Pharmacokinetics of ampicillin-sulbactam in serum and synovial fluid samples following regional intravenous perfusion in the distal portion of a hind limb of adult cattle

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OBJECTIVE  
To describe concentration-over-time data for ampicillin and sulbactam in the digital and systemic circulations and synovial fluid (SYN) of cattle following a single injection of ampicillin-sulbactam as a regional IV perfusion (RIVP).

ANIMALS  
6 healthy adult nonlactating Jersey-crossbred cows.

PROCEDURES  
The right hind limb of each cow was aseptically prepared. A tourniquet was applied around the midmetatarsal region, and 1.0 g of ampicillin with 0.5 g of sulbactam in a combined formulation was administered as an RIVP into the dorsal common digital vein (DCDV). Blood samples from the DCDV and jugular vein and SYN samples from the metatarsophalangeal joint of the prepared limb were collected immediately before and at predetermined times for 24 hours after RIVP. One blood sample was obtained from the abaxial proper plantar vein of the lateral digit of the prepared limb 0.25 hours after RIVP. Serum and SYN ampicillin and sulbactam concentrations were determined by high-performance liquid chromatography.

RESULTS  
Mean ± SD maximum concentration of ampicillin in SYN and serum obtained from the abaxial proper plantar and jugular veins was 1,995 ± 1,011 µg/mL.  
SERUM: 5,422 ± 1,953 µg/mL.  
SYN: 2.5 ± 1.6 µg/mL.  
Corresponding serum and SYN concentrations of sulbactam were lower but followed the same pattern over time as those for ampicillin. Synovial fluid ampicillin concentration remained above 8 µg/mL for a mean time of 18.9 hours.

CONCLUSIONS AND CLINICAL RELEVANCE  
Potentially therapeutic concentrations of ampicillin were achieved in regional serum and SYN samples; SYN concentrations remained at potentially therapeutic values for > 12 hours following RIVP of 1.5 g of ampicillin-sulbactam in the hind limb of healthy cows. (Am J Vet Res 2017;78:1372–1379)

Infections of the distal portion of the limbs are an important cause of lameness and pose welfare and production concerns for both dairy and beef cattle. Potential septic foci within the deep tissues of the bovine digit include septic arthritis, septic tenosynovitis, and septic pedal osteitis or osteomyelitis and are collectively referred to as DDS. In cattle, lesions associated with DDS are some of the most severe and painful causes of lameness.

Treatment goals for DDS include debridement of the lesion, provision of adequate antimicrobial concentrations at the site of infection, and pain management. Antimicrobial treatment can be achieved by use of various techniques that include systemic or local administration. Cefiofur, tulathromycin, oxytetracycline, and florfenicol are antimicrobials approved for systemic use in cattle for the treatment of interdigital necrobacillosis (an infection that may result in DDS). Compared with systemic administration of an antimicrobial, local administration of an antimicrobial has the advantage of resulting in high regional concentrations of the drug, which may increase drug penetration to poorly perfused or diseased tissues and decrease systemic drug concentrations, which in turn reduces the total amount of drug used per ani-
mal, limits the potential for toxicosis and treatment costs, and decreases the potential for antimicrobial residues in meat and milk from treated cattle. Injection of an antimicrobial directly into the digital circulation via RIVP can be a clinically effective method for providing local antimicrobial treatment in a diseased limb of various large animal species. Regional IV perfusions of drugs such as cefazolin, ceftiofur, tetracycline, florfenicol, and amikacin have been investigated in large animals, and results of those studies indicate that RIVP of those drugs results in local drug concentrations that are dramatically greater than those achieved after systemic administration. Regional IV perfusions do not require direct synovial access, are associated with fewer signs of pain and complications than intraosseous perfusions, are fairly easy to perform, and do not require specialized equipment or implants.

In fact, RIVP requires only a safe way to restrain the animal and facilitate immobilization of the affected limb similar to that used for examination of a digit, a tourniquet, and supplies necessary for an IV injection.

The most common bacterial species isolated from adult cattle with septic arthritis and DDS is Trueperella pyogenes. Another important pathogen of bovine digital tissues is Fusobacterium necrophorum. Although many other pathogenic bacteria have been isolated from DDS lesions in cattle, we consider T. pyogenes and F. necrophorum to be the target species for the initial antimicrobial treatment due to their frequency and clinical importance. However, resistance to penicillin with a narrow spectrum of activity includes T. pyogenes, F. necrophorum, streptococci, staphylococci, and some Enterobacteriaceae.

A combination of ampicillin and sulbactam was selected for investigation in the study reported here because of its potential for greater activity against β-lactamase-producing strains of T. pyogenes. Concurrent administration of sulbactam with ampicillin does not affect the pharmacokinetics of ampicillin. A potential clinical advantage of the ampicillin-sulbactam formulation selected is its compatibility with lidocaine, which is often administered as an RIVP for regional anesthesia of digits affected by DDS in conjunction with antimicrobials.

In the United States, there are currently no drugs approved by the FDA for administration as an RIVP in cattle; therefore, RIVP of any drug to cattle represents extralabel drug use. Some antimicrobials administered by RIVP to cattle in other studies are prohibited from extralabel use in cattle or pose a high risk for violative milk residues if administered to lactating dairy cattle in the United States. However, extralabel administration of ampicillin-sulbactam to cattle is allowed in the United States under AMDUCA. Clinically, ampicillin has been administered as an RIVP for the treatment of DDS in various large animal species, but minimal data are available in the peer-reviewed literature regarding the pharmacokinetics of ampicillin when administered as an RIVP to cattle.

The purpose of the study reported here was to describe concentration-over-time data for ampicillin and sulbactam in the digital and systemic circulations and SYN of healthy cattle following a single injection of ampicillin-sulbactam as an RIVP in the distal portion of a hind limb. It was believed that the data generated could be compared with the MIC data for common bacterial pathogens of the distal portion of bovine limbs to provide targeted information that could be used to guide treatment intervals.

### Materials and Methods

#### Animals

The study was approved by the Institutional Animal Care and Use Committee at The Ohio State University. Six university-owned nonlactating, nonpregnant, adult Jersey-crossbred cows with a mean weight of 376 kg (range, 330 to 449 kg) that were free of systemic illness, lameness, and digital infection were used for the study. Cows were housed at The Ohio State University Veterinary Medical Center and provided ab libitum access to water and grass-alfalfa hay throughout the duration of the study. Additionally, approximately 3 kg of grain/cow/d was provided at predetermined sampling times to facilitate animal handling. Prior to study initiation, cows had been housed at an off-campus university facility for at least 6 months, during which no pharmaceuticals containing ampicillin or sulbactam were administered.

#### Catheter placement

A minimum of 24 hours prior to RIVP, catheters were placed in each cow for administration of the
RIVP and collection of blood and SYN samples. Each cow was restrained in left lateral recumbency on a hydraulic tilt chute and sedated with xylazineb (20 mg, IV). A 14-gauge, 5.25-inch catheterc was aseptically placed in a jugular vein. Hair from the distal portion of the right hind limb (distal limb) was clipped from the midmetatarsal region distally to the coronary band, and the underlying skin was aseptically prepared. A tourniquetd was placed around the proximal portion of the metatarsus, and 30 mL of a 2% lidocaine solutione was infused as a ring block around the midmetatarsal region to provide regional anesthesia of the distal limb. Aseptic preparation of the distal limb was then repeated. An 18-gauge, 1.5-inch needle was inserted into the cranialateral aspect of the metatarsophalangeal joint, and the joint was distended with 40 mL of sterile saline (0.9% NaCl) solution. Then, the caudalateral aspect of the joint pouch was palpated, and a No. 15 scalpel blade was used to make a 0.5-cm stab incision through the skin. A 19-gauge Tuohy needlef was inserted through the incision and into the joint. A 20-gauge, 90-cm polyurethane catheterg was inserted through the needle until the distal end was approximately 2 cm within the joint; then, the Tuohy needle was removed. A 16-gauge needle was used to make a subcutaneous tunnel from a point 4 cm proximal to the stab incision. The proximal end of the polyurethane catheter tubing was passed through the 16-gauge needle, and the needle was removed. The polyurethane catheter was cut 3 cm from its exit point from the skin. An injection port was placed on the cut end, and the catheter was sutured in place. To further secure the injection port, white medical tape was placed around the port, and the free ends of the tape were stapled to the skin with skin staples. Finally, a cut-down procedure was used to aseptically place an 18-gauge, 2-inch over-the-wire cathetere in the DCDV. A short extension set and injection port were placed on the catheter, sutured into place, and further secured with adhesive. The catheter and extension set were flushed with 3,000 U of heparin to form a heparin lock. The distal limb was then bandaged to further protect the catheters and catheter sites (Figure 1).

**RIVP**

Each cow was restrained in left lateral recumbency on a hydraulic tilt chute for the RIVP. A wide rubber tourniquetd was applied around the right hind limb at the midmetatarsal region proximal to the bandage. The tourniquet was applied with standard manual tension to each cow by the same investigator (KMS). A strip of rubber-tire inner tubing with a width similar to that of the tourniquet was wrapped over the tourniquet to provide stabilization of and protection to the tourniquet. The ampicillin-sulbactam solution was prepared immediately before RIVP by reconstitution of a 1.5-g vial of the combined drug formulationf (1 g of ampicillin and 0.5 g of sulbactam) with 3.2 mL of sterile saline solution to yield a total volume of 4 mL. That dose of ampicillin-sulbactam was selected on the basis of information in the scientific literature, which recommends the use of 1 g of ampicillin as a regional limb perfusion for the treatment of deep digital infections of the bovine foot. Additionally, the 1-g dose of ampicillin was convenient because the ampicillin-sulbactam formulation used was commercially sold in vials containing that amount and the formulation had to be used within 1 hour after reconstitution. Therefore, the content of an entire vial was reconstituted and used for each cow. However, the 1-g dose of ampicillin used was much lower than the systemic dose of ampicillin (22 mg/kg) recommended for cattle, which would have equated to approximately 8.3 g of ampicillin/cow for this study. The 4-mL volume of the reconstituted ampicillin-sulbactam solution used for the RIVP was the lowest volume recommended for injection by the product manufacturer. The reconstituted drug was administered via the catheter in the DCDV, then the catheter was flushed with 5 mL of heparinized saline solution (approx 10 U of heparin/mL of saline solution) to ensure the entire dose was delivered. The tourniquet was left in place for 45 minutes, after which it was removed, and the cow was returned to a standing position with access to food and water for the remaining duration of the sampling period.

**Sample collection**

Samples were collected from all 3 catheters (jugular vein, DCDV, metatarsophalangeal joint) at 0 (immediately before), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, and 24 hours after RIVP. The cow was restrained in left lateral recumbency on a hydraulic tilt chute and sedated with xylazine (20 mg, IV).
24 hours after RIVP. The jugular and DCDV catheters were flushed with 5 mL and 1.2 mL of heparinized saline solution, respectively, after each sample collection. Approximately 0.5 to 1.0 mL of SYN was aspirated from the catheter in the metatarsophalangeal joint at each time. Approximately 12 mL of blood was collected from the catheter in the jugular vein, and 5 mL of blood was collected from the catheter in the DCDV at each time. The volume of blood collected from each catheter included a waste sample of blood and residual flush solution for discard, as determined by the catheter and extension volume, followed by the blood sample collection for analysis. At 0.25 hours after RIVP, a single 1- to 2-mL blood sample was obtained from the right APPV via standard phlebotomy with a 19-gauge butterfly needle. All blood and SYN samples were placed on ice in a cooler immediately after collection and remained refrigerated until the blood samples were centrifuged within 24 hours after collection. Serum was harvested from each blood sample following centrifugation and placed in plastic microcentrifuge tubes. The serum and SYN samples were then stored at -80°C until analysis. All catheters were removed following collection of the final blood and SYN samples 24 hours after RIVP.

**Determination of serum and SYN ampicillin and sulbactam concentrations**

Ampicillin and sulbactam concentrations in serum and SYN samples were determined by use of HPLC. The HPLC method used was validated with a blank (control) matrix from cattle that had not received either ampicillin or sulbactam. Ampicillin sodium was used as the reference standard for ampicillin and was dissolved in distilled water to prepare a spiking solution for calibration-curve and QC samples. Sulbactam analytic reference material was used as the reference standard for sulbactam and was likewise dissolved in distilled water to prepare a spiking solution for calibration-curve and QC samples. The calibration curve for measurement of serum ampicillin concentrations consisted of a zero (blank) concentration and 8 fortified serum samples with ampicillin concentrations that ranged from 0.05 to 10 µg/mL. The calibration curve for measurement of serum sulbactam concentrations consisted of a blank concentration and 6 fortified serum samples with sulbactam concentrations that ranged from 0.1 to 100 µg/mL. All incurred samples, calibration-curve samples, and QC samples were processed in the same manner.

To determine the ampicillin concentration in each serum sample, a solid-phase extraction column was conditioned with methanol and distilled water in accordance with the manufacturer’s instructions, then 300 µL of serum was added to the column. The sample was eluted with methanol and evaporated under a stream of air at 40°C until a dry residue was obtained. The sample was then reconstituted with 200 µL of the mobile phase, which consisted of 10% acetonitrile and 90% phosphate buffer (0.05M), and 30 µL of the reconstituted sample was injected into the HPLC system. Retention time for ampicillin was approximately 5 to 5.2 minutes.

To determine the serum sulbactam concentration, 400 µL of a serum sample and 400 µL of acetonitrile were added to a clean microcentrifuge tube. The tube was vortexed and centrifuged for 10 minutes, then 500 µL of the supernatant was transferred to a clean tube and evaporated under a stream of air at 40°C. The dry residue was reconstituted with 200 µL of the mobile phase, which consisted of 96% phosphate buffer and 4% acetonitrile adjusted to a pH of 5.5, and 40 µL of the reconstituted sample was injected into the HPLC system. Retention time for sulbactam was 3 to 3.2 minutes.

Both ampicillin and sulbactam could be detected during the same run for the SYN samples, which were treated with hyaluronidase prior to analysis. Briefly, 10 µL of hyaluronidase was added to 200 µL of each SYN sample, then the samples were vortexed and centrifuged. For each sample, 15 µL of the resulting supernatant was injected directly into the HPLC system. A calibration curve consisted of a zero concentration and 5 fortified SYN samples with sulbactam concentrations that ranged from 10 to 100 µg/mL and ampicillin concentrations that ranged from 10 to 500 µg/mL. The retention time for ampicillin was approximately 5 to 5.2 minutes, and that for sulbactam was approximately 3 to 3.2 minutes.

For both serum and SYN samples, separation of peaks was achieved at 40°C with a 4.6 X 150-mm reverse-phase column. The system consisted of a quaternary solvent delivery system, autosampler, UV detector with absorbance set at 229 nm, and built-in software suite for data collection and analysis. Mobile phases for both ampicillin and sulbactam were prepared fresh and filtered and degassed prior to use. The flow rate was 1 mL/min.

For both ampicillin and sulbactam in each matrix (serum and SYN), the calibration curves had to have a linear concentration range with an $R^2 \geq 0.99$, and the respective drug concentrations for the calibration samples had to be back calculated to within 15% of the nominal concentration for the curve to be accepted. Fresh calibration curves were prepared for each day’s run. The lower LOQ was defined as the lowest concentration of analyte (ampicillin or sulbactam) that could be quantified with acceptable precision and accuracy, and was established as the lowest point on a linear calibration curve that produced a signal-to-noise ratio of at least 6. Serum and SYN samples that had an ampicillin or sulbactam concentration greater than the upper limit of the appropriate calibration curve were diluted with the appropriate mobile phase and reanalyzed until the drug concentration was within the range of the calibration curve. A linear response for diluted samples was confirmed by testing of diluted fortified samples.

**Data analysis**

Data were analyzed by use of a pharmacokinetic-pharmacodynamic add-in program for a commercial spreadsheet program. Similar to other studies of RIVP in large animals, a noncompartmental model provided the best fit for the data. The $T_{\text{SYNC/MIC}}$ was determined by calculating the x-axis intercept for the
line of best fit for the terminal elimination phase of the concentration-time curve of ampicillin for each cow when various MIC values were used for the y-axis.

**Results**

**Cows**

All 6 cows had signs of moderate discomfort during placement of the tourniquet or administration of the RIVP, which subsided as soon as the tourniquet was removed. The cows were monitored for a minimum of 2 weeks after the study, and no additional adverse events were reported during or after sample collection.

**Serum and SYN ampicillin and sulbactam concentrations**

The lower LOQ for ampicillin was 0.05 µg/mL and 5 µg/mL for serum and SYN samples, respectively. The lower LOQ for sulbactam was 0.1 µg/mL and 5 µg/mL for serum and SYN samples, respectively.

Low concentrations of ampicillin and sulbactam were detected in some serum samples obtained from the DCDV and jugular vein prior to RIVP. At 24 hours after RIVP, ampicillin was undetectable in the systemic circulation for 3 of the 6 cows, and the serum ampicillin concentration was either near (n = 2) or below (1) the lower LOQ for the remaining 3 cows. Ampicillin was not detected in the systemic circulation at any time for 1 cow. At 8 hours after RIVP, sulbactam was undetectable in the systemic circulation for 2 of the 6 cows, and the serum sulbactam concentration was below the lower LOQ for 1 cow. The serum sulbactam concentration was near the lower LOQ at 24 hours after RIVP for the remaining 3 cows.

The mean serum ampicillin concentration over time for blood samples obtained from the DCDV and jugular vein was summarized (Figure 2). Similarly, the mean serum ampicillin and sulbactam concentrations over time for the cows of Figure 2. Synovial fluid samples were collected from the metatarsophalangeal joint of the right hind limb at the same times that blood samples were collected. See Figure 2 for remainder of key.

**Table 1**—Summary pharmacokinetic data for ampicillin and sulbactam in SYN and serum obtained from blood samples collected from the jugular vein, DCDV, and APPV of 6 healthy adult nonlactating Jersey-crossbred cows that received 1.5 g of ampicillin-sulbactam (1 g of ampicillin and 0.5 g of sulbactam) as an RIVP in the DCDV of the right hind limb.

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Sample type</th>
<th>Drug</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0–t&lt;/sub&gt; (µg/mL•h)</th>
<th>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (µg/mL•h)</th>
<th>MRT&lt;sub&gt;0–∞&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metatarsophalangeal joint of right hind limb SYN</td>
<td>Ampicillin</td>
<td>1,995 ± 1,011</td>
<td>1.0 ± 0.32</td>
<td>4,233 ± 930</td>
<td>4,342 ± 866</td>
<td>3.1 ± 0.98</td>
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<tr>
<td></td>
<td>Sulbactam</td>
<td>885 ± 320</td>
<td>1.0 ± 0.32</td>
<td>1,823 ± 406</td>
<td>1,892 ± 421</td>
<td>3.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>DCDV of right hind limb Serum</td>
<td>Ampicillin</td>
<td>4,827 ± 1,833</td>
<td>0.25 ± 0</td>
<td>4,939 ± 1,343</td>
<td>4,941 ± 1,344</td>
<td>0.73 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulbactam</td>
<td>4,456 ± 1,337</td>
<td>0.25 ± 0</td>
<td>4,034 ± 888</td>
<td>4,034 ± 888</td>
<td>0.6 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Jugular vein Serum</td>
<td>Ampicillin</td>
<td>2.5 ± 1.57</td>
<td>5.4 ± 4.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulbactam</td>
<td>1.4 ± 0.51</td>
<td>1.6 ± 0.20</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>APPV of lateral digit of right hind limb* Serum</td>
<td>Ampicillin</td>
<td>5,422 ± 1,953</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulbactam</td>
<td>5,261 ± 2,038</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the mean ± SD. Unless otherwise noted, blood and SYN samples were collected at 0 (immediately before), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, and 24 hours after RIVP.

— = Not calculated. AUC<sub>0–t</sub> = Area under the concentration-time curve from time 0 to the final sample collection. AUC<sub>0–∞</sub> = Area under the concentration-time curve from time 0 extrapolated to infinity. C<sub>max</sub> = Maximum concentration. MRT<sub>0–∞</sub> = Mean residence time extrapolated to infinity. T<sub>max</sub> = Time at maximum concentration.

*Only 1 blood sample was collected from the APPV at 0.25 hours after RIVP.
the mean SYN ampicillin and sulbactam concentrations over time (Figure 3) were summarized. The SYN sulbactam concentration over time followed a similar trend as the SYN ampicillin concentration over time, and the sulbactam-to-ampicillin ratio remained near or above 1:2. Summary pharmacokinetic data for both ampicillin and sulbactam in serum and SYN samples (Table 1) and the mean ± SD T_{[SYN] > MIC} for various MICs (Table 2) were summarized.

Discussion

Results of the present study indicated that administration of 1.5 g of an ampicillin-sulbactam combination formulation as an RIVP via a DCDV to cattle was a fairly safe procedure that achieved prolonged and potentially therapeutic drug concentrations in the regional circulation and SYN of the metatarsophalangeal joint of the injected limb. The high concentrations of both ampicillin and sulbactam achieved in the digital circulation (APPV and DCDV) and SYN indicated that both drugs diffused from the site of administration into the area distal to the tourniquet. Ampicillin is a time-dependent antimicrobial; therefore, treatment efficacy is dependent on maintaining ampicillin concentrations greater than the MIC for the pathogen of interest at the site of infection for ≥ 50% of the dosing interval.\(^{35,41}\) The SYN ampicillin concentration remained above 8 µg/mL, the CLSI breakpoint for ampicillin-susceptible bacterial isolates of human origin, for approximately 19 hours and remained above other CLSI large animal breakpoints (0.5 and 0.25 µg/mL) for ampicillin-susceptible bacterial isolates for > 24 hours. Thus, the duration of a presumably therapeutic concentration of ampicillin in SYN following RIVP of ampicillin-sulbactam for the cows of the present study was greater than that in the serum of calves and sheep following systemic (IV or IM) administration of the same ampicillin-sulbactam combination or in the SYN of calves following IM administration of a similar antimicrobial, ampicillin trihydrate.\(^{43}\)

The detection of low concentrations of ampicillin and sulbactam in some serum samples obtained from blood collected from the DCDV and jugular vein prior to the RIVP was unexpected and surprising because none of the cows had received any pharmaceuticals containing ampicillin or sulbactam within the 6 months prior to RIVP. We believe those findings were the result of the samples being contaminated with 1 drug or both drugs. Although the source of that contamination was not identified, it may have been caused by crossover contamination during HPLC analysis because some of the calibration-curve and QC samples contained very high drug concentrations and were analyzed before the serum samples collected immediately before RIVP were analyzed. A similar problem was described by investigators of a study\(^{36}\) in which cefazolin was used as an RIVP in cattle. Nevertheless, the low serum ampicillin and sulbactam concentrations in samples obtained prior to RIVP did not affect the pharmacokinetic analysis or our conclusions.

Another unexpected finding of the present study was the increase in both the mean SYN ampicillin and sulbactam concentrations for samples collected 18 hours after RIVP, compared with those for samples collected 12 hours after RIVP. The cause for this was unknown.

The mean maximum concentrations of ampicillin and sulbactam in serum samples obtained from blood collected from the jugular vein were significantly lower than those in serum samples obtained from blood collected from the DCDV and APPV as well as those in SYN samples collected from the metatarsophalangeal joint of the injected limb. Although the collected data were insufficient to reliably calculate other pharmacokinetic parameters for serum samples obtained from the jugular vein, the low mean maximum concentrations for both drugs and the inability to detect or quantify either drug in samples collected at the later sample acquisition times suggested that systemic exposure to the ampicillin-sulbactam combination was lower than regional exposure following RIVP. Tissue drug concentrations were not determined in the present study, and the study cows were not lactating. Therefore, we could not estimate a slaughter (meat) or milk withdrawal interval following RIVP of ampicillin-sulbactam in the distal limb of a cow.

In the present study, DCDV blood samples were acquired from the same catheter used to administer the RIVP of ampicillin-sulbactam. That is less than ideal because the catheter can become contaminated with the drug, which can introduce error for blood samples collected after drug administration. The catheter in the DCDV was flushed with heparinized saline solution after drug administration and between each blood sample collection in an attempt to minimize such error. Potential consequences of that sampling technique include blood sample dilution or artificially high drug concentrations from contamination of blood samples with drug residue remaining in the catheter. We believe the high serum ampicillin and sulbactam concentrations de-

\[\text{Table 2—Mean ± SD } T_{[\text{SYN} > \text{MIC}} \text{ and percentage of a 24-hour period that the } T_{[\text{SYN} > \text{MIC}} \text{ for various MICs as determined by use of data obtained for the cows of Table 1.}\]

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>T_{[\text{SYN} &gt; \text{MIC}} (h)</th>
<th>Percentage of a 24-hour period that T_{[\text{SYN} &gt; \text{MIC}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>33.5 ± 6.7</td>
<td>140</td>
</tr>
<tr>
<td>0.50</td>
<td>30.6 ± 6.1</td>
<td>128</td>
</tr>
<tr>
<td>1.00</td>
<td>27.7 ± 5.5</td>
<td>115</td>
</tr>
<tr>
<td>2.00</td>
<td>24.8 ± 5.0</td>
<td>103</td>
</tr>
<tr>
<td>4.00</td>
<td>21.8 ± 4.5</td>
<td>91</td>
</tr>
<tr>
<td>8.00</td>
<td>18.9 ± 4.0</td>
<td>78</td>
</tr>
<tr>
<td>16.00</td>
<td>16.0 ± 3.7</td>
<td>67</td>
</tr>
<tr>
<td>32.00</td>
<td>13.1 ± 3.4</td>
<td>55</td>
</tr>
</tbody>
</table>

The T_{[\text{SYN} > \text{MIC}} was determined by calculating the x-axis intercept for the line of best fit for the terminal elimination phase of the concentration-time curve of ampicillin for each cow when various MIC values were used for the y-axis. See Table 1 for remainder of key.
ected in serum obtained from DCDV blood samples were accurate because they were of similar magnitude to the ampicillin and sulbactam concentrations detected in serum obtained from APPV blood samples and SYN samples. Another consequence of the blood-sampling technique used in the present study was potential removal of the drug from local circulation before removal of the tourniquet; that could have led to sampling-induced drug elimination while the drug was still being distributed to the tissues distal to the tourniquet.

The small volume (4 mL) of ampicillin-sulbactam administered was selected to ensure that the entire dose could be administered to all cows without drastically increasing the pressure within the DCDV or causing catheter leakage. Moreover, the right hind limb was not exsanguinated prior to the RIVP, and a large volume of perfusate could decrease the efficacy of the tourniquet.

In the present study, high and potentially therapeutic concentrations of ampicillin and sulbactam were achieved in regional serum and SYN samples obtained distal to the tourniquet; SYN concentrations remained at potentially therapeutic values for > 12 hours following RIVP of 1.5 g of an ampicillin-sulbactam combination formulation in the DCDV of the right hind limb of healthy adult cows. Further research is necessary to determine the efficacy of RIVP administration of ampicillin-sulbactam for the treatment of cattle with DDS and establish appropriate milk and slaughter withdrawal intervals for treated cattle.

Acknowledgments

This study was performed at The Ohio State University Veterinary Medical Center.

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Footnotes

b. Xylazine HCl (20 mg/mL), Lloyd laboratories, Shenandoah, Iowa.
c. Milacath extended use, 14-gauge X 13-cm catheter, Mila International Inc, Erlander, Ky.
e. Lidozaine, VetOne, Boise, Idaho.
f. Epidural pain management kit (20-gauge catheter, 18-gauge X 7.5-cm Touhy needle), Mila International Inc, Erlander, Ky.
g. Surflo IV catheter, Teruma Medical Corp, Somerset, NJ.
h. Superglu Duro, Pacer Technology Inc, Rancho Cucamonga, Calif.
i. Heparin sodium (10,000 U/10 mL), NOVAPLUS, Schaumburg, Ill.

References


