calves from which M. bovis was isolated, and 4 of those calves had a positive outcome. The positive outcome for those calves was likely associated with the prolonged administration of an antimicrobial that was effective against or labeled for the treatment of M. bovis infections and with thorough debridement of intra-articular fibrin. The fairly high prevalence of calves with M. bovis–induced septic arthritis (9/64 [14%]) in the present study may have been the result of selection bias because calves that fail to respond to traditional first-line treatments on the farm of origin were more likely to be referred to a tertiary hospital. The prevalence of M. bovis–induced septic arthritis in the calves of this study may also have been affected by the inclusion of M. bovis–specific PCR assay results in the analysis. Mycoplasmas are atypical bacteria and require special media and conditions for culture, which are notoriously laborious.44,45 The M. bovis–specific PCR assay detects M. bovis DNA and does not require cultivation of live organisms. Results of other studies46,47 suggest that the M. bovis–specific PCR assay may be more sensitive than mycoplasmal culture.

In our hospital, β-lactams, such as ampicillin sodium, are the primary antimicrobials used to treat calves with septic arthritis. Mycoplasmas lack a cell wall; therefore, they have a natural resistance to β-lactams. In Canada and the United States, there are currently few antimicrobials approved for the treatment of cattle with infections caused by M. bovis, and of those that are approved, fluoroquinolones, tetracyclines, and macrolides have the best distribution into synovial structures and fluid.48 Therefore, those classes of drugs should be considered for the treatment of calves with septic arthritis that are refractory to or failed to respond to first-line treatments. For dairy calves with M. bovis–induced septic arthritis, we typically use enrofloxacin49 when the calves have...
concomitant respiratory tract disease or tulathromycin in combination with another antimicrobial when calves do not have concomitant respiratory tract disease; we use tetracycline and spectinomycin less commonly owing to the acquired resistance of *M. bovis* to those 2 antimicrobials. Furthermore, clinical data are needed to confirm the microbiologic efficacy of macrolides within the synovial fluid of cattle.

It is important that all local and federal regulations be followed whenever treatment protocols are developed for food-producing animals. Extralabel administration of fluoroquinolones, such as enrofloxacin, to food animals is prohibited in the United States but not in Canada where this study was conducted. Enrofloxacin is labeled for the treatment or control of respiratory tract disease associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *M. bovis* in beef cattle and nonlactating dairy cattle < 20 months old. Although extralabel use of enrofloxacin in cattle is not prohibited in Canada, the only calves of the present study that received that drug were those with concomitant respiratory tract disease, and all label directions regarding dose, route of administration, and frequency were followed. Some calves of the present study received antimicrobials, such as trimethoprim-sulfadaxone, that are available and labeled for the treatment of cattle in Canada but not in the United States. However, the primary purpose of this study was to evaluate the clinical features and diagnostic test findings for calves with septic arthritis and to use those data to identify variables that might be useful prognostic indicators. It was not intended to evaluate the effectiveness of specific treatments or antimicrobials. All drugs received by the calves of the present study were administered in accordance with Canadian regulations.

Cytologic evaluation of synovial fluid can be used to confirm diagnosis of septic arthritis. To our knowledge, the present study was the first in which synovial fluid cytologic variables of calves with septic arthritis were assessed for their usefulness as prognostic indicators. Multiple studies involving foals with septic arthritis have failed to identify a significant association between synovial fluid TNCC or total protein concentration and the likelihood of survival. In the present study, results of both the univariate analysis and multivariable logistic regression analysis indicated that synovial fluid TNCC was positively associated with a positive outcome. A similar association between synovial fluid TNCC and outcome has been described in foals < 6 months old with suspected infectious polyarthritis.

Many factors, such as pathogen virulence and host immune system status, can affect the outcome for patients with septic arthritis. It has been postulated that synovial invasion by bacteria is more likely to cause destruction of articular cartilage than modification of synovial fluid. Although WBCs release substances that degrade collagen within articular cartilage, a high synovial fluid TNCC may not be entirely detrimental, but rather an indication of a strong host immune response to an infection. The positive association between synovial fluid TNCC and a positive outcome was supported by the finding that calves with a synovial fluid TNCC > 72,125 cells/µL were more likely than calves with a lower synovial fluid TNCC to be treated with only 1 instead of multiple antimicrobials, which suggested that those calves had a better response to treatment. However, we could not exclude the possibility that synovial fluid TNCC was a proxy for a confounding variable that was not included in our multivariable analysis, and the positive association between synovial fluid TNCC and a positive outcome for calves with septic arthritis warrants further validation.

The prognosis for a positive outcome following treatment for septic arthritis does not appear to vary significantly between adult cattle and calves. Treatment success for adult cattle with septic arthritis ranges from 33% to 90% and is dependent on treatment administered. In another study, joint lavage was considered a successful treatment for 16 of 20 (80%) calves with septic arthritis, and although the long-term outcome was not reported for those calves, success rate was similar to the hospital discharge rate (53/64 [83%]) for the calves of the present study.

The main limitation of the present study was its retrospective nature. The accuracy and completeness of the medical records were inconsistent. For many calves that were euthanized, it was difficult to ascertain whether they were euthanized because of financial reasons, a guarded prognosis, or lack of genomic value. This could have resulted in misclassification of the outcome for those calves. The small number of calves evaluated limited the statistical power of the study, and it is possible that additional associations might have been identified had a larger population been evaluated. Calves were separated into 2 groups on the basis of their age at hospital admission rather than their age at the onset of clinical signs, which may have contributed to the lack of significant differences identified between the 2 groups. Follow-up data were not available for all calves, and the interval between hospital discharge and the follow-up telephone survey may have resulted in false information, particularly in regard to culled animals, because of recall bias.

Results of the present study indicated that gram-positive catalase-negative cocci was the most frequently isolated bacterial category, and *Streptococcus* was the most frequently isolated genus from synovial specimens of calves with septic arthritis. This information may be beneficial for empirical antimicrobial treatment of calves with septic arthritis when results of bacterial cultures are negative or pending. The prognosis for calves < 6 months old with septic arthritis is fair following hospitalization and treatment at a referral hospital. This study also revealed a positive association between synovial fluid TNCC and a positive outcome; thus, synovial fluid TNCC may be a useful prognostic indicator and help guide treatment decisions for calves with septic arthritis.
Acknowledgments

The authors thank Dr. Isabelle Masseau for review and evaluation of radiographic images, Guy Beauchamp for statistical analysis, Dr. Emma Marchionatti for technical assistance, and Dr. Becky Gilday for assistance with manuscript preparation.

Footnotes


b. Multitran system collection and transport system for virus, Chlamydia and Mycoplasma, Starplex Scientific Inc, Etobicoke, ON, Canada.

c. BBL Port-A-Cul Tubes, Becton, Dickinson and Co, Sparks, Md.


e. Bactron anaerobic environment system, Sheldon Manufacturing Inc, Cornelius, Ore.

f. Qiagen Fast Cycling PCR Kit, QIAgen Inc, Valencia, Calif.

g. Vacutainer blood collection tubes, BD Vacutainer, Franklin Lakes, NJ.

h. EDTA Monoject lavender stopper blood collection tubes, Tyco Healthcare Group LP, Mansfield, Mass.

i. TS meter, Reichert Inc, Depew, NY.

j. Neubauer hemocytometer, VWR, Mannheim, Germany.

k. Microsoft Excel, Microsoft Corp, Redmond, Wash.

l. SAS, version 9.4, SAS Institute Inc, Cary, NC.

m. Baytril 100 Injectable, Bayer Corp, Shawnee Mission, Kan.


References


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