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Florfenicol concentrations in ovine tear fluid following intramuscular and subcutaneous administration and comparison with the minimum inhibitory concentrations against mycoplasmal strains potentially involved in infectious keratoconjunctivitis

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Objective—To measure florfenicol concentrations in ovine tear fluid after IM and SC administration and determine minimum inhibitory concentrations (MICs) of florfenicol against field isolates of *Mycoplasma* organisms potentially involved in infectious keratoconjunctivitis.

Animals—9 healthy adult Lacaune ewes.

Procedures—Animals received an IM and SC administration of florfenicol (20 mg/kg) in a 2-way crossover design. Samples of blood and tear fluid were collected before and for 24 hours after administration. Concentrations of florfenicol in plasma and tear fluid were measured via high-performance liquid chromatography. The MIC of florfenicol for various *Mycoplasma* strains cultured from sheep and goats was determined via an agar dilution method.

Results—Mean florfenicol concentration in tear fluid for the 24-hour period was significantly higher after IM administration (0.70 µg/mL) than after SC administration (0.22 µg/mL) and was maintained for a longer duration. The lacrimal fluid-to-plasma concentration ratio was not different between the 2 routes of administration, with mean values of 40.2% and 32.5% after IM and SC administration, respectively. The MIC for *Mycoplasma agalactiae*, *Mycoplasma conjunctivae*, and *Mycoplasma mycoides* isolates ranged from 0.5 to 8 µg of florfenicol/mL. Two strains of *M. agalactiae* could be considered resistant to florfenicol.

Conclusions and Clinical Relevance—Florfenicol readily penetrated the preocular tear fluid of sheep after IM and SC administration. For both routes of administration, doses > 20 mg/kg would be necessary to achieve tear fluid concentrations of florfenicol greater than the MICs for most strains of *Mycoplasma* organisms. (*Am J Vet Res* 2013;74:268–274)

Infectious keratoconjunctivitis (contagious ophthalmia) is a contagious ocular disease that affects sheep,^{1–3} goats, and wild small ruminants.^{4,5} The disease manifests with acute clinical signs of conjunctivitis and, in its later stages, keratitis with corneal edema, vascularization, and possibly ulceration.^{6,7} Spread through a flock is rapid, and the most severely affected eyes can progress to extensive corneal abscesses and panoph-

ABBREVIATIONS

AUC _{plasma}	Area under the drug concentration–time curve in plasma
AUC _{tears}	Area under the drug concentration–time curve in tear fluid
C _{max}	Maximum concentration
MIC	Minimum inhibitory concentration
T _{max}	Time of maximum concentration

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thalmitis.⁸ Although infectious keratoconjunctivitis in sheep is ascribed to various agents such as *Branhamella ovis* and *Chlamydia* spp,^{6,9} data in the literature suggest that *Mycoplasma* spp play a primary causal role in the disease.^{10–12} *Mycoplasma conjunctivae* is a common isolate in outbreaks of the disease, and ocular instillations of pure cultures of this organism can reproduce the disease.^{2,11–15} Other *Mycoplasma* spp such as *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp *mycoides* have been isolated from sheep with keratocon-

junctivitis and other manifestations of the disease.^{7,16} Topically or parenterally administered antimicrobials, such as tetracycline or oxytetracycline, are used in severely affected animals to shorten the clinical course of the disease and reduce the risk of permanent corneal opacification.^{6,7,9}

The rationale for systemic administration of antimicrobials in the treatment of infectious keratoconjunctivitis in sheep has been based on the observation that some antimicrobial agents can diffuse into the lacrimal fluid after parenteral administration¹⁷ and that this route of administration can be clinically effective.^{9,18} Florfenicol, a monofluorinated analogue to thiamphenicol, has greater in vitro activity against pathogenic bacteria than does chloramphenicol or thiamphenicol.^{19,20} In sheep, the drug has a high bioavailability after IM administration,^{21,22} a prolonged half-life of elimination after IM and SC administration,^{22,23} and a large volume of distribution (1.86 L/kg), which suggests good diffusion into body tissues.²² This last feature partly results from the low amount of binding to plasma proteins at therapeutic concentrations,^{24,25} which is an important determinant for diffusion into tears.^{26,27} Excretion of parenterally administered florfenicol in the tear fluid is not confirmed, but studies^{28–30} have found that IM administration of florfenicol is effective in the treatment of infectious keratoconjunctivitis in cattle. Given the possible use of florfenicol in the treatment of infectious keratoconjunctivitis in sheep, the objective of the study reported here was to evaluate the drug's penetration in ovine tears after a single IM and SC administration and determine its MIC for various *Mycoplasma* spp potentially involved in this disease.

Materials and Methods

Animals—Nine healthy Lacaune sheep that weighed 45 to 57 kg and were 2 to 3 years old were included in the study. All animals were nonpregnant females that were allowed to acclimate to their surroundings for at least 2 weeks prior to the start of the study. During the acclimation and study periods, the ewes were housed separately in metabolism cages. They were provided hay and water ad libitum and fed a commercially prepared concentrate mixture once daily. A general physical examination and an ophthalmic examination were performed on each ewe during the acclimation period to rule out preexisting systemic and ocular abnormalities. All animal experiments were performed in compliance with institutional and national guidelines in accordance with the European Community Council directive 86/609/EEC. The experimental protocol was approved by the INRA Toulouse/ENVT ethics committee.

Experimental design—The study was conducted in a crossover design, in which each sheep received florfenicol by the 2 routes of administration in a random order and with a washout period of at least 1 week between the 2 administrations. Florfenicol^a was administered at a dose of 20 mg/kg for each route of administration; all treatments were administered between 9:00 AM and 9:30 AM. The drug was administered IM and SC in the right gluteal muscle mass and in the right lateral

abdominal region (flank), respectively, with a 19-gauge needle.

For each route of administration, blood and tear fluid samples were collected from each sheep before (time 0) and 0.25, 0.50, 1, 2, 3, 4, 6, 8, 10, and 24 hours after florfenicol administration. Blood samples (3 mL) were collected via a 17-gauge polyethylene catheter implanted in the left jugular vein 1 day before drug administration and sample collection. The catheter was flushed with saline (0.9% NaCl) solution containing heparin (10 µg/mL) after each sample. Blood samples were transferred into heparinized tubes, and the plasma was separated by centrifugation (1,000 × g for 10 minutes at 4°C) and stored frozen (−20°C) in polypropylene tubes until analysis. Tear fluid specimens were collected without the use of topical anesthesia, as described elsewhere,^{31,32} with preweighed Schirmer tear strips placed in the tear fluid between the middle and inner third of the lower lid of 1 randomly selected eye of each sheep. Collection was limited to 30 seconds but was stopped before then if the strip became saturated with at least 20 mm of fluid. Each strip was then returned to its corresponding vial and was immediately reweighed. Tear samples were stored frozen at −20°C until analysis.

Analytic procedures—Samples of plasma and tear fluid were analyzed for florfenicol concentrations via high-performance liquid chromatography. An internal standard (prednisolone [0.2 µg/mL])^b and florfenicol were extracted from plasma samples via solid-phase extraction. The high-performance liquid chromatography apparatus consisted of a pump system equipped with an automatic injector and a UV detector set at a wavelength of 224 nm. Separation was achieved via a reverse-phase column^c with a guard column.^d The mobile phase consisted of a mixture of distilled water and acetonitrile (74 parts distilled water:26 parts acetonitrile) and was used at a flow rate of 0.7 mL/min. For these conditions, florfenicol and prednisolone were eluted at retention times of 7.9 and 10.3 minutes, respectively. The quantification limit of the method was 0.2 µg/mL. Accuracy of the assay ranged from 96% to 102%, and coefficients of variation for interday and intraday precision were < 5% for both plasma and tear fluid.

For determination of florfenicol concentrations in tear fluid, 1 mL of internal standard (prednisolone [0.2 µg/mL]) in acetonitrile was added to vials containing the preweighed Schirmer strips impregnated with standard solutions of florfenicol or tear fluids. After evaporation, the dry extract was redissolved in 40 µL of acetonitrile, and 20 µL of the solution then was injected. For the tear fluid, accuracy was 97% to 104% for intraday variation and < 10% for interday variation; the limit of quantification was 0.25 µg/mL. Results obtained for the tear fluid were expressed as micrograms per milliliter, assuming a tear specific gravity of 1, as reported elsewhere.³³

Pharmacokinetic analysis—Pharmacokinetic parameters, except for terminal half-life, were determined via noncompartmental analysis. Terminal half-life after IM administration was estimated via linear regression. The C_{max} and T_{max} were obtained from

the raw data. The AUC and mean residence time were calculated via the arithmetic trapezoidal rule for the experimental period (0 to 24 hours). Drug concentrations in tear fluid were analyzed via a noncompartmental approach. Calculated values included the AUC_{tears} estimated from time 0 to the last measurable concentration in tear fluid, C_{max} in tear fluid, and T_{max} in tear fluid. The extent of penetration of florfenicol into the tear fluid was calculated by dividing AUC_{tears} by the corresponding AUC_{plasma} and was reported as a percentage.

MIC of florfenicol for *Mycoplasma* isolates from small ruminants—Five strains of *M. mycoides* subsp. *mycoides* large colonies, 5 strains of *M. conjunctivae*, and 22 strains of *M. agalactiae* isolated from sheep or goats were used in the study. One reference strain of each species was also evaluated.

Strains were grown in Friis broth medium containing a dye indicator (ie, phenol red), which allowed detection of the metabolic activity of *Mycoplasma* organisms and therefore their growth.³⁴ Strains were incubated at 37°C until there was a relevant color change (from pink to orange-yellow). After addition of 20% (vol/vol) sterile glycerol,^c the cultures were separated into aliquots and stored at -70°C. These cultures were subsequently titrated on Friis agar medium to determine the number of CFUs per milliliter.

The antimicrobial solutions for MIC determinations were prepared by dissolving florfenicol^f in absolute alcohol and subsequently diluting that solution in

water. Solutions were sterilized by filtration through filters with a pore size of 0.22 μm .^g

The MICs were determined by an agar dilution method on Friis medium.^{35,36} Florfenicol concentrations ranged between 0.03 and 64 $\mu\text{g}/\text{mL}$. A multipoint inoculator^h was used to deposit 1 μL of mycoplasmal suspension (10^5 CFUs/mL) on each florfenicol concentration. In addition, the strains were incubated on an agar medium that did not contain antimicrobials. Plate contents were incubated at 37°C in a 5% CO_2 atmosphere for 3 to 5 days (depending on the rapidity of growth of the strains tested). The MIC was defined as the lowest concentration for which no visible growth was observed. Strains were considered susceptible to florfenicol when the MIC was ≤ 4 $\mu\text{g}/\text{mL}$.

Statistical analysis—Data were reported as mean \pm SD. Differences between the pharmacokinetic variables for the IM and SC routes of administration were compared via paired *t* tests. For all comparisons, values of *P* < 0.05 were considered significant.

Results

No adverse effects were observed after florfenicol administration, and all sheep remained healthy throughout the study. Plasma florfenicol kinetic profiles after IM and SC administrations were plotted (Figure 1). The profiles revealed that the absorption rate of florfenicol was significantly faster and plasma drug concentrations were significantly higher for the IM route than for the SC route. A mean \pm SD C_{max} of 6.23 ± 6.16 $\mu\text{g}/\text{mL}$ was detected in plasma at 55 ± 26 minutes after IM administration, compared with a mean C_{max} of 1.32 ± 0.54 $\mu\text{g}/\text{mL}$ at 130 ± 58 minutes after SC administration. The mean terminal half-life was 544 ± 104 minutes after IM administration and was longer after SC administration, but it could not be calculated for SC administration because the terminal portion of the curve was flat. Other major pharmacokinetic determinants derived from data collected after IM and SC administrations were summarized (Table 1).

Tear fluid concentration-time profiles for florfenicol after IM and SC administrations were plotted (Figure 1). Pharmacokinetic parameters in tear fluid determined after IM and SC administration of florfenicol were summarized (Table 2). Overall, florfenicol concentrations in tear fluid were higher and persisted longer after IM administration than after SC administration. A mean \pm SD C_{max} of 2.74 ± 2.72 $\mu\text{g}/\text{mL}$ was detected in tear fluid after IM administration, which

Table 1—Mean \pm SD values describing the disposition of florfenicol in plasma obtained from 9 sheep after a single IM or SC administration at a dose of 20 mg/kg.

Variable	IM administration	SC administration
C_{max} ($\mu\text{g}/\text{mL}$)	6.23 ± 6.16	$1.32 \pm 0.54^*$
T_{max} (min)	55 ± 26	$130 \pm 58^*$
AUC_{plasma} ($\mu\text{g} \cdot \text{min}/\text{mL}$)	$2,544 \pm 1,041$	$990 \pm 434^*$
$MRT_{0-\text{last}}$ (min)	438 ± 74	$575 \pm 121^*$

*Value differs significantly (*P* = 0.01) from the value for IM administration.
 $MRT_{0-\text{last}}$ = Mean residence time from the time of florfenicol administration (time 0) to the time of the last measurable concentration.

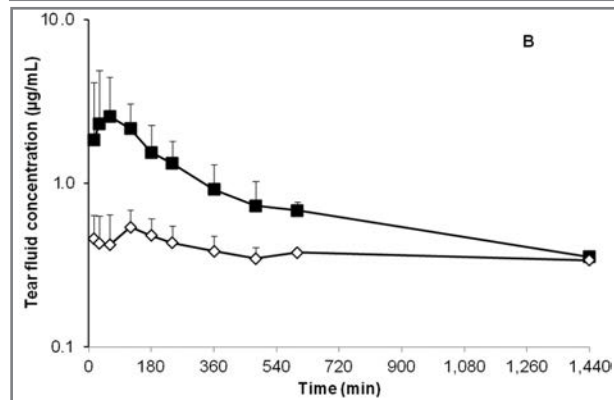
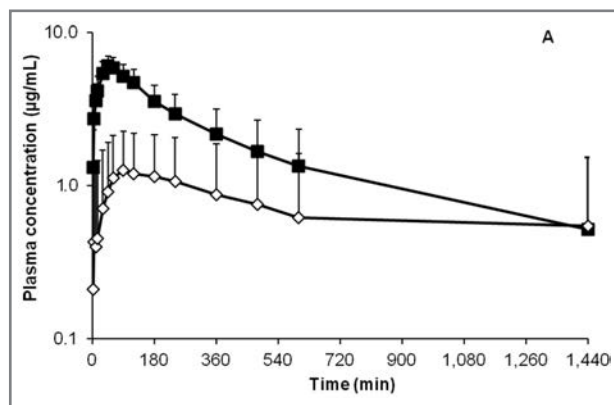


Figure 1—Mean \pm SD florfenicol concentrations in plasma (A) and tear fluid (B) obtained from 9 sheep before (time 0) and at various times after IM (black squares) and SC (white diamonds) administration of 20 mg of florfenicol/kg.

Table 2—Mean \pm SD values describing the disposition of florfenicol in tear fluid obtained from 9 sheep after a single IM or SC administration at a dose of 20 mg/kg.

Variable	IM administration	SC administration
C _{max} (μ g/mL)	2.74 \pm 2.72	0.56 \pm 0.19*
T _{max} (min)	90 \pm 37	127 \pm 56
AUC _{tears} (μ g \cdot min/mL)	1,009 \pm 355	314 \pm 243*
T _{last} (min)	1,053	787
C _{last} (μ g/mL)	0.45	0.34
Mean concentration in tear fluid (μ g/mL)	0.70 \pm 0.24	0.22 \pm 0.17*
AUC _{tears} :AUC _{plasma} ratio	0.40 \pm 0.08	0.32 \pm 0.26

*Value differs significantly ($P < 0.05$) from the value for IM administration.
 C_{last} = Last measurable drug concentration in tear fluid. T_{last} = Time of last measurable drug concentration in tear fluid.

Table 3—The MICs (μ g/mL) for florfenicol against field and type strains of 3 *Mycoplasma* spp isolated from small ruminants in France.

<i>Mycoplasma</i> spp	Type strains*	Field strains		
		Range	MIC ₅₀	MIC ₉₀
<i>Mycoplasma mycoides</i> subsp <i>mycoides</i> large colonies (n = 6)	4	4	4	4
<i>Mycoplasma conjunctivae</i> (n = 6)	2	2–4	2	4
<i>Mycoplasma agalactiae</i> (n = 23)	0.5	1–8	1	4

*Type strains were Y Goat for *M mycoides* subsp *mycoides* large colonies, National Collection of Type Cultures 10123 for *M conjunctivae*, and ATCC 25834 for *M agalactiae*.
 MIC₅₀ = The MIC required to inhibit growth of 50% of strains.
 MIC₉₀ = The MIC required to inhibit growth of 90% of strains.

was significantly higher than that of $0.56 \pm 0.19 \mu\text{g/mL}$ detected after SC administration. By contrast, the T_{max} in tear fluid was not significantly different between the 2 routes of administration, with mean values of 90 ± 37 minutes and 127 ± 56 minutes after IM and SC administration, respectively. A mean AUC_{tears} of $1,009 \pm 355 \mu\text{g}\cdot\text{min/mL}$ was calculated for IM administration, which was significantly higher, compared with that of $314 \pm 243 \mu\text{g}\cdot\text{min/mL}$ calculated for SC administration. The mean drug concentration in tears over the 24-hour period was significantly higher after IM administration ($0.70 \pm 0.24 \mu\text{g/mL}$) than after SC administration ($0.22 \pm 0.17 \mu\text{g/mL}$). The mean time of the last measurable drug concentration in tear fluid was 1,053 minutes (range, 480 to 1,440 minutes) and 787 minutes (range, 240 to 1,440 minutes) after IM and SC administration, respectively. No significant difference was found between the mean florfenicol tear penetration calculated for IM (40.2%) and SC (32.5%) administration.

All the *M conjunctivae* and *M mycoides* strains were susceptible to florfenicol, with MICs between 2 and 4 $\mu\text{g/mL}$ (Table 3). Results obtained for *M agalactiae* were less homogenous, with MICs ranging from 0.5 $\mu\text{g/mL}$ for the type strains to 8 $\mu\text{g/mL}$ for 2 recently isolated field strains.

Discussion

Antimicrobials that can be used to treat infectious keratoconjunctivitis in sheep must have a broad spectrum of activity to cover the wide variety of causative agents, in-

cluding *Mycoplasma* spp, and must provide effective concentrations in the tear fluid after systemic administration. The study reported here was designed to assess the secretion of florfenicol into tear fluid after parenteral administration in sheep and correlate these drug concentrations with the MICs against *Mycoplasma* spp to make predictions on the therapeutic value of systemic administration of florfenicol for the treatment of infectious keratoconjunctivitis in sheep by use of this pharmacokinetic-pharmacodynamic relationship. Although florfenicol is not labeled for use in sheep, pharmacokinetics after IM and SC administration in this species with doses ranging from 20 to 40 mg/kg have been reported.^{21–23} The differences in the blood kinetic behavior of florfenicol between the IM and SC routes of administration in the present study are in agreement with results of those studies.^{21–23} The disposition of florfenicol after IM administration was characterized by a more rapid absorption, a higher plasma C_{max}, and a higher relative bioavailability, compared with the disposition after SC administration. The mean terminal half-life and mean residence time after IM administration were in agreement with findings in other studies,^{21,22} which suggested that IM injection should be the selected mode for florfenicol administration in sheep.

The data in the present study also illustrated the ability of parenterally administered florfenicol to be secreted into the tear fluid of sheep. The mean penetration of florfenicol into ovine tear fluid was 40.2% and 32.5% after IM and SC administration, respectively. By comparison, the amount of tear secretion of kanamycin after IM administration in calves was 9%,³⁷ and mean tear plasma ratios of 6.2%, 9.4%, 8.4%, and 4.3% have been reported in humans after IV administration of gentamicin, tobramycin, amikacin, and netilmicin, respectively.³⁸ Similarly, parenteral administration of a long-acting formulation of oxytetracycline led to low drug concentrations in tear fluid of sheep and calves.^{17,39} Oxytetracycline concentrations in lacrimal fluid were one-seventh to one-tenth those in serum after IV administration in sheep¹⁷ and were $< 1 \mu\text{g/mL}$ even at serum C_{max} following IM administration in calves.³⁹

The ability of florfenicol to distribute into tears may be related to its large volume of distribution, lipophilicity, and low protein binding, given that these features are indicators of diffusion of systemically administered drugs in the eyes and CNS.^{27,38} A study⁴⁰ of CSF obtained from calves after IV administration of florfenicol (20 mg/kg) revealed that the ratio between the area under the curve in CSF and plasma was 46%, a value close to the value of 40.2% calculated in the present study for the AUC_{tears}:AUC_{plasma} ratio after IM administration.

The Schirmer strip technique has been used for tear collection to study the pharmacokinetic profile of ofloxacin, ciprofloxacin, norfloxacin, and tobramycin in human tear fluid^{31,32} and was found to have a comparable accuracy and precision to collection of tears with capillary glass tubes or surgical sponges for quantifying drug concentrations in tear fluid.³³ The Schirmer strip technique was used in the present study because it is easy to perform, can be performed rapidly, and does not cause adverse ocular effects when used repeatedly to obtain samples for pharmacokinetic evaluations.²⁷

Strains isolated from sheep and goats were used for MIC determination because closely related strains circulate between these 2 species in field conditions.^{41,42} The MIC results were similar between sheep and goat strains (data not shown). Currently, there are no MIC breakpoint values approved by the Clinical Laboratory Standard Institute for *Mycoplasma* infections of domestic animals and humans,⁴³ and few studies^{44–47} have been conducted on antimicrobial susceptibility of *M. agalactiae* and *M. mycoides* subsp. *mycoides* large colonies. Strains of these 2 *Mycoplasma* spp were susceptible to various antimicrobials in vitro,^{45–47} but isolates of *M. agalactiae* with elevated MICs have also been identified.⁴⁷

To our knowledge, antimicrobial susceptibilities of *M. conjunctivae* strains have not been investigated. In the study reported here, the MICs of florfenicol for *M. agalactiae*, *M. conjunctivae*, and *M. mycoides* subsp. *mycoides* isolates ranged from 0.5 to 8 µg/mL. Florfenicol MICs obtained in the present study for *M. mycoides* subsp. *mycoides* large colonies are in accordance with values reported for this organism.^{44–46} Although there are no clear guidelines to define effective MICs for *Mycoplasma* spp, interpretative criteria derived from those validated for other pathogens have been proposed.⁴³ For example, it was speculated that the critical breakpoints for determining florfenicol efficacy against respiratory pathogens of cattle (ie, *Pasteurella multocida*, *Mannheimia haemolytica*, and *Haemophilus somnus*) could also represent interpretative data of *Mycoplasma bovis* susceptibility to florfenicol; thus, the argument could be made that when susceptibility data for *Mycoplasma* spp are close to those of other pathogens, they are considered predictive of potential usefulness for treatment.^{43,48} As such, an MIC ≤ 2 µg/mL would be indicative of susceptibility of *Mycoplasma* organisms to that antimicrobial, a value between 2 and 4 µg/mL would be indicative of intermediate susceptibility, and a value ≥ 8 µg/mL would be indicative of resistance to that antimicrobial.^{43,44} According to this breakpoint value, most strains of *M. conjunctivae* and *M. agalactiae* in the present study could be considered susceptible to florfenicol, and 2 strains of *M. agalactiae* could be considered resistant; however, the *M. mycoides* subsp. *mycoides* isolates had intermediate susceptibility.

A rational use of florfenicol to treat infectious keratoconjunctivitis in sheep should be based on sound pharmacokinetic data in the target biophase (ie, the preocular tear fluid) and pharmacodynamic data of the potential pathogens (ie, *Mycoplasma* spp). Florfenicol is a time-dependent antimicrobial, and the surrogate marker that best predicts its clinical efficacy is the time the concentration is higher than the MIC is during 50% to 80% of the dosing interval.⁴⁹ In the context of the present study, this pharmacokinetic-pharmacodynamic index would rely on both the bioavailability in tear fluid after parenteral administration and terminal half-life in the tear fluid. This implies that a florfenicol dosage regimen should be selected that maintains drug concentrations in tear fluid greater than the MICs of *Mycoplasma* spp for at least 50% of the dosing interval to optimize drug exposure for therapeutic efficacy. In the present study, only the IM route of administration achieved flo-

rfenicol concentrations of at least 2 µg/mL in the tear fluid, and analysis of the tear concentration–time curve revealed that these concentrations were maintained for approximately 160 minutes. Thus, analysis of the data in this sample population of healthy sheep indicated that a dose of 20 mg/kg, IM, every 24 hours would be unable to maintain effective drug concentrations in the tear fluid for at least 50% of the dosing interval (ie, 720 minutes) and hence would be inadequate for treatment of infectious keratoconjunctivitis caused by *Mycoplasma* strains.

In cattle, florfenicol can be used for the treatment of infectious keratoconjunctivitis caused by *Moraxella bovis* with a single SC administration of 40 mg/kg or 2 IM administrations of 20 mg/kg 48 hours apart.²⁸ Considering that *M. bovis* (MIC required to inhibit growth of 90% of strains, ≤ 0.5 µg/mL) are more susceptible to florfenicol than are *Mycoplasma* spp and that plasma clearance of florfenicol in cattle (1.5 mL/kg/min) is approximately half that in sheep (2.6 mL/kg/min),^{22,24} the plasma exposure to florfenicol in sheep would be one-half that in cattle for the same total bioavailable dose. Consequently, the florfenicol dose should be doubled in sheep to achieve the same plasma florfenicol exposure as in cattle. Assuming that florfenicol disposition is linear, as suggested by pharmacokinetic data after administrations of 20 and 30 mg/kg in sheep,²² administration of a 40 mg/kg dose to sheep would allow florfenicol concentrations in tear fluid to be maintained at > 2 µg/mL for approximately 320 minutes (ie, 160 minutes × 2) after IM administration. The amount of time that the concentration is greater than the MIC should be at least 50% of the dosing interval; therefore, on the basis of the previous assumptions, florfenicol could be administered IM at 40 mg/kg every 12 hours for microbiological effectiveness against the most susceptible *Mycoplasma* isolates that cause infectious keratoconjunctivitis in sheep.

In the study reported here, florfenicol was administered IM and SC and readily penetrated the tear fluid in sheep, but analysis of the data suggested that a dose of 20 mg/kg was unlikely to be effective against the pathogenic *Mycoplasma* organisms involved in infectious keratoconjunctivitis in sheep. On the basis of tear concentration–time curve data and MICs of common mycoplasmal isolates, IM administration of florfenicol at a rate of 40 mg/kg every 12 hours would appear appropriate for the treatment of infectious keratoconjunctivitis in sheep. Additional studies with diseased animals and an efficacy study are needed to provide adequate treatment information. In addition, considering that we are recommending extralabel drug use, a substantially extended withdrawal period prior to marketing of milk or other edible products should be established via appropriate scientific information or veterinarians should take appropriate measures to ensure that appropriate timeframes for the withdrawal period are met to avoid violative residues.

- a. Nuflor, 300 mg/mL, MSD Santé Animale-Intervet, Beaucazoué, France.
- b. Prednisolone Sigma P-6004, Saint Quentin Fallavier, France.
- c. Inertsil ODS 3, 5 µm, 150 × 4.6 mm, Interchim, Montluçon, France.

- d. Inertsil ODS3, 5 µm, 10 × 3.0 mm, Interchim, Montluçon, France.
- e. Glycerol, Sigma-Aldrich Inc, Saint Quentin Fallavier, France.
- f. Florfenicol, Sigma-Aldrich Inc, Saint Quentin Fallavier, France.
- g. Millipore, Saint Quentin en Yvelines, France.
- h. Denley multipoint inoculator, Fischer Scientific Labosi, Elancourt, France.

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