Comparative pharmacokinetics of two florfenicol formulations following intramuscular and subcutaneous administration to sheep

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OBJECTIVE
To compare the pharmacokinetics of 2 commercial florfenicol formulations following IM and SC administration to sheep.

ANIMALS
16 healthy adult mixed-breed sheep.

PROCEDURES
In a crossover study, sheep were randomly assigned to receive florfenicol formulation A or B at a single dose of 20 mg/kg, IM, or 40 mg/kg, SC. After a 2-week washout period, each sheep was administered the opposite formulation at the same dose and administration route as the initial formulation. Blood samples were collected immediately before and at predetermined times for 24 hours after each florfenicol administration. Plasma florfenicol concentrations were determined by high-performance liquid chromatography. Pharmacokinetic parameters were estimated by noncompartmental methods and compared between the 2 formulations at each dose and route of administration.

RESULTS
Median maximum plasma concentration, elimination half-life, and area under the concentration-time curve from time 0 to the last quantifiable measurement for florfenicol were 3.76 µg/mL, 13.44 hours, and 24.88 µg•h/mL, respectively, for formulation A and 7.72 µg/mL, 5.98 hours, and 41.53 µg•h/mL, respectively, for formulation B following administration of 20 mg of florfenicol/kg, IM, and 2.63 µg/mL, 12.48 hours, and 31.63 µg•h/mL, respectively, for formulation A and 4.70 µg/mL, 16.60 hours, and 48.32 µg•h/mL, respectively, for formulation B following administration of 40 mg of florfenicol/kg, SC.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that both formulations achieved plasma florfenicol concentrations expected to be therapeutic for respiratory tract disease caused by Mannheimia haemolytica or Pasteurella spp at both doses and administration routes evaluated. (Am J Vet Res 2018;79:107–114)

Respiratory tract disease causes substantial morbidity and death on commercial sheep-raising operations and was responsible for 5.1% of nonpredator-related deaths of adult sheep in 2014.1,2 Mannheimia haemolytica and Pasteurella multocida, the bacte-rial pathogens most frequently associated with lower respiratory tract disease in sheep, are also associated with pneumonia in cattle.3,4 Thus, antimicrobials approved by the US FDA for the treatment of BRD in cattle may also be effective for the treatment of pneumonia in sheep. There are at least 16 antimicrobial compounds approved by the FDA for use in cattle for the treatment, prevention, or control of BRD, available in over 100 commercially available products in various concentrations and formulations and labeled for various routes of administration.5 In contrast, there are only 5 antimicrobials (ceftiofur sodium, tilmicosin, penicillin G procaine, oxytetracycline hydrochloride, and neomycin) approved by the FDA for the treatment, prevention, or control of respiratory tract disease in sheep.6–9

Florfenicol is a fluorinated derivative of thiamphenicol. It is a bacteriostatic antimicrobial with a broad spectrum of activity against gram-negative and gram-pos-

ABBREVIATIONS

\( \lambda_e \) Elimination rate constant

BRD Bovine respiratory disease

\( C_{\text{max}}(\text{obs}) \) Observed maximum plasma concentration

MIC Minimum inhibitory concentration

MIC_{50} Minimum antimicrobial concentration that inhibits growth of 50% of organisms

MIC_{90} Minimum antimicrobial concentration that inhibits growth of 90% of organisms

\( t_{1/2} \) Elimination half-life

\( T_{\text{high}}(\text{obs}) \) Observed duration during which the plasma drug concentration was greater than the MIC (1.0 µg/mL)

\( t_{\text{max}}(\text{obs}) \) Time at which the maximum plasma concentration was observed

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itive bacteria including *M. baemolytica*, *P. multocida*, and some isolates of *Escherichia coli*, *Staphylococcus aureus*, and *Trueperella pyogenes*. The Clinical Laboratories Standards Institute has established only a few breakpoints for florfenicol in livestock. In cattle, the florfenicol breakpoint is ≤ 2 µg/mL for susceptible isolates of *M. baemolytica*, *P. multocida*, and *Histophilus somni*. In swine, the florfenicol breakpoint is ≤ 4 µg/mL for susceptible isolates of *Salmonella enterica* serotype Cholerasuis and ≤ 2 µg/L for susceptible isolates of *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, and *Bordetella bronchiseptica*. Florfenicol has good penetration of CSF in calves, tear fluid in sheep, and synovial fluid in cattle. It is approved by the FDA for parenteral administration to beef and nonlactating dairy cattle for the treatment and control of BRD associated with *M. baemolytica*, *P. multocida*, and *H. somni* as well as for treatment of bovine interdigital phlegmon (foot rot) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*. Florfenicol is also used for the treatment of nonrespiratory tract diseases in sheep including *Campylobacter jejuni*-induced abortion and foot rot and has been suggested for use as treatment for infectious keratoconjunctivitis in sheep.

Currently, there are 2 formulations of florfenicol commercially available for parenteral administration to livestock in the United States. One formulation contains florfenicol (300 mg/mL) in the excipient N-methyl-2-pyrrolidone (formulation A) and is labeled for the treatment and prevention of BRD associated with *M. baemolytica*, *P. multocida*, and *H. somni* and treatment of foot rot associated with *F. necrophorum* and *B. melaninogenicus* in cattle at a dosage of 20 mg/kg, IM, twice with a 48-hour interval between doses or 40 mg/kg, SC, once. The other formulation contains florfenicol (300 mg/mL) in the excipient 2-pyrrolidone and triacetin (formulation B) and is labeled for the treatment of BRD associated with *M. baemolytica*, *P. multocida*, *H. somni*, and *Mycoplasma bovis* in beef and nonlactating dairy cattle at a dosage of 40 mg/kg, SC, once.

The pharmacokinetics of formulation A has been determined following IV, IM, and SC administration to sheep, goats, and camels. Limited pharmacokinetic research has been performed for formulation B, although the pharmacokinetics of formulations A and B have been compared following administration to feedlot calves for the treatment of BRD. To our knowledge, the pharmacokinetics of formulations A and B following administration to sheep has not been compared. The objective of the study reported here was to compare the pharmacokinetics of those 2 formulations following administration of a single dose of 20 mg/kg, IM, and 40 mg/kg, SC, to healthy adult sheep.

**Materials and Methods**

**Animals**

The study was approved by the University of California-Davis Institutional Animal Care and Use Committee (protocol No. 15471) and was conducted at the University of California–Davis Hopland Research and Extension Center. Sixteen adult mixed-breed sheep (8 ewes and 8 wethers) with ages ranging from 12 to 24 months and a median weight of 37.5 kg (range, 32 to 44 kg) were used for the study. Prior to study initiation, all sheep were determined to be healthy on the basis of results of a physical examination. The sheep were housed in a single pen that included a shelter and outdoor access, were fed alfalfa hay, and had ad libitum access to water.

**Study design**

The study had a 2-way crossover design. Initially, sheep were divided into 2 groups on the basis of sex; then, the sheep within each group were stratified on the basis of weight. The study population was then resorted into 4 subgroups, each of which contained 2 ewes and 2 wethers and had a similar weight distribution. Each subgroup was randomly assigned by pulling numbers from a hat to receive florfenicol formulation A (florfenicol [300 mg/mL] with the excipient N-methyl-2-pyrrolidone) or B (florfenicol [300 mg/mL] with the excipient 2-pyrrolidone and triacetin) by the IM or SC route such that 1 group received formulation A (florfenicol dose, 20 mg/kg) by the IM route, 1 group received formulation B (florfenicol dose, 20 mg/kg) by the IM route, 1 group received formulation A (florfenicol dose, 40 mg/kg) by the SC route, and 1 group received formulation B (florfenicol dose, 40 mg/kg) by the SC route. Following a 2-week washout period, each group was administered the opposite formulation at the same dose and by the same route as the initial formulation administered. Both formulations were injected into the cervical musculature or region during IM and SC administration, respectively. For each sheep, the volume of the assigned formulation required was calculated and preloaded into a syringe. To ensure dosing accuracy, syringes used for florfenicol administration were weighed prior to filling, after they were filled with the calculated volume, and after the drug was injected.

**Blood sample collection**

Florfenicol administration was designated as day 1. For each sheep on day 0 prior to administration of the first formulation, a catheter was aseptically placed in a jugular vein for blood sample collection. The catheter was flushed with 6 mL of heparinized saline solution (0.9% NaCl solution with 1% sodium heparin) immediately after placement and every 6 hours thereafter until blood sample collection was initiated and after each blood sample collection. It was removed immediately after collection of the last blood sample. The process was repeated for administration of the second formulation, except the catheter was placed in the opposite jugular vein.

For each sheep, a blood sample (10 mL) was obtained and placed into a blood collection tube containing lithium heparin as an anticoagulant immediately before (0 hours) and at 0.25, 0.50, 0.75, 1.0, 1.5,
2, 4, 6, 8, 12, 18, and 24 hours after florfenicol administration. Prior to each sample collection, 10 mL of blood was withdrawn from the catheter and discarded to ensure that the sample collected for analysis was not diluted with any residual heparinized saline solution from the preceding catheter flush. Blood samples were centrifuged at 2,000 × g at 37°C for 15 minutes. Plasma was harvested from each sample and stored at approximately -18°C for up to 36 hours until transport to the analytical laboratory, after which they were stored at -80°C until analysis.

Determination of plasma florfenicol concentration

The florfenicol concentration in each plasma sample was determined as described.22 Briefly, plasma samples were deproteinized with acetone and then extracted with ethyl acetate. The ethyl acetate was dried, and the residue was dissolved in a mobile phase that consisted of 35% acetonitrile in water for analysis. Sample analysis was performed with high-performance liquid chromatography6 and UV detection at 224 nm on a dual-wavelength absorbance detector. A 5-μm, 4.6 × 250-mm column with a 5-μm, 4.6 × 12.5-mm precolumn6 was used. The mean recovery of florfenicol was 96.2%, the limit of quantification was 0.05 μg/mL, and the limit of detection was 0.02 μg/mL. The pharmacokinetic assay was linear within florfenicol concentrations of 0.05 to 5 μg/mL, and the intraday and inter-day coefficients of variation were 7.7% and 13.4%, respectively.

Pharmacokinetic analysis

Individual plasma florfenicol concentration-time data were tabulated for all sheep, and the data were plotted by use of a commercially available program.1 A semilogarithmic scale was used to visually evaluate concentration-time data for the 2 formulations and routes of administration. The C max(obs), t max(obs), and T high(obs) were determined directly from the concentration-time data. The area under the plasma concentration-time curve from time 0 to infinity, area under the plasma concentration-time curve from time 0 to the last quantifiable measurement, λ z, and t 1/2β of florfenicol were estimated by use of noncompartmental methods performed by a commercially available software program.1

Statistical analysis

The data distribution for each pharmacokinetic parameter was evaluated for normality by means of the Shapiro-Wilk test and use of a commercial software program.1 Results indicated that none of the pharmacokinetic parameters were normally distributed. The data underwent a logarithmic transformation, were rechecked for normality, and still had nonparametric distributions. Therefore, pharmacokinetic parameters were compared between formulations A and B by use of a Mann-Whitney test performed with a statistical software program.8 and results were reported as the median (range). For all analyses, values of P < 0.05 were considered significant.

MIC data

The MICs of florfenicol for ovine bacterial isolates identified between January 1, 2011, and December 31, 2016, at 4 veterinary diagnostic laboratories (University of California-Davis Veterinary Medical Teaching Hospital Clinical Microbiology Laboratory, South Dakota Animal Disease Research and Diagnostic Laboratory, University of Missouri Veterinary Medical Diagnostic Laboratory, and Iowa State Veterinary Diagnostic Laboratory) were compiled. All MICs were determined by use of the broth microdilution technique1 in accordance with guidelines described by the Clinical Laboratory Standards Institute.31 The MIC 50 and MIC 90 were calculated when data were available for ≥ 10 isolates of a particular pathogen. On the basis of those data and results of antimicrobial susceptibilities for respiratory tract pathogens reported in previous surveys,34 a plasma florfenicol concentration of 1.0 μg/mL was selected as the target MIC required to effectively treat disease caused by M haemolytica and P multocida in sheep.

Figure 1—Mean ± SD plasma florfenicol concentration over time for 8 healthy adult mixed-breed sheep following IM administration of a single dose (20 mg/kg) of formulation A (florfenicol [300 mg/mL] with the excipient N-methyl-2-pyrrolidone; white circles) and B (florfenicol [300 mg/mL] with the excipient 2-pyrrolidone and triacetin; black circles). The study had a crossover design. Sheep were separated into 2 groups (4 sheep/group) and then randomly allocated to receive either formulation A or B. After a 2-week washout period, each group received the opposite formulation. Notice the y-axis has a logarithmic scale.
Results

Pharmacokinetics

In 1 sheep, the plasma florfenicol concentration peaked twice (18 µg/mL at 0.25 hours and 53.3 µg/mL at 1 hour) after IM administration of formulation B. In another sheep, the t1/2 was exceptionally long (100.7 hours) following SC administration of formulation B, compared with that for the other sheep in the same treatment group. The data from those 2 sheep were included in the plasma florfenicol concentration plots, but were excluded from pharmacokinetic analyses. The mean ± SD plasma florfenicol concentrations over time following IM (Figure 1) and SC (Figure 2) administration of florfenicol formulations A and B were plotted.

![Mean ± SD plasma florfenicol concentration over time for 8 healthy adult mixed-breed sheep following SC administration of a single dose (40 mg/kg) of formulation A (white circles) and B (black circles). See Figure 1 for remainder of key.](image)

Table 1—Median (range) values for pharmacokinetic parameters for florfenicol formulations A and B following IM administration of a single dose (20 mg/kg) to each of 8 healthy adult mixed-breed sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formulation A</th>
<th>Formulation B*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax(obs) (µg/mL)</td>
<td>3.76 (2.40–6.50)</td>
<td>7.72 (3.82–10.25)</td>
<td>0.005</td>
</tr>
<tr>
<td>tmax(obs) (h)</td>
<td>0.99 (0.50–1.53)</td>
<td>0.78 (0.51–1.03)</td>
<td>0.96</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>8.06 (5.69–20.68)</td>
<td>13.48 (7.64–15.78)</td>
<td>0.23</td>
</tr>
<tr>
<td>AUC0–t (µg•h/mL)</td>
<td>24.88 (21.91–40.51)</td>
<td>41.53 (31.84–48.25)</td>
<td>0.002</td>
</tr>
<tr>
<td>AUC0–∞ (µg•h/mL)</td>
<td>36.75 (27.80–63.04)</td>
<td>47.77 (39.42–52.00)</td>
<td>0.02</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>13.44 (8.27–24.11)</td>
<td>5.98 (1.50–14.58)</td>
<td>0.02</td>
</tr>
<tr>
<td>λz (h⁻¹)</td>
<td>0.05 (0.03–0.08)</td>
<td>0.12 (0.05–0.46)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Formulation A consisted of florfenicol (concentration, 300 mg/mL) with the excipient N-methyl-2-pyrrolidone, and formulation B consisted of florfenicol (concentration, 300 mg/mL) with the excipient 2-pyrrolidone and triacetin. The study had a crossover design. Sheep were separated into 2 groups (4 sheep/group) and then randomly allocated to receive either formulation A or B. After a 2-week washout period, each group received the opposite formulation. Pharmacokinetic parameters were estimated by noncompartmental methods. None of the parameters were normally distributed; therefore, a Mann-Whitney test was used for all comparisons between the 2 formulations. Values of P < 0.05 were considered significant.

AUC0–t = Area under the plasma concentration-time curve from time 0 to the last quantifiable measurement.

AUC0–∞ = Area under the plasma concentration-time curve from time 0 to infinity.

*The plasma florfenicol concentration in 1 sheep peaked twice following administration of formulation B, and the data from that sheep were excluded from the pharmacokinetic analysis. Therefore, only 7 sheep contributed to the median (range) values in this column.

Comparisons of pharmacokinetic parameters between formulations A and B following IM (Table 1) and SC (Table 2) administration were summarized. The median Cmax achieved for formulation B was significantly greater than that for formulation A, whereas the median tmax did not differ between the 2 formulations, regardless of whether the formulations were administered at 20 mg/kg, IM, or 40 mg/kg, SC. The median T1/2 for formulation B was greater than that for formulation A at both doses and administration routes, but that difference was not significant.

MIC data

During the 6-year search period, a total of 367 ovine isolates originating from sheep in 13 states were identified and had an MIC for florfenicol determined (Table 3). The MIC range, MIC0–, and MIC0– reported for florfenicol were ≤ 0.25 to 2.0, 0.5, and 1 µg/mL, respectively, for M baemolytica isolates and ≤ 0.25 to 1.0, 0.25, and 0.5 µg/mL, respectively, for P multocida isolates. Those data supported our selection of a target MIC for florfenicol of 1.0 µg/mL for the treatment of bacterial infections in sheep. Respiratory tract bacterial pathogens with a reported MIC0– > 1.0 µg/mL included Campylobacter spp (2.0 µg/mL) and Salmonella spp (2.0 µg/mL).

Discussion

Results of the present study indicated that some pharmacokinetic parameters were comparable, whereas others differed significantly, for the 2 florfenicol formulations approved by the FDA for parenteral administration to livestock in the United States when
they were administered by the IM and SC routes to healthy sheep. Nevertheless, the mean plasma florfenicol concentration achieved following administration of both formulations A (florfenicol [300 mg/mL] with the excipient N-methyl-2-pyrrolidone) and B (florfenicol [300 mg/mL] with the excipient 2-pyrrolidone and triacetin) at a dose of 20 mg/kg, IM, or 40 mg/kg, SC, was > 1 µg/kg (the target MIC of florfenicol required to treat respiratory tract disease caused by *M. haemolytica* and *P. multocida* in sheep selected on the basis of antimicrobial susceptibility data for ovine respiratory tract pathogens isolated at 4 veterinary diagnostic laboratories). The The 4 laboratories were the University of California-Davis Veterinary Medical Teaching Hospital Clinical Microbiology Laboratory, South Dakota Animal Disease Research and Diagnostic Laboratory, University of Missouri Veterinary Medical Diagnostic Laboratory, and Iowa State Veterinary Diagnostic Laboratory. The antimicrobial administration history was unavailable for the sheep from which the bacterial isolates were obtained.

The distribution of florfenicol concentration achieved following administration of a single dose (40 mg/kg) to each of 8 healthy adult mixed-breed sheep is provided in Table 2. The mean C\text{max}(obs) for formulation A was significantly lower than that for formulation B at both doses (20 mg/kg, IM, and 40 mg/kg, SC) evaluated. This could be clinically relevant because florfenicol has concentration-dependent activity against certain organisms. Distribution of florfenicol to the central compartment was rapid (within 2011 to 2016 and previous reports\(^{1,2}\)) for a period of time; thus, both formulations could be effective for the treatment of respiratory tract disease in sheep, although regional differences in antimicrobial susceptibility of pathogens should always be considered.

In the present study, the mean C\text{max}(obs) for formulation B was significantly greater than that for formulation A at both doses (20 mg/kg, IM, and 40 mg/kg, SC) evaluated. This could be clinically relevant for the treatment of respiratory tract disease in sheep because florfenicol has concentration-dependent activity against certain organisms.\(^{32}\)

Table 2—Median (range) values for pharmacokinetic parameters for florfenicol formulations A and B following SC administration of a single dose (40 mg/kg) to each of 8 healthy adult mixed-breed sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formulation A</th>
<th>Formulation B*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{max}(obs) (µg/mL)</td>
<td>2.63 (1.61–4.55)</td>
<td>4.70 (4.65–9.86)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>t\text{max} (h)</td>
<td>3.00 (0.29–6.00)</td>
<td>1.00 (0.76–3.03)</td>
<td>0.27</td>
</tr>
<tr>
<td>T\text{max} (h)</td>
<td>13.78 (8.28–23.81)</td>
<td>17.50 (12.98–23.94)</td>
<td>0.12</td>
</tr>
<tr>
<td>AUC\text{Cmax} (µg•h/mL)</td>
<td>31.63 (19.32–49.51)</td>
<td>48.32 (35.59–78.42)</td>
<td>0.01</td>
</tr>
<tr>
<td>AUC\text{Cinf} (µg•h/mL)</td>
<td>43.48 (22.50–64.06)</td>
<td>73.58 (54.40–104.80)</td>
<td>0.004</td>
</tr>
<tr>
<td>t\text{1/2} (h)</td>
<td>12.48 (7.51–19.75)</td>
<td>16.60 (6.59–33.76)</td>
<td>0.28</td>
</tr>
<tr>
<td>λ\text{e} (h(^{-1}))</td>
<td>0.06 (0.04–0.092)</td>
<td>0.04 (0.02–0.11)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*The t\text{1/2} for 1 sheep was exceptionally long (100.7 hours) following SC administration of formulation B, compared with that for the other sheep in the same treatment group, and the data for that sheep were excluded from the pharmacokinetic analysis. Therefore, only 7 sheep contributed to the median (range) values in this column.

See Table 1 for remainder of key.

Table 3—Summary florfenicol MIC data for ovine bacterial isolates identified between January 1, 2011, and December 31, 2016, at 4 veterinary diagnostic laboratories.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of isolates</th>
<th>MIC\text{50} (µg/mL)</th>
<th>MIC\text{90} (µg/mL)</th>
<th>MIC range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. trehalosi</em></td>
<td>16</td>
<td>1</td>
<td>8</td>
<td>0.5 to 8</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>45</td>
<td>1</td>
<td>2</td>
<td>0.5 to 8</td>
</tr>
<tr>
<td><em>C. pseudotuberculosis</em></td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>0.5 to 2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>41</td>
<td>4</td>
<td>8</td>
<td>2 to &gt; 8</td>
</tr>
<tr>
<td><em>M. haemolytica</em></td>
<td>117</td>
<td>0.5</td>
<td>1</td>
<td>≤ 0.25 to 2</td>
</tr>
<tr>
<td><em>P. multocida</em></td>
<td>50</td>
<td>0.25</td>
<td>0.5</td>
<td>≤ 0.25 to 1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12</td>
<td>0.5</td>
<td>1</td>
<td>≤ 0.25 to 1</td>
</tr>
<tr>
<td><em>S. spp</em></td>
<td>38</td>
<td>1</td>
<td>2</td>
<td>1 to &gt; 8</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>11</td>
<td>4</td>
<td>8</td>
<td>2 to &gt; 8</td>
</tr>
</tbody>
</table>

The 4 laboratories were the University of California-Davis Veterinary Medical Teaching Hospital Clinical Microbiology Laboratory, South Dakota Animal Disease Research and Diagnostic Laboratory, University of Missouri Veterinary Medical Diagnostic Laboratory, and Iowa State Veterinary Diagnostic Laboratory. The antimicrobial administration history was unavailable for the sheep from which the bacterial isolates were obtained. Data are provided only for the pathogens that had > 10 isolates identified.

Table 4—Summary of pharmacokinetic parameters for florfenicol following IM or SC administration to sheep and cattle as reported in the veterinary literature.

<table>
<thead>
<tr>
<th>Reference No.</th>
<th>Species</th>
<th>No. of animals</th>
<th>Formulation</th>
<th>Dose (mg/kg)</th>
<th>Route of administration</th>
<th>C\text{max} (µg/mL)</th>
<th>t\text{max} (h)</th>
<th>AUC\text{Cmax} (µg•h/mL)</th>
<th>t\text{1/2} (h)</th>
<th>λ\text{e} (h(^{-1}))</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21(^{1})</td>
<td>Sheep</td>
<td>6</td>
<td>A</td>
<td>20</td>
<td>IM</td>
<td>1.94 ± 0.10</td>
<td>1.44 ± 0.16</td>
<td>49.56 ± 5.51</td>
<td>3.25 ± 0.20</td>
<td>65.82 ± 6.71</td>
<td></td>
</tr>
<tr>
<td>23(^{1})</td>
<td>Sheep</td>
<td>5</td>
<td>A</td>
<td>20</td>
<td>IM</td>
<td>4.13 ± 0.29</td>
<td>1.45 ± 0.16</td>
<td>67.95 ± 0.61</td>
<td>10.34 ± 1.11</td>
<td>0.07 ± 0.01</td>
<td>89.04</td>
</tr>
<tr>
<td>25(^{1})</td>
<td>Sheep</td>
<td>5</td>
<td>B</td>
<td>20</td>
<td>IM</td>
<td>4.66 ± 0.21</td>
<td>2.0 ± 0.05</td>
<td>50.51 ± 1.51</td>
<td>3.01 ± 0.28</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>22(^{1})</td>
<td>Sheep</td>
<td>10</td>
<td>A</td>
<td>40</td>
<td>SC</td>
<td>2.64 ± 1.07</td>
<td>2.0 ± 1.0</td>
<td>42.2 ± 12.4</td>
<td>34.7 ± 9.6</td>
<td>0.02 ± 0.005</td>
<td>40.2 ± 16.2</td>
</tr>
<tr>
<td>30(^{1})</td>
<td>Cattle</td>
<td>24</td>
<td>A</td>
<td>40</td>
<td>SC</td>
<td>4.69 ± 0.73</td>
<td>6.0 (10–11)</td>
<td>141.78 (27)</td>
<td>51.79 (42)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>30(^{1})</td>
<td>Cattle</td>
<td>24</td>
<td>B</td>
<td>40</td>
<td>SC</td>
<td>5.93 ± 0.28</td>
<td>5.0 (2.0–2.0)</td>
<td>150.2 (20.9)</td>
<td>37.67 (27.3)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Results reported as mean ± SE. | AUC\text{Cinf} | Results reported as mean ± SD. | Systemic bioavailability from time 0 to infinity. | Results reported as mean (% coefficient of variation) or median (range).

— = Not reported. | AUC = Area under the plasma concentration-time curve. | F = Systemic availability. | λ\text{e} = Elimination rate constant.

See Table 1 for remainder of key.
1 and 3 hours after IM and SC administration, respectively) for both formulations. The $C_{\text{max,obs}}$ (median, 3.76 µg/mL; range, 2.40 to 6.50 µg/mL) and $t_{\text{max,obs}}$ (median, 0.99 hours; range, 0.50 to 1.53 hours) for formulation A following IM administration of the 20-mg/kg dose were similar to the mean ± SD $C_{\text{max}}$ and $t_{\text{max}}$ reported following IM administration of the same dose to sheep of other studies21,23,25,33 (Table 4). Following SC administration of the 40-mg/kg dose, the median $C_{\text{max,obs}}$ for both formulations A (2.63 µg/mL; range, 1.61 to 4.55 µg/mL) and B (4.70 µg/mL; range, 4.65 to 9.86 µg/mL) for the sheep of the present study was fairly similar to that reported for formulations A (4.69 µg/mL) and B (5.93 µg/mL) in cattle30; however, the median $t_{\text{max,obs}}$ for the sheep of this study (formulation A, 3 hours; formulation B, 1 hour) was less than that for the cattle of that study30 (formulation A, 6 hours; formulation B, 5 hours).

For the sheep of the present study, the median area under the plasma concentration-time curve from time 0 to infinity and area under the plasma concentration-time curve from time 0 to the last quantifiable measurement for formulation B were significantly greater than those for formulation A, regardless of whether the drug was administered by the IM or SC route. This finding was likely an artifact of flip-flop kinetics and the fact that only a small number of samples were collected during the elimination phase of the concentration-time curve. In the present study, the florfenicol concentration-time curve plateaued during the elimination phase in a manner similar to that reported following florfenicol administration to sheep of other studies.22–24 A persistent plateau in the concentration-time curve also occurs following IV administration of florfenicol, which further supports the occurrence of flip-flop kinetics owing to the continuous absorption and redistribution of florfenicol from the site of injection.24

The median plasma $t_{1/2,\beta}$ of florfenicol was significantly shorter and the median $\lambda_z$ was significantly longer for formulation B, compared with those for formulation A, following IM administration; however, the median $t_{1/2,\beta}$ and $\lambda_z$ did not differ significantly between the 2 formulations following SC administration. The median plasma $t_{1/2,\beta}$ of florfenicol for both formulations A (12.48 hours) and B (16.60 hours) following SC administration was shorter than that (34.7 hours) reported in another study22; however, the median $t_{1/2,\beta}$ of florfenicol for formulations A (13.44 hours) and B (5.98 hours) following IM administration was 2 to 5 times that reported following IM administration of the same dose to sheep of 2 other studies21,23 (Table 4). The median $t_{1/2,\beta}$ for both formulations A and B following SC administration to the sheep of this study was substantially shorter than that following SC administration of the same dose (40 mg/kg) of formulations A (51.79 hours) and B (37.67 hours) to cattle.30 The median $t_{1/2,\beta}$ of florfenicol in cattle following IM administration of formulation A at a dose of 20 mg/kg (18.3 hours)15 was 1.4 and 3 times that following IM administration of the same dose of formulations A and B, respectively, to the sheep of the present study. The reasons for the differences in the $t_{1/2,\beta}$ and $\lambda_z$ between the sheep of this study and cattle of those other studies21,30 are unknown. Unfortunately, information regarding the metabolism of florfenicol in animals is scarce. Results of a study involving calves indicate that florfenicol is excreted in its parent form via urine, which suggests the drug is cleared by the kidneys. However, to our knowledge, the metabolism of florfenicol in sheep has not been elucidated. Some differences in the pharmacokinetic parameters for florfenicol observed among the present study and other studies might be attributable to the use of less sensitive assay methods21 or individual variation among animals.22 Furthermore, although the $\lambda_z$ was estimated on the basis of the plasma florfenicol concentration at a minimum of 3 points during the elimination phase, a more optimal estimate would have been obtained had more blood samples been acquired during the elimination phase.

The MICs of florfenicol reported for ovine M. haemolytica and P. multocida isolates identified at 4 veterinary diagnostic laboratories during the 6-year period from 2011 to 2016 were similar to those reported in multiple studies.5,32,35,36 This finding indicated that florfenicol is active against various bacterial pathogens in vitro and suggested that it may be an effective treatment for sheep with respiratory tract disease or other diseases caused by susceptible organisms. However, the antimicrobial susceptibility pattern for some pathogens can vary among regions, and veterinarians should evaluate local antimicrobial susceptibility trends prior to using florfenicol to optimize the probability of treatment success. Additionally, the antimicrobial administration history was unavailable for the sheep from which the bacterial isolates described in this study were obtained (Table 3), and prior antimicrobial administration could have affected the MICs reported.

Although florfenicol is generally considered to have a time-dependent mechanism of action, it has a concentration-dependent mechanism of action against certain organisms32; therefore, the duration that the plasma florfenicol concentration is greater than the target MIC should be at least 50% of the dosing interval to achieve optimal inhibition of bacterial growth.37 On the basis of that guideline, the veterinary literature suggests that, in sheep, administration of florfenicol at a dose of 20 to 30 mg/kg, IM,5,25 or 40 mg/kg, SC,53 daily for 3 days is necessary to achieve and maintain plasma florfenicol concentrations that will be effective against most bacterial pathogens. That dosing interval is shorter than the FDA-approved dosing interval (once or every 48 hours for SC and IM administration, respectively) for florfenicol in cattle.17,20 Further studies involving diseased sheep are necessary to evaluate the accumulation of and potential adverse effects associated with administration of multiple doses of florfenicol, especially in regard to injection site safety. In the
In the present study, the median $T_{\text{high(obs)}}$ was $> 12$ hours for formulation B following administration of both the IM (13.48 hours) and SC (17.50 hours) doses; however, it did not differ significantly from that for formulation A following administration of either dose, and was substantially less than the $T_{\text{high(obs)}}$ reported for sheep following IM administration of formulation A at a dose of 20 mg/kg in another study²⁻²⁵ (24 hours). Because the median $T_{\text{high(obs)}}$ did not differ between formulations A and B following administration by either the IM or SC route, altering the dosing interval of florfenicol to sheep on the basis of formulation might be unnecessary. Nonetheless, IM administration of formulation A (20 mg/kg) resulted in the shortest median $T_{\text{high(obs)}}$ (8 hours) in the present study, and the $T_{\text{high(obs)}}$ should be considered when patients fail to respond to treatment. Practitioners are also encouraged to use regional MIC data to calculate appropriate dosing intervals for florfenicol to optimize treatment efficacy.

In the United States, administration of florfenicol to sheep is considered extralabel drug use, which is permitted as long as it is in accordance with the AMDUCA, and requires practitioners to determine an appropriate withdrawal interval. Results of a study²⁻²⁵ in which sheep were administered 40 mg of formulation A/kg, SC, daily for 3 days indicate that treated sheep have florfenicol residues present in adipose tissue for a prolonged period necessitating a withdrawal interval for meat of at least 42 days. To our knowledge, tissue florfenicol residues in sheep following administration of formulation B have not been investigated. In the United States, veterinarians can contact the Food Animal Residue Avoidance Databank³⁸ to obtain scientifically based withdrawal intervals for drugs administered in an extralabel manner in accordance with AMDUCA.

In the present study, the median $C_{\text{max(obs)}}$ of florfenicol in sheep following administration of formulation B was significantly greater than that following administration formulation A, regardless of whether the 2 formulations were administered at a dose of 20 mg/kg, IM, or 40 mg/kg, SC, and that difference may be clinically relevant and affect treatment outcome. Although the median $T_{\text{high(obs)}}$ of florfenicol did not differ between the 2 formulations at the doses and administration routes evaluated, it was shortest following administration of formulation A at a dose of 20 mg/kg, IM. The MICs of florfenicol for ovine isolates of $M$ hemolytica and $Pasteurella$ spp, common respiratory tract pathogens, ranged from 0.25 to 1.0 µg/mL. Given the plasma florfenicol concentrations achieved in the sheep of the present study, both florfenicol formulations evaluated may be effective against those and other bacterial pathogens of sheep. However, antimicrobial susceptibility results for bacterial pathogens can vary regionally, and local antimicrobial susceptibility trends should be evaluated prior to use of florfenicol to optimize the probability of treatment success. Even though the median $t_{1/2}$ was estimated for each of the 4 treatments evaluated in the present study, a multiple-dose study in which plasma florfenicol concentrations are determined more frequently during the elimination phase than in this study is warranted as is a tissue residue study following administration of formulation B to sheep.

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The authors declare that there were no conflicts of interest.

Footnotes

a. Nuflor injectable solution, Schering-Plough Animal Health, Kenilworth, NJ.
b. Nuflor Gold injectable solution, Intervet Schering-Plough Animal Health, Roseland, NJ.
c. Milacath, Mila International Inc. Erlanger, Ky.
d. 0.9% Sodium chloride injection USP, Baxter Healthcare Corp, Deerfield, III.
e. Heparin sodium injection USP (1000 U/mL), APP Pharmaceuticals LLC, Schaumburg, Ill.
g. Waters 2690 Alliance System, Waters Corp, Milford, Mass.
h. Zorbax SB-C18, 5 µm, 4.6 X 250 and 4.6 X 12.5 mm, Agilent Technologies Inc. Wilmington, Del.
i. SAS, version 9.4, SAS Institute Inc, Cary, NC.
k. Prism, version 7.0, GraphPad Software Inc. La Jolla, Calif.
l. Sensititre, Thermo Fisher Scientific, Oakwood Village, Ohio.

References


